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PKPD relationships and dose rationale in analgesic drug development : towards the prediction of target engagement

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PKPD RELATIONSHIPS AND DOSE RATIONALE IN ANALGESIC DRUG DEVELOPMENT-TOWARDS THE PREDICTION OF TARGET ENGAGEMENT



Amit Taneja

**PKPD RELATIONSHIPS AND DOSE RATIONALE IN ANALGESIC
DRUG DEVELOPMENT-TOWARDS THE PREDICTION
OF TARGET ENGAGEMENT**

Amit Taneja

COLOFON

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**PKPD RELATIONSHIPS AND DOSE RATIONALE IN ANALGESIC
DRUG DEVELOPMENT-TOWARDS THE PREDICTION
OF TARGET ENGAGEMENT**

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Take risks in your life, If u win you can lead, if you lose, you can guide

-Swami Vivekananda

To Aanchal and Avnay

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SECTION I

**GENERAL INTRODUCTION - TRANSLATIONAL
PHARMACOLOGY OF DRUG EFFECTS IN CHRONIC PAIN**

CHAPTER 1

Challenges in translational drug research in neuropathic and inflammatory pain: Towards a new paradigm

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Submitted

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ABSTRACT

Ongoing research through the past decades has led to an improved understanding of molecular mechanisms involved in pain. Yet there have been negligible tangible gains, and existing analgesic drugs remain limited in terms of efficacy in chronic conditions, with opioid analgesics and NSAIDs still being the mainstay of analgesic therapy. Pharmaceutical R&D activities have in most cases identified new drugs that suppress symptoms, despite the efforts and rationale for treatments that alter the underlying disease processes.

Multiple molecular and cellular mechanisms act concurrently to produce pain symptoms, which in turn are non-specific manifestations of the underlying nociceptive mechanisms. At the biological level, these manifestations can be divided into neuropathic and inflammatory pain. There is however, some overlap amongst the two categories, with inflammatory mechanisms as a common trigger for symptoms in both types of pain.

Despite the evidence for inflammatory components, the assessment of drug effects on neuropathic pain has relied primarily on overt behavioural measures. This situation contrasts with the use of mechanistic biomarkers in inflammatory pain, which has provided the pharmacological basis for dose selection and evaluation of NSAIDs in the treatment of acute and chronic pain.

A new paradigm is required for the identification of relevant targets and candidate molecules in which pain is coupled to the cause of sensorial signal processing dysfunction rather than to the symptom. Furthermore, it must become evident that any behavioural measure of response involves cortical components, which may be unrelated to the neuropathological dysfunction that leads to pain symptoms. Biomarkers are required that enable characterisation of drug binding and target activity. Here we show how a biomarker-guided approach can provide the basis for future pain therapy research. In addition, we show how such biomarkers can be integrated in a systematic manner by the use of pharmacokinetic-pharmacodynamic modelling, enabling the characterisation of exposure-response relationships and consequently of the level of target engagement required in patients.

INTRODUCTION

Chronic pain remains a debilitating condition with high morbidity and heavy impact on the quality of life of patients who experience it. Yet, currently marketed analgesic drugs are at best moderately effective, in that not all patients respond to treatment accordingly [1, 2]. In addition, some drugs are known to cause debilitating side-effects or have been linked to long term safety issues [1, 2]. The search for effective and safe compounds remains therefore a challenge for pharmaceutical R&D.

The current landscape for the development of analgesic drugs

Ongoing research throughout the past decades has led to an improved understanding of molecular mechanisms involved in pain. This is evidenced by the rising number of publications in the aforementioned period which numbered 171,400 in the period between 2000 and 2009. Nevertheless the mainstay of pain treatment continues to focus on opioids and non-steroidal anti-inflammatory mechanisms, with very few novel selective mechanisms effective in clinical practice (e.g., opioids, triptans and coxibs) [3]. In addition, rapid progress has been made in pain genetics, which has led to a better understanding of potential sources of variability in pain perception and nociceptive response [4, 5]. Despite these developments, drug research continues to rely on traditional experimental models of pain which adequately reproduce symptoms, but clearly lack construct validity [6]. In fact, it can be stated that the available models are sensitive enough to detect analgesia, but pain is mostly evoked by external stimuli, leading to response that involves non-specific substrates and consequently to the selection of false positive compounds. One example of such non-specificity is illustrated by the development of aprepitant, an NK1 antagonist which was effective in preclinical species but failed in clinical studies [7].

In the following paragraphs we provide an overview of the issues underpinning the challenges for the development of novel analgesic drugs. Of particular interest is the insight into the molecular mechanisms of pain signalling. We will highlight how further understanding of the pathways and of the reversibility of the mechanisms leading to sensorial dysfunction are critical for the identification of effective treatments. These points are then complemented by a detailed description of the experimental protocols and approaches currently used in the assessment of pain behaviour, which focus primarily on pain perception rather than pain signalling. We conclude the discussion by shedding light on the so-called translational challenge, which has prevented the development of suitable compounds for neuropathic pain. In this context, we emphasise the role of biomarkers and in particular of the need to understand target engagement, reversibility of the underlying dysfunction as well as of the timing of the intervention. An integrated approach is proposed in which not only are treatments aimed at the underlying mechanisms, but diagnosis also takes place before nociception evolves into pain symptoms.

PATHWAYS INVOLVED IN THE ONSET AND MAINTENANCE OF PAIN

The process from tissue injury and inflammation to signal transduction reflects multiple molecular and cellular pathways involved in the processing and perception of pain. This is illustrated Figure 1.1 where the role of known pathways is schematically depicted.

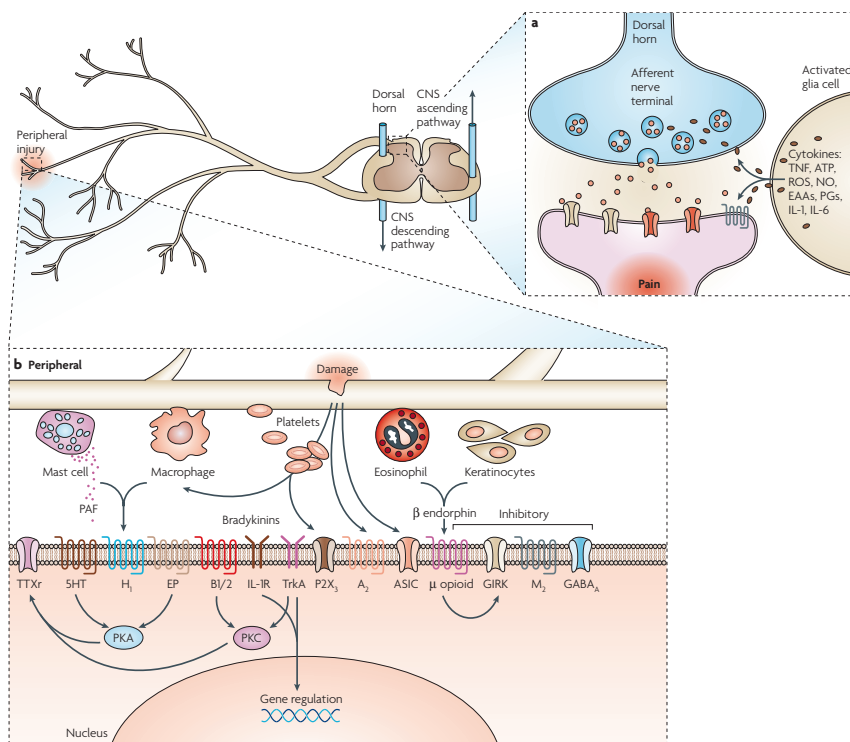


Figure 1.1 : a) Upper panel: Following nerve injury, neurochemical modulation of synaptic transmission occurs in the dorsal horn, post-synaptic receptors and ion channels are activated by excitatory amino acids released pre-synaptically and further sensitised by cytokines from activated glial cells. b) Lower panel: Peripheral mediators of pain transduction after tissue injury. Following tissue injury, mast cells, macrophages and other injured cells directly or indirectly release numerous chemicals that alter sensitivity of receptors and ion channels on peripheral nerve endings. These receptors release secondary messengers such as protein kinase A and C that can activate other membrane bound receptors and gene transcription. A2 =adenosine 2 receptor, ASIC=acid sensing channels, B1/2 =bradykinin receptors, CNS= central nervous system; EAA= excitatory amino acids; EP= prostaglandin E receptor, GABA= γ amino butyric acid; GIRK= G protein coupled inwardly rectifying K⁺; H₁ = histamine receptor; 5HT=5 hydroxy-tryptamine; IL 1/2=interleukins 1/2; M₂= muscarinic 2 receptor; NO= nitric oxide; P₂X₃= purinergic receptor X₃; PAF= platelet activating factor; PGs= prostaglandins; ROS= reactive oxygen species; TNF= tumour necrosis factor; TTXr= tetrodotoxin receptor; TrkA= tyrosine receptor protein kinase A. *Adapted with permission from*[4].

Following cellular or tissue injury, there is an inflammatory reaction which leads to the release of inflammatory mediators that sensitise sensory receptors on peripheral nerve endings. These receptors are known to release secondary messengers such as protein kinase A and C,

which activate other membrane-bound receptors and trigger gene transcription. As shown in the diagram, both the peripheral sensitisation and transduction processes described above can progress into central sensitisation, which reflects a functional and histological change in the afferent fibres that are present in the dorsal horn of the spinal cord.

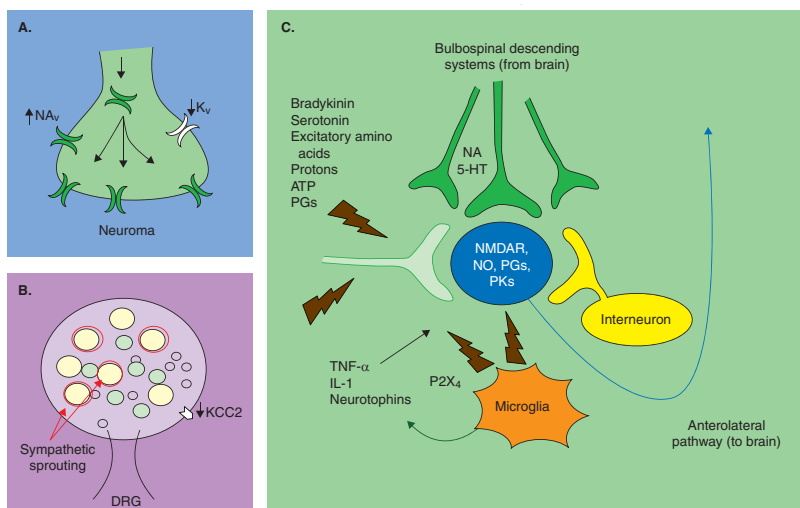


Figure 1.2: NP arises following nerve injury or dysfunction. a): Following nerve damage, transcription and axonal trafficking of Na⁺ channels to the site of injury is increased, with concomitant attenuation of K⁺ channels. The altered expression of ion channels results in hyperexcitable neurons and the generation of ectopic activity, which is thought to lead to the genesis of spontaneous and paroxysmal pain. b) At the cell body of primary afferent neurons within the dorsal root ganglia (DRG), sympathetic neuronal sprouting occurs and may account for sympathetically maintained pain. c) Peripheral nerve injury causes a multitude of changes in gene transcription and activation of various kinases and proteins including enhanced N-methyl-D-aspartate (NMDA) receptor activity. However, nerve injury also elicits hypertrophy and activation of glial cells, including neuroglia within the grey matter of the spinal cord. Microglia expresses P₂X₄ receptors allowing them to be activated by adenosine triphosphate (ATP). Following activation, microglia releases various pronociceptive cytokines, such as interleukin-1(IL-1), tumour necrosis factor (TNF-α), and neurotrophins, including brain-derived neurotrophic factor, which in turn exacerbates nociceptive transmission and contributes to the sensitisation and maintenance of NP. Aβ=A beta neuron, Aδ=A delta neuron, C=C nociceptor, 5 HT=serotonin, KCl₂=chloride transporter, NA=noradrenaline, Nav=sodium channel, NO=nitric oxide, Kv=potassium channel, PGs=prostaglandin, PKs=protein kinases, P₂X₄=purinergic receptors. *Adapted with permission from*[9].

Whilst many of the mechanisms discussed above are applicable to acute and chronic pain conditions, certain important differences need to be considered when evaluating neuropathic pain. The complex pathways involved in the initiation, transmission and maintenance of neuropathic pain (NP) are shown in Figure 1.2. Among many of the changes associated with central hypersensitisation, trafficking of Na channels is increased whilst K channel activity is reduced. Together these changes lead to neuronal hyper-excitability and irregular firing. At the cell bodies of afferent neurons in the dorsal root ganglion, sympathetic neuronal

sprouting occurs and may account for sympathetically mediated pain. Peripheral nerve injury also causes enhanced NMDA activity, glial cell activation and hypertrophy within the spinal cord. Activated microglia expresses P2X₄ receptors and releases pro-nociceptive cytokines such as IL1, TNF- α , neurotrophins, which exacerbate nociceptive transmission and ultimately sustain the symptoms of hypersensitisation. See Figure 1.3.

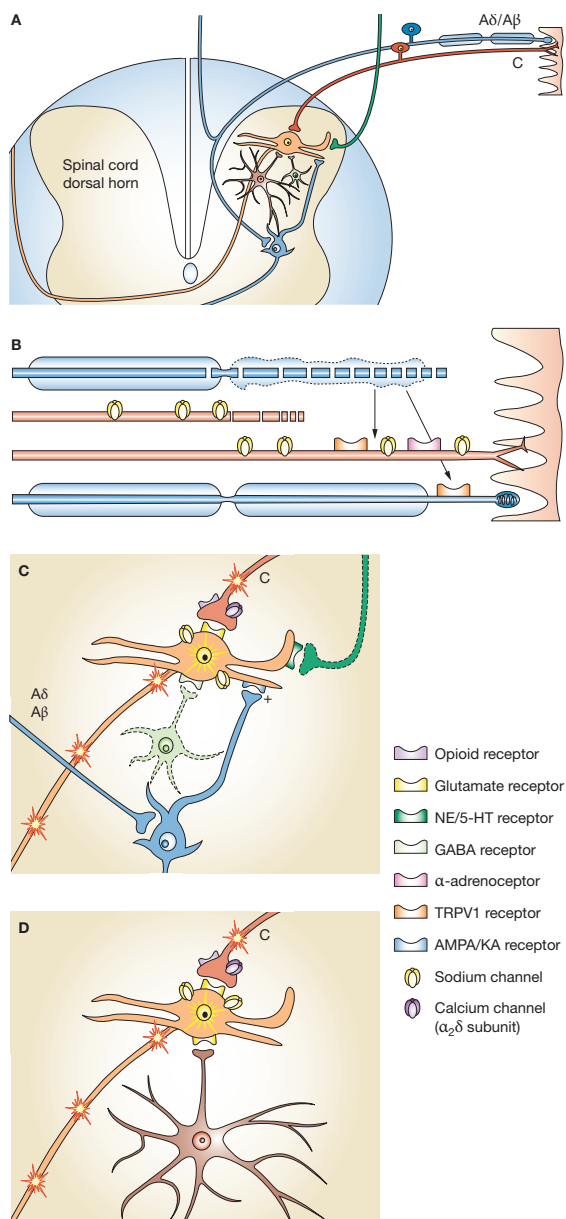


Figure 1.3: Mechanisms of peripheral and central sensitisation in NP a) Primary afferent pathways and their connections in the dorsal horn of the spinal cord. Nociceptive fibres terminate at the spinothalamic projection neurons in the superficial laminae whereas non- nociceptive myelinated A fibres project to deeper laminae. Second-order order projection neurons (WDR) receive direct synaptic input from nociceptive terminals and also from myelinated A fibres. GABA releasing interneurons exert inhibitory synaptic input on the WDR neurons. b) Peripheral changes at primary afferent neurons. Some neurons are damaged and degenerate after partial nerve lesion while others are intact. The lesion triggers the expression of Na⁺ channels on damaged C fibres. Nerve growth factor triggers the expression of Na⁺ channels, TRV₁ receptors, and adrenoceptors on uninjured fibres. c) Spontaneous activity in C nociceptors induces secondary changes in central sensory processing leading to spinal cord hyperexcitability. This causes input from A fibres (light touch and punctuate stimuli) to be perceived as allodynia. Inhibitory interneurons and descending modulation are dysfunctional following nerve lesions. d) Cytokine and glutamate release after peripheral injury further enhances excitability in WDR neurons. *Adapted with permission from*[10].

Clearly, the multiplicity of signalling pathways involved in the onset and maintenance of pain cannot be ignored when devising novel pharmacological interventions. Given the nature and irreversibility of some of the pathophysiological processes, it can be anticipated that effective treatments may not be achievable unless both the timing of intervention and level of engagement of the target(s) are considered.

The molecular pathophysiology of pain

From a pathophysiological standpoint, chronic pain may be subdivided as neuropathic (NP) and inflammatory pain (IP). In the following paragraphs we discuss the various mechanisms associated with pain signalling and perception in chronic pain.

Peripheral and central sensitisation in neuropathic pain

Peripheral sensitisation can result from the sensitisation of nociceptors by inflammatory mediators (e.g., ATP, PGE₂, 5-HT, bradykinin, epinephrine, adenosine), by neurotrophic factors released during tissue damage (e.g., nerve growth factor (NGF)) or by pro-inflammatory cytokines (e.g., interleukin-1 α , 1 β , tumour necrosis factor- α (TNF- α) and COX-2). Peripheral sensitisation is also associated with intense, repeated, or prolonged action potential generation in primary sensory afferents that is mediated by altered expression and activity of voltage-gated sodium and calcium channels [10, 11]. One of the consequences of peripheral sensitisation is a lowering of the activation threshold of nociceptors and an increase in their firing rate, which results in symptoms such as allodynia and hyperalgesia. In addition, these peripheral processes also play an important role in the development and maintenance of central sensitisation [12], which ultimately causes irreversible increased neuronal excitability [13].

While both peripheral and central sensitisation play a role in chronic pain, central sensitisation clearly plays a key role in neuropathic pain. In fact, it explains why established pain is more difficult to suppress than acute pain [11, 12]. Interestingly, not only neurons, but also glial cells (e.g. astrocytes and microglia), as well as infiltrating mast cells are involved in the generation and maintenance of central sensitisation [10]. Among the various mechanisms, four processes should be mentioned that seem to determine the continuous, chronic nature of the symptoms, namely: 1) Release of pro inflammatory neurotransmitters by pathologically sensitised C fibres; 2) Over expression of voltage gated Na-Ca-channels, 3) Loss of supraspinal inhibitory control maintained by γ -aminobutyric acid (GABA) releasing interneurons and 4) Loss in function of descending serotonergic pathways (see Figure 1.2 and Figure 1.3). Central sensitisation is also associated with expansion of dorsal horn neuron receptive fields, reduction in central inhibition and long-lasting spontaneous dorsal horn neuron activity [10, 14]. Such activity leads to sensory response to low intensity stimuli

(altered neural connections following sprouting of A β fibres to superficial laminae). In addition, these changes cause pain signalling to spread to uninjured tissue, i.e., secondary hyperalgesia. This process is known as “wind-up” in that the response of sensitised dorsal horn neurons is exaggerated relative to the normal situation [10, 12].

As mentioned previously, the sensitisation of the nervous system in response to chronic pain involves the alteration and/or activation of many neurotransmitter systems [11, 15]. Clearly, these changes are responsible for a shift in the balance between excitatory and inhibitory systems, which leads to the disruption of normal intracellular signalling cascades. Taken together chronic pain results from a large variety of deranged patterns of neurotransmission with considerable target redundancy. Consequently, even in the absence of sustained injury, chronic pain can progress as a pathophysiological condition or disease in itself. An overview of the inflammatory mediators and neurotransmitters involved in central hypersensitisation is presented in Table 1.1.

Plasticity and other changes in pain processing

Plasticity is a term used to refer to changes that occur in neuronal structure; connections between neurons; and alterations in the quantity and properties of neurotransmitters, receptors, and ion channels that can ultimately result in increased functional activity and ultimately in increased pain. Tissue injury, inflammation, and disease can all induce neuronal plasticity and increased pain by means of increased excitatory or decreased inhibitory mechanisms. An important feature of plasticity is that long-term changes may be permanent. Compelling evidence suggests that plasticity in nociceptors contributes substantially to the increased pain one feels in the presence of injury [14]. Moreover, imaging studies demonstrate fundamental changes in the somatosensory cortical representation and excitability in patients with phantom limb pain, complex regional pain syndromes (CRPS) and central pain syndrome, as well as in experimental pain models [16-19]. Interestingly, these alterations appear to correlate with the intensity of the perceived pain and wane after successful treatment of the pain [20, 21].

Mechanisms of chronic inflammatory pain

Although NP and IP have been clinically and biologically defined as distinct entities, Omoiquei hypothesises that, all pain originates from inflammation, with the different substrates underlying hyperexcitability such as wind-up, neuroplasticity and central sensitisation being considered a continuum from injury to persistent inflammatory response [22]. Obviously, in contrast to neuropathic pain, chronic inflammatory pain (IP) does not involve primary damage to neuronal tissue. It is defined as pain that lasts longer than the expected time that is needed for healing, or pain caused by progressive, non-malignant disease. Typically, inflammatory mediators, originating from arachidonic acid degradation are released from

the site of injury resulting in the transduction of painful stimuli. Although other pathways have been shown to contribute to the onset and maintenance of pain symptoms, it is cyclooxygenase that triggers the production of prostacyclins and thromboxanes. These pathways have determined most of the research activity involving non-steroidal anti-inflammatory drugs.

An overview of the inflammatory cascade is shown in Figure 1.4. Similarly to the phenomenon of central hypersensitisation in which neuronal activity is up-regulated, cyclooxygenase 2 activity is greatly augmented in response to tissue injury. The initial step in the inflammatory cascade is the conversion of arachidonic acid to prostaglandin H_2 (PGH₂), which is the precursor of other prostaglandins and thromboxanes [22]. Increased levels of these lipids leads to various physiological and pathophysiological responses associated with inflammation and pain signalling, including fever. In addition, they are also responsible for the regulation of renal function and maintenance of the mucosal integrity in the stomach. In fact, these homeostatic functions seem to be a differentiating factor between the role of mediators in neuropathic and inflammatory pain. A more comprehensive description of the pathophysiology and mechanisms of inflammatory pain is beyond the scope of this review, but details can be found elsewhere [23].

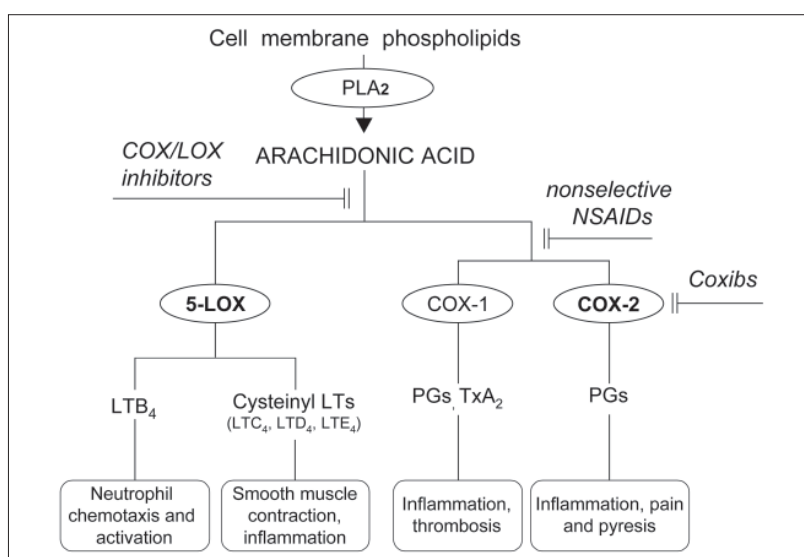


Figure 1.4: Main metabolic pathways associated with arachidonic acid degradation during the inflammatory response. Known targets for anti-inflammatory drugs are also shown. COX=cyclo-oxygenase, 5-LOX=5-lipoxygenase, LTs=leukotrienes, PGs= prostaglandins, PLA₂=phospholipase A₂, TxA₂= thromboxane A₂. Adapted with permission from [22].

In view of the common biochemical substrates for inflammatory and neuropathic pain, it is reasonable to assume that the pathways involved in pain signalling and processing may show significant overlap. In this context, it should be noted that chronic pain must be considered the result of a preceding dysfunction in sensory signalling. The identification of effective treatments requires therefore further insight into the reversibility of the underlying dysfunction as well as the timing of intervention relative to the onset of the disease. These aspects will form the basis for the requirements for translation of drug effects from pre-clinical species to humans.

Pain: from aetiology to syndrome

Despite current focus on the assessment of pain relief and pain intensity, it is the dysfunction in signalling pathways that needs to be characterised and targeted by novel therapeutic interventions. The concept of an underlying dysfunction prior to diagnosis and overt symptoms is not strange to medical practice and is best illustrated by the progression of diabetes into diabetic peripheral neuropathy (DPN), in which the symptoms of neuropathy are clearly a consequence of the underlying disease. In this case, it has been established that both hyperglycaemia and the duration of disease are predisposing factors in the development of DPN[24].

Likewise, dysfunction in pain signalling and subsequent changes due to neuroplasticity is known to precede the appearance of the symptoms of neuropathic pain. Furthermore, it is important to emphasise that the delay between the onset of the disease and overt symptoms is associated with irreversible changes in neuronal activity, which makes the timing of any therapeutic intervention a key factor for the success or failure of treatment.

As can be seen in Figure 1.5, current guidelines for diagnosis and treatment rely on evidence of persistent allodynia and/or hyperalgesia, a phenomenon which develops after the occurrence of sprouting and other relevant changes induced by hypersensitisation and neuroplasticity. Diagnosis and therapeutic interventions at that stage of the disease will be sub-optimal given that the pathophysiological and functional changes that have taken place are likely to be irreversible or cannot be reset by further neuronal remodelling.

Such irreversible changes are common in the course of progressive disease and have triggered the need for different intervention strategies in other therapeutic areas. For instance, the use of imaging has become a powerful diagnostic tool in rheumatoid arthritis and oncology, whilst inflammatory or genetic markers have been used to guide treatment in Crohn's disease and cystic fibrosis. Such an approach does not apply to the diagnosis of neuropathy. In the absence of a well-defined diagnosis, prophylaxis is therefore barely considered; current pharmacological targets cannot offer more than symptomatic relief.

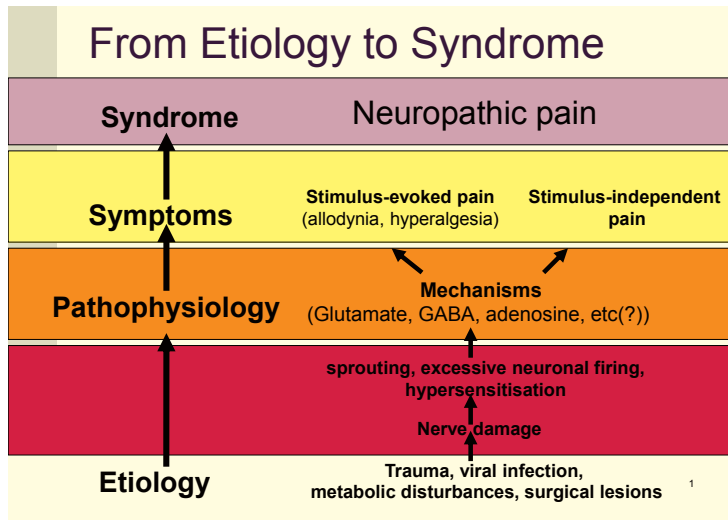


Figure 1.5: A flow diagram showing the progression of an underlying aetiology to the ultimate clinical/outward manifestations of NP. Adapted with permission from [25].

The role of early diagnosis, timing of the intervention and reversibility of the underlying processes cannot be disentangled from each other. The identification of effective targets and therapies must account for these factors. This line of reasoning also contributes to further understanding of the efficacy of treatment in acute inflammatory pain following injury. In the majority of cases, diagnosis (inflammatory reaction) is reasonably immediate relative to onset of the underlying dysfunction, which allows interventions to be initiated before pathophysiological activity induces irreversible changes, such as fibrosis.

Clearly there is a gap between diagnosis, target selection and therapeutic intervention that needs to be addressed to ensure further advancement of the field. The role of functional imaging and other relevant biomarkers describing the underlying pathophysiological changes needs to be considered in the evaluation of efficacy.

These considerations also have major implications for drug discovery, which relies on a paradigm that mimics current standard of care in neuropathic pain. Pre-clinical models of neuropathic pain rely primarily on the suppression of symptoms and on behavioural measures of pain to define efficacious doses. Undeniably, such an approach contrasts with the evolving understanding of disease and creates a paradox or gap in the discovery process in which despite extensive research efforts novel therapies cannot be delivered [26]. In this context, lessons can be learned from other therapeutic areas, in particular from oncology research, where processes associated with tumorigenesis and metastasis has been defined at genetic, cellular, organ and system level [27, 28]. Based on the use of hallmarks as an organizing principle for rationalizing the complexities of neoplastic disease,

six biological capabilities have been identified that describe the multistep development of human tumours. The changes in normal cell function are captured in modules which comprise proliferative signalling, evasion of growth suppression, resistance to cellular death, replicative immortality, angiogenesis, and invasion and metastasis. In addition to the multilevel elements underlying each of the hallmarks, the concepts introduced by Hanahan facilitate the link between biological processes to outcome. From a pharmacological modelling point of view, these elements integrate the time course of disease with drug action [29]. This approach resonates with the point made previously about the importance of the timing of interventions in relation to symptoms and disease progression.

Based on the aforementioned, one would need to approach the treatment of pain in a more mechanistic manner, taking into account the possibility for pre-emptive treatments and prophylactic interventions. Any dysfunction in nociceptive signalling will likely involve sequential recruitment of different inherently dynamic pathways and neurobiological components, including multiple sensory pathways originating in the spinal cord which project to different areas of the brain and requiring cortical activation to determine descending modulation of nociceptive activity [30].

Hence, we defend the view that the earliest hallmark for effective intervention in neuropathic pain is the acknowledgement that dysfunctional signalling is a disease entity in itself [1]. In this sense, it is worth mentioning that a commonly held view was that the nociceptive system was activated in the periphery only by nociceptors in response to an adequate noxious stimulus. Although this is true of nociceptive pain (pain evoked by a noxious stimulus) in normal circumstances, it is certainly incorrect for pain hypersensitivity or spontaneous pain, where different afferent channels can lead to the pain symptoms [6, 25].

The use of dysfunctional signalling as hallmark for the treatment and prevention of pain symptoms entails a different strategy for target identification, screening and selection of compounds in drug discovery. In the next paragraphs we will highlight how current processes and methods contribute to R&D's inability to bridge the gap between our basic understanding of disease and the clinical implications of an intervention.

SCREENING AND SELECTION OF COMPOUNDS IN DRUG DEVELOPMENT

A drug discovery programme begins with target selection, often followed by high-throughput screening and generation of lead compounds. Subsequently, lead optimisation starts taking into account pre-defined developability criteria that are aimed at assessing the drugability of the molecule as well as its safety profile (Figure 1.6) [1]. This approach is primarily aimed at identifying drugs with greater specificity for the target without taking into account the heterogeneity of pain mechanisms or their relative contribution to the progression of underlying signalling dysfunction.

From a conceptual perspective, the aforementioned imposes an approach that accounts for the timing, reversibility and diversity of pathways involved in the onset, progression and maintenance of neuropathic pain symptoms. In practical terms, in addition to early diagnosis and availability of functional markers, this means that drug combinations or molecules with action on different targets and pathways may be required to ensure efficacy in patients [2, 4]. Based on current practice, this requirement also implies that screening procedures will often face high rates of false positive and/or false negatives, even when animal models show some degree of construct validity.

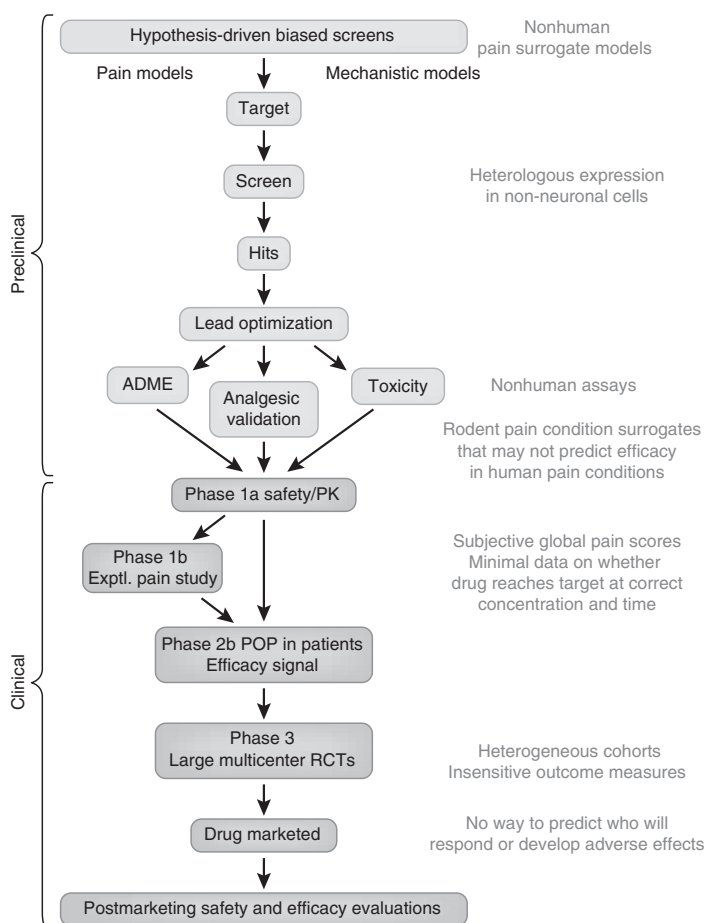


Figure 1.6: Sequential steps used in the discovery and development of analgesic drugs. Typically, R&D efforts start with target selection and end with regulatory approval for the indication in the target patient group. Clearly, failures in phases 2 or 3 are a major cause of attrition, and represent the bulk of losses/expenses in this therapeutic area. Clinical programmes will always fail without informative, predictive models during the screening phase. The lack of construct validity of preclinical models currently used in drug screening, the irreversibility of changes induced by signalling dysfunction and the absence of early diagnostic tools lead to different pharmacological effects in animals and humans. Adapted with permission from [1].

In addition to the aforementioned shortcomings, it should be noted that dose selection in early human studies are based primarily on an empirical criteria, such as the maximum tolerated dose without taking into consideration how differences in exposure correlate with pharmacodynamics and most importantly how systemic drug exposure relates to target engagement. The deficiencies arising from these early clinical studies are further amplified in Phase 2, given that the mechanisms associated with pain in patients may differ considerably from those by which the pain symptoms are induced in animal models of disease [1, 6]. These differences are likely to explain why most failures in Phase 2 are due to lack of efficacy and possibly to limited target engagement [31]. Inadequate exposure at the target site (biophase) is mostly overlooked, as systemic pharmacokinetics may not reflect drug levels in the CNS and the use of functional imaging or positron emission tomography with radiolabelled ligands is not routine practice [1].

Lastly, it should be emphasised that patient inclusion criteria as well as the selection of clinical endpoints to detect pain relief after treatment also play an important role in the attrition observed in the late phases of clinical development. Many of the clinical scales are be insensitive to the underlying pharmacological effects or lack precision to enable accurate dose selection [1]. In addition, factors such as gender, ethnicity, age, temperament and genetic differences are known to contribute to wide inter- and intraindividual variation in pain response [32, 33]. These covariates affect not only pain perception but also alter the tolerance to painful stimuli.

From behavioural measures to functional markers of pain signalling

As can be inferred from the previous paragraphs, the successful identification of efficacious candidate molecules will depend on a number of factors and processes, which should ultimately contribute to clear insight into the nature of the signalling dysfunction, its reversibility and the extent of target engagement observed upon administration of the drug. Such a scrutiny has however never been considered as the basis for the development of analgesic drugs, which has traditionally relied on suppression of behavioural measures of pain. Huntjens *et al* have argued that such measures lack the sensitivity to be able to discriminate between compounds with different pharmacological properties. Also these measures may not necessarily correlate with the time course of inflammatory response [34]. They further argue that behavioural endpoints of pain such as those measured in preclinical models represent a qualitative rather than a quantitative measure of drug effect *in vivo* with little correlation to the underlying mechanisms of action [35]. These views are further corroborated by Woolf, who has eloquently stated that while many pain assessment tools have been developed, they are mainly designed to measure pain intensity and not its identity [1].

Furthermore, laboratory animal models of pain have been essentially designed to mimic pain in humans. Experimental studies are often considered ‘behavioural studies’ in which responses to graded-strength mechanical, thermal, or chemical stimuli (nociceptive) are measured. However, pain measurements are based on the detection of a change in the threshold or response to an applied stimulus, making them unsuitable for the quantification of spontaneous pain, a major feature of disease in humans [25, 36]. In this regard, observed behavioural measures such as reduction of spontaneous activity characteristic of pain as in the formalin induced pain (FIP) [37] or the reduction in spontaneous activity by adjuvant (RSAA) models [38] represent an advantage but yet do not map the changes in spontaneous behaviour to the underlying biological substrates.

Although there are a number of potential mediators associated with neuronal firing and hypersensitisation, identification of the pathway(s) determining the progression of disease remains elusive. Consequently, in the absence of easily measurable markers of signalling dysfunction, behavioural measures have remained the endpoint of choice in the development of analgesic drugs. These difficulties may explain why NK₁ antagonists have shown clear efficacy in preclinical models but failed in clinical trials [7].

The predictive value of animal models of pain

The predictive value of any animal model resides in our ability to understand which mechanisms are involved and which endpoints are measured, so that one can accurately assess and interpret correlations between pharmacokinetics and pharmacodynamics. Yet, there is no unanimity on how well a compound should be expected to perform in animal models before it should be selected for study in patients [39, 40]. Translational studies in animal models and human subjects have identified an association between pathological mechanisms and symptoms such as tactile allodynia in the non-inflamed area and central sensitisation. However, it is not clear if this association represents a mechanistic underpinning for this particular symptom. Thus a critical path analysis is missing to explore if the tactile allodynia is always a consequence of central sensitisation or may also result from other related pathological processes such as sprouting of low threshold afferent terminals in the dorsal horn. A recent critique by van Der Worp *et al.* conclude that whilst animal models have contributed to our understanding of disease mechanisms, in most cases they may not be deemed suitable to inform clinical trials. They attribute the translational failure across species to the methodological flaws in preclinical protocols which cause a systematic bias in the evaluation of drug effects [41].

Apart from considerations of how translatable the preclinical models of disease are, findings from these studies are often confounded by poor experimental design. Meta-analyses of over 100 published studies have revealed that random allocation of treatment was done in less than 28% of the studies, while observer blinding was done in less than 2% of these

publications. Often no formal sample size calculations are done a priori to determine the appropriate number of animals given the expected effect size. In other cases, unplanned interim analyses are performed and experimental protocols continued when interim results are in favour of the working hypothesis. When results show a promising trend, additional data are collected, a practice commonly referred to ‘sampling to a foregone conclusion’[41].

Shortcomings of clinical measures of pain response

As discussed previously for pre-clinical models, the lack of appropriate tools to detect and quantify signalling dysfunction imposes the use of symptoms for diagnosis and pain management in the clinic. Global pain scores which quantify symptom severity provide evidence of the problem, but not its nature[1]. Patients are assessed according to symptom clusters under the assumption that common mechanisms underlie many if not all of the diverse etiological factors eliciting pain. Despite these limitations, subjective pain scales are still considered the gold standard to evaluate pain responses in clinical trials. The assessment of pain symptoms imposes some additional constraints to the evaluation of efficacy above and beyond the fact that the underlying pathophysiological processes may be irreversible. It creates a distortion of the magnitude of the symptoms. A typical visual analogue scale (VAS) is based on a continuous metric ranging from no pain to worst imaginable pain. Peak pain sensation for each individual is based on his/her previous experience which differs widely. As seen in Figure 1.7 a standard VAS would distort this difference by equating the maximum pain for all individuals irrespective of their different subjective experiences[42].

In analgesic trial reports it is also customary to report mean outcomes of global pain rating scales, as these studies are based on a hypothesis testing approach[25]. The differences in mean responses of apparently homogenous populations of patients are construed as evidence of clinical benefit. This is counter-intuitive to the wide inter-individual variability alluded to in the preceding paragraphs. Subsequently, such a ‘group’ response is used as the basis for dose selection and formal assessment of efficacy. The lack of attention to inter individual differences and the concept of a ‘one-dose-fits-all’ means that analgesia is achieved in some patients, in others the same dose could either be ineffective or even toxic. In fact, in many cases such interindividual variability may be caused by differences in the underlying biological substrate. Lee *et al.* showed that variability in gene expression for COX 2 (PTGS2) correlated with pain responses to different analgesics. Subjects homozygous for the gene had a better response to rofecoxib, while the heterozygote responded better to ibuprofen on VAS [43].

Lastly it should become clear to the reader that interindividual variability in pain response may be also explained by differences in target or even systemic exposure to the drug. The lack of pharmacokinetic sampling and sensitive measures of exposure thwart most attempts

to establish exposure-response relationships. In contrast to situations such as anaesthesia, in which clinical response (nociception) is closely linked to systemic levels of an anaesthetic drug, nonlinearity and other time-variant processes make instantaneous circulating concentrations in plasma inappropriate metrics of drug exposure.

In summary, the absence of tools for early diagnosis and the lack of a dose rationale based on target engagement give rise to a chain reaction which prevents the identification of appropriate targets and compounds capable of restoring or blocking the progression of the underlying signalling dysfunction. These limitations are compounded by the fragmented process used throughout the various phases of development. There is little or no opportunity for the enforcement of a learning and confirming paradigm [44]

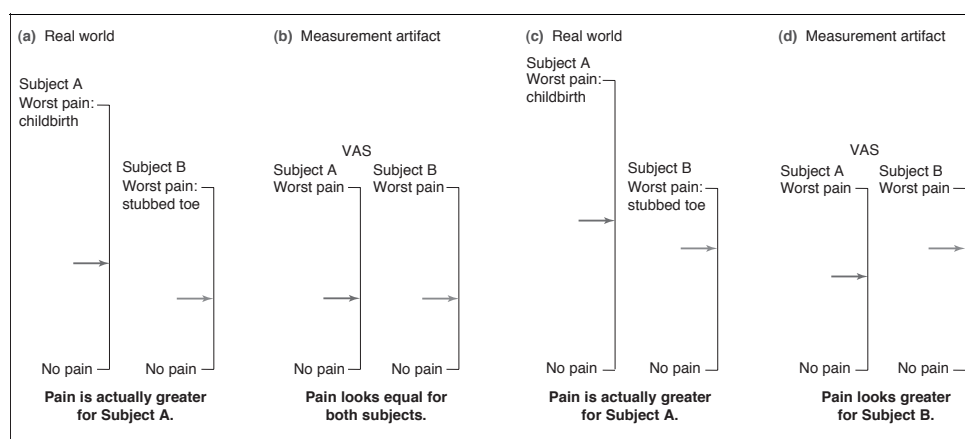


Figure 1.7: Fallacies of pain comparisons using the VAS. If one subject's worst pain is childbirth and another's is a stubbed toe, rating the same point on a scale would result in a discrepancy between the actual magnitude of pain experienced and that reported on a conventional VAS. Thus, as depicted in (a), subject A has experienced greater magnitude of pain than B, it appears that the pain intensity is the same for both subjects. In (c) the discrepancy is compounded. Subject A experiences pain that is only slightly greater than that of subject B. When maximum pain is treated as it were the same for both subjects, the pain depicted by the arrows in (d) erroneously suggests greater pain for B than for A. This is referred to as reversal artefact. Thus a conventional VAS anchored by 'no pain' and 'worst pain imaginable' can conceal real differences in pain intensity across subjects. *Adapted with permission from [32].*

TOWARDS A NEW PARADIGM

The focus of this review was a critical appraisal of the reasons why analgesic drug development is plagued by high failure rates. Despite the few landmark publications in which a roadmap is proposed for the development of analgesic drugs [1, 4, 45], most of the new strategies overlook some of the conceptual elements highlighted in the various sections of this review. Our purpose is not to dispute the proposals put forth in the aforementioned publications, but focus on a few workable and practical aspects which are urgently required even in the current drug development paradigm.

The role of biomarkers

Morgan *et al* have summarised three elements that need to be demonstrated for a development candidate to survive all phases of development. These are 1) exposure at the target site of action over a desired period of time; 2) binding to the pharmacological target as expected for its mode of action and 3) expression of pharmacological activity commensurate with the demonstrated targeted exposure and target binding [31]. In conjunction with integrative techniques, such as mathematical modelling, we envisage that a biomarker guided strategy can play a central role in dose selection and in the screening of new candidate molecules.

A biomarker as defined by the Biomarkers Definitions Working Group is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. They can be classified as predictive markers or markers of pharmacology, when used early on in development and as prognostic markers or markers of disease/clinical response, when used in the later phases [46]. In early development the availability of markers of pharmacology can provide evidence of target engagement and activation. Such biomarkers can be used as the basis for establishing exposure–response relationships, especially for progression from Phase 1 to Phase 2 studies.

In a concept allied to the three pillars of survival, Hargreaves *et al.* have categorised biomarkers into target, mechanism and clinical response. They stress that biomarkers should be deployed as early as possible first to confirm target engagement and then to test whether target engagement alters the pathophysiological processes downstream and subsequently whether this mechanism affects the clinical response [47]. In addition to this functional classification, Danhof *et al.* have proposed a seven point mechanistic classification based on the location in the chain of events from underlying subject genotype or phenotype through to clinical scales [48]. An example of the concept is the KRAS mutation in advanced colorectal cancer which has been demonstrated in multiple trials to predict a lack of effect of monoclonal antibodies [46]. An application of such biomarkers is to optimize patient selection, wherein only those patients predicted to benefit most are enrolled in the clinical trial, i.e., in this case patients with HER2/*neu* positive gastric cancer are most likely to respond to trastuzumab therapy [46].

It should also be noted that in conjunction with mathematical modelling techniques, these classifications provide a framework for defining and discriminating drug from system-specific properties. Such information can be used for inferences, extrapolations and hypothesis generation when evaluating novel molecules or exploring the efficacious dose range. An inherent challenge here is the level of evidence available to demonstrate the correlations between biomarker and response are causative and biologically consistent across different stages of disease [49]. This challenge is very pertinent to the assessment of the underlying signalling dysfunction which precedes the symptoms of neuropathic pain.

Ideally, analogously to the use of thromboxane B_2 and prostaglandins E_2 as biomarkers for the evaluation of anti-inflammatory drugs acting on the arachidonic acid cascade, mediators or other functional measures are required that describe target engagement in nociceptive pathways. Such markers can subsequently serve as a tool for differentiating the sensitivity of other physiological and behavioural measures arising from signalling dysfunction. In fact, Huntjens *et al.* have given examples of how drug effects on biomarkers unravel differences in the sensitivity of behavioural measures to the selectivity of COX inhibitors [35].

In contrast to the developments observed in the evaluation of anti-inflammatory drugs, potential biomarkers such as glutamate, endocannabinoids, GABA or cyclo-oxygenase were identified but ultimately failed to provide qualitative and quantitative information on underlying processes[2]. None of these markers appear to satisfy the essential requirements for establishing a biomarker i.e. expression of the pharmacology or pathophysiology, feasibility, clinical relevance and ease of use [47]. Notwithstanding these failures, promising results have been observed with functional imaging techniques, such as functional magnetic resonance (fMRI), which allows characterisation of nociceptive phenotypes and positron emission tomography (PET), which yields reliable measures of receptor occupancy. Challenge models have also been considered as an alternative to the evaluation of disease processes under controlled conditions, such as the induction of secondary allodynia and hyperalgesia following subcutaneous or topical administration of capsaicin [45]. However, none of these markers have yet been adopted as mainstream technologies for the development of analgesic drugs. Their application in drug development requires similar efforts in medical practice, as clinical criteria will have to consider early diagnosis and prophylaxis. Similar awareness has evolved in the evaluation of drugs for Alzheimer's disease, where interventions aimed at improving cognitive function are probably unlikely to prevent or mitigate the impact of brain tissue loss [50].

Modelling and simulation

A discussion on biomarkers cannot be complete without highlighting their role in model-based drug development. In contrast to empirical evidence, the central focus of model-based drug development is to use mathematical and statistical models that describe biological system and drug properties in a quantitative manner. Hierarchical or population models are among the various approaches currently used. An important property of hierarchical models is the ability to describe variability at individual level by identifying stochastic distributions that describe within and between-subject differences. Subsequently, these models can be used for inferences about the role of distinct components of a biological system as well as for making predictions about treatment effects and disease progression.

Prior to any modelling activities, efforts are required to clearly identify the modelling goals, understand the statistical requirements and evaluate the most suitable parameterisation to solve the questions relevant to the modelling exercise[44]. This is an iterative process which

consists of the following steps: knowledge gathering, parameterisation and model building, parameter estimation, model validation and prediction or extrapolation by simulation or simulation scenarios [51]. At the simplest level of implementation, pharmacokinetic-pharmacodynamic (PKPD) models provide the ability to relate the drug exposure to the time-course of the pharmacological effects (or side-effects) [52]. Given the role of absorption and distribution processes as well as the presence of functional barriers, pharmacokinetic equilibration models can be incorporated into the analysis to ensure accurate description of drug disposition properties, enabling inferences about drug exposure at the biophase (target site). Furthermore, models also allow correlations to be established when nonlinear processes are required to describe signal transduction or disease progression, both of which are associated with delays between the pharmacological effect and the time course of drug concentrations. Overall, one of the major advantages of a model-based approach is the opportunity to leverage prior information and integrate historical data in a more robust manner. Existing scientific knowledge may be incorporated in the analysis of experimental data through deterministic or stochastic parameters (e.g., informative prior probability distributions) ,(see Figure 1.8)[51].

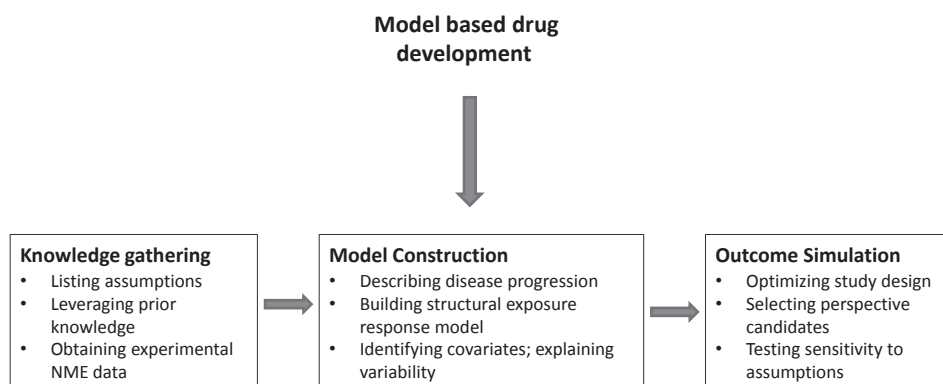


Figure 1.8: Main steps for the implementation of model-based approaches in drug development. NME=New molecular entity. *Adapted with permission from [51].*

Pertinent to the utilisation of biomarkers in drug discovery and development is the role of mechanism-based PKPD models, which contain specific expressions to characterise, in a strictly quantitative manner, processes on the causal path between drug administration and effect. This includes distribution to the target site, interaction with and activation of the target, transduction and influence of in vivo homeostatic feedback mechanisms [48]. Of particular relevance is that mechanism-based models facilitate the integration of information, including pooling of data from different experimental conditions. Using the appropriate choice of parameterisation it is possible not only to distinguish drug from

disease specific properties, but also to evaluate the impact of influential covariates on pharmacokinetics, pharmacodynamics and disease.

Another important dimension of model-based approaches is the use of models as a design and optimisation tool. In conjunction with other relevant statistical models, it is possible to explore trial or protocol-related issues such as, sampling requirement, drop-out, compliance as well as the statistical power to detect a predetermined treatment effect size [53].

One must also realise that not all experimental protocols are equally informative, irrespective of how accurate they are. In addition to the use of prior information, the use of a hierarchical model enables one to cope with experimental limitations, such as design imbalance and sparse sampling. In pain research, pharmacokinetic information is barely considered due to the potential interference pharmacokinetic sampling represents to behavioural experiments. Information from a satellite cohort can be complemented with very sparse samples from the actual treatment group providing evidence of differences in individual exposure, instead of relying on the dose or satellite data to describe pain response and the underlying exposure-response relationships [48].

The same principles apply to experimental design issues in clinical development. Efficacy trials with analgesic drugs barely take systemic or target tissue exposure into account. Attempts have been made to establish a dose-response relationship, without full understanding of the implication of intra and interindividual variability in pharmacokinetics and pharmacodynamics. The use of hierarchical models allows pharmacokinetic data from phase 1 studies to be integrated with sparse blood sampling from efficacy trials. This approach contributes to greater understanding of the role of pharmacokinetic variability on the observed individual differences in biomarkers and clinical response.

The availability of a validated PKPD model also provides the basis for further optimisation of experimental protocols by exploring what-if scenarios. In contrast to meta-analysis, clinical trial simulation (CTS) allows for the investigation of a range of design characteristics on the power to detect a treatment effect prior to exposing patients to an experimental drug. In a field where most clinical trials have a conservative design, this methodology offers a unique opportunity to evaluate innovative designs.

In general, CTS utilises two types of models. First, a drug-action (PKPD) model is considered, which comprises pharmacokinetic and pharmacodynamic factors. In chronic diseases the model also accounts for disease progression. Unfortunately, the lack of knowledge about the mechanisms underlying treatment response in many therapeutic indications has prevented the development of mechanistic PKPD models, as is the case for chronic pain populations. Secondly, CTS requires a trial execution model. These models simulate other important aspects of the trial, such as dropout and protocol deviations. Thereby, one can determine all possible outcomes under candidate trial designs. It is also important to stress that CTS allows investigation of factors that cannot be scrutinised by meta-analysis or empirical design. First, designs which have not been implemented cannot be included in

a meta-analysis. Second, it is difficult to separate the influence of multiple design factors, whereas CTS allows evaluation of a single factor at a time.

One of the main advantages of such a virtual or statistical experiment is the possibility to predict 'trial performance' and so to identify potential limitations in study and protocol design prior to its implementation. Regrettably, PKPD modelling and clinical trial simulations have been applied only sporadically in pain research. Data in the published literature suggests that such efforts were made to answer specific research questions, rather than used as the basis for a new paradigm or strategy [54].

CONCLUSIONS

There are several methodological issues that hinder the development of novel medications for the treatment of chronic pain. Essentially those issues can be clustered around a common denominator in that they are related to the construct validity of the experimental protocols used to assess drug effects. Multiple molecular and cellular mechanisms act concurrently to produce pain symptoms, which in turn are non-specific manifestations of the underlying nociceptive mechanisms. Most pain research has focused on transient behavioural models of pain that do not necessarily reflect what is occurring in a chronic pain patient. It is important to understand the changes in the nervous system that result in the pain experience and consider the need for interventions before symptoms evolve. It follows that the appropriate measures of patient response are crucial in establishing a pattern of response, or lack thereof. On the other hand, studies focusing solely on chronic pain have overlooked the fact that such conditions may require prophylaxis rather than symptomatic intervention only. There are certainly missed opportunities whereby central sensitisation can be interrupted, effectively halting the metamorphosis of acute injury to chronic pain.

A new paradigm is required for the identification of relevant targets and candidate molecules in which pain is coupled to the cause of sensorial signalling dysfunction rather than to the symptoms. Furthermore, biomarkers are required that enable characterisation of drug binding and target activity. We envisage the development of a biomarker-guided approach, by which target engagement is used as the basis for future pain research. Given that experimental limitations in this field cannot be completely eradicated, the success of such a biomarker-guided approach will also depend on scientific efforts to incorporate inferential methods by mathematical and statistical modelling and simulation. Biomarkers can be integrated in a systematic manner by pharmacokinetic-pharmacodynamic modelling, enabling the characterisation of exposure-response relationships and consequently providing a mechanistic underpinning be it for the purpose of interspecies translation or determination of the therapeutic dose levels in patients.

Table 1.1:Functional Components of neuropathic pain pathways,key anatomical substrates and their importance.

| Process and underlying mechanism | Major neurotransmitter/s (target /tissue) | Time of release / activation | Consequences | Importance/ Remarks |
|---|--|---|--|---|
| <i>Pain signalling/peripheral sensitisation at primary afferent neurons</i> | | | | |
| -Peripheral nociceptor sensitisation/hyperexcitability | Substance P (Receptors on peripheral terminals (NK1 receptors), plasma membrane of cell bodies, dendrites of non-stimulated neurons)[1, 2] | Early in the induction of NP. | Sensitisation of peripheral terminals increased firing rate. Induction of NP state | Provides a basis for hyperalgesia by causing central sensitisation in the FCA model, highest concentrations were seen at day 21, following algogen injection [3, 4] |
| - Release of excitatory amino acids (EAA) | | | Release of TNF- α | |
| -Following tissue injury is released by macrophages and nerve cell | Cytokines (Receptors on blood monocytes) | Early, within 24 hrs of the inflammatory response | Mediates the septic state | Ectopic hyper-excitability due to increase in nerve cell communication resulting in a vicious cycle of inflammation |
| -Inflammation (active macrophage infiltrate) | TNF- α | | Activation of endothelium and release of platelet derived growth factor (PGDF) | |
| -Activation of phospholipase A ₂ enzyme on cell membranes causes release of arachidonic acid from the cell membrane phospholipid | | | Increase in PG concentrations which in turn increase production of excitatory neurotransmitter glutamate | TNF- α is the primary inflammatory mediator involved in certain nerve injuries as lumbar disc herniation |
| -This triggers 2 competing pathways the cyclo-oxygenase (COX) and the lipo-oxygenase (LOX) | COX pathway Prostaglandins (Peripheral nociceptors, PGE2 receptors in smooth muscle) Thromboxane (TXA2 receptors on platelets) | Peak concentrations in the FCA model are seen around day14-25 following algogen injection | Sensitisation of peripheral nociceptors, localized pain, hypersensitivity in uninjured tissue | |

| Process and underlying mechanism | Major neurotransmitter/s (target /tissue) | Time of release / activation | Consequences | Importance/ Remarks |
|--|---|---|---|---|
| -Release of the interleukins | LOX pathway Leukotrienes (receptors on smooth muscle) | | LT ⁺ -Induction of platelet activation and constriction of smooth muscle Increased vascular permeability and leukocyte attraction | |
| | Others Interleukins (IL- β , IL-6, IL-8, IL-10) [5](Osteoclasts, local pain receptors) | Within the first few hours of tissue injury | Stimulation of the production of pro-inflammatory mediators such as PGE ₂ , COX-2, matrix metallo-proteases (MMP) Bone resorption and Cartilage damage correlated with intensity of mechanical allodynia following nerve injury in experimental animals | While IL-6 is the primary chemical mediator in bone inflammation and pain, IL-10 is a natural anti-inflammatory cytokine. The net inflammatory effect is the resultant of these opposing effects. |
| <i>Pain processing</i> | | | | |
| Central sensitisation (spinal cord) | Glutamate (Presynaptic; μ , glutamate receptors) | Unknown | Dynamic mechanical allodynia | Spread of spinal hyper-excitability |
| Phenotypical switch Nociceptor peptides normally expressed by A δ and C fibres are expressed by large myelinated A α s fibres | Substance P (Calcium channels-(α_2 - δ)) Protein kinase C (NMDA receptors) | | Punctate mechanical allodynia | Expansion of neuronal fields[6, 7] |
| | Calcitonin gene-related peptide, substance P (dorsal horn receptors) | Unknown | Input from mechanoreceptor A fibres is perceived as pain (dynamic and punctuate allodynia) | Increased synaptic transmission (Most important/pivotal steps in the critical pathway)[8] |
| Descending dysinhibition | GABA (GABA receptors) Endogenous opioids (μ receptors) | | Loss of inhibitory synaptic currents | Selective apoptotic loss of GABAergic neurons in superficial dorsal horn of the spinal cord |

| Process and underlying mechanism | Major neurotransmitter/s (target /tissue) | Time of release / activation | Consequences | Importance/ Remarks |
|--|---|---------------------------------|---|--|
| Functional degeneration of interspinal inhibitory interneurons Decreased supraspinal descending modulation Descending facilitation | Serotonin/NE/DA (α -2, 5-HT Dorsal horn inhibitory interneurons) Glutamate(respective receptors) | | Enhanced signal transmission in the DRG | Inhibition or prevention of this apoptotic loss could provide disease modifying effect in NP structures in the mesencephalic reticular formation – possibly the nucleus cuneiformis and the periaqueductal gray area are involved in central sensitisation in neuropathic pain. [8] Interestingly, advanced functional MRI (fMRI) techniques show that the same brainstem structures are active in humans with allodynia |
| <i>Pain perception/plasticity in the brain</i> | | | | |
| | | | Maintain synaptic plasticity. Develop and maintain inflammatory hyperalgesia | Similar changes occur in the brain, particularly in the cortex and can be measured experimentally and by functional magnetic resonance Imaging or PET. Dramatic alterations in cortical spatial maps can be detected after nerve injury that may contribute to phantom pains[9] |

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CHAPTER 2

Translation of drug effects from experimental models of neuropathic pain and analgesia to humans

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ABSTRACT

Neuropathic pain research remains a challenging undertaking due to the i) lack of understanding of the underlying disease processes and ii) poor predictive validity of the current models of evoked pain used for the screening of novel compounds. Common consensus is that experimental models replicate symptoms, i.e., have face validity but no construct validity. Another issue that requires attention is the sensitivity of endpoints to discriminate drug effects which are relevant to the disease in humans. In this paper we provide an overview of the pre-clinical models which can be used in conjunction with a model-based approach to facilitate the prediction of drug effects in humans. Our findings strongly suggest that evidence of the concentration-effect relationships is necessary for translational purposes.

BACKGROUND

Physiologic pain usually arises as a result of the activation of nociceptive afferents by noxious stimuli which can lead to tissue damage, as e.g., during inflammation. Pain may also occur in the absence of or by sub-threshold stimulation of peripheral nociceptors. This type of pain is called neuropathic pain (NP). The International Association of the Study of Pain (IASP) defines NP as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [1]. Following tissue injury, pro-nociceptive mediators are released that sensitise peripheral nerve terminals, which in turn trigger neurochemical and phenotypic alterations of the sensory nerves and increased excitability of spinal dorsal horn neurons (central sensitisation) [2]. Common causes of nerve damage and subsequent pain response include metabolic diseases, infection, ischemia, traumatic injury, malignancy, adverse drug reaction and toxins. However, the cause of neuropathic pain symptoms may not be easily linked to a cause in the majority of patients who experience it [3]. The lack of a clear aetiology has major consequences for the selection of treatments. In addition, it underlies some of the main hurdles in target identification and compound screening in drug discovery. Current efforts in research and development (R&D) therefore predominantly focus on the suppression of symptoms, rather than on the treatment of the primary cause(s) of pain [4]. In addition, these drugs often produce unacceptable side effects, making the clinical management of pain syndromes a major challenge [5].

The hallmark of NP is impaired sensation, with sensory dysfunction manifesting itself as hypo or hyperaesthesia for one or more modalities [6]. As a matter of fact, heightened pain perception to harmful painful stimuli (hyperalgesia) or to normally painless stimuli (allodynia) are often accompanied by spontaneous pains such as burning or electric shock-like pain, paroxysms and dysaesthesia [7, 8]. Clinically, symptoms are classified into positive and negative. This classification, which was primarily developed for diagnostic purposes, has also driven the choice of endpoints in clinical research protocols. Positive symptoms include mechanical and thermal allodynia and hyperalgesia, temporal and spatial summation, whilst negative symptoms are indicative of loss of sensory and motor function [9, 10].

Despite the symptomatic nature of drug therapy, existing treatments are suboptimal, with effective reduction in pain by an average of 40-50% from baseline in only 30 to 40% of patients [4, 11]. Available treatment options include opioids, anticonvulsants and antidepressants, all of which have a direct or indirect suppressant effect on neuronal activity. Besides limited efficacy, these compounds often cause dose limiting toxicities that, along with impairment in the quality of life, prevent titration to effective dose ranges [4]. From a drug discovery perspective, these findings are associated with a high attrition rate in the progression of candidate molecules. Whilst the overall probability of a candidate in development being approved is approximately 11%, the figure for NP candidates is as low as 3-5% [12].

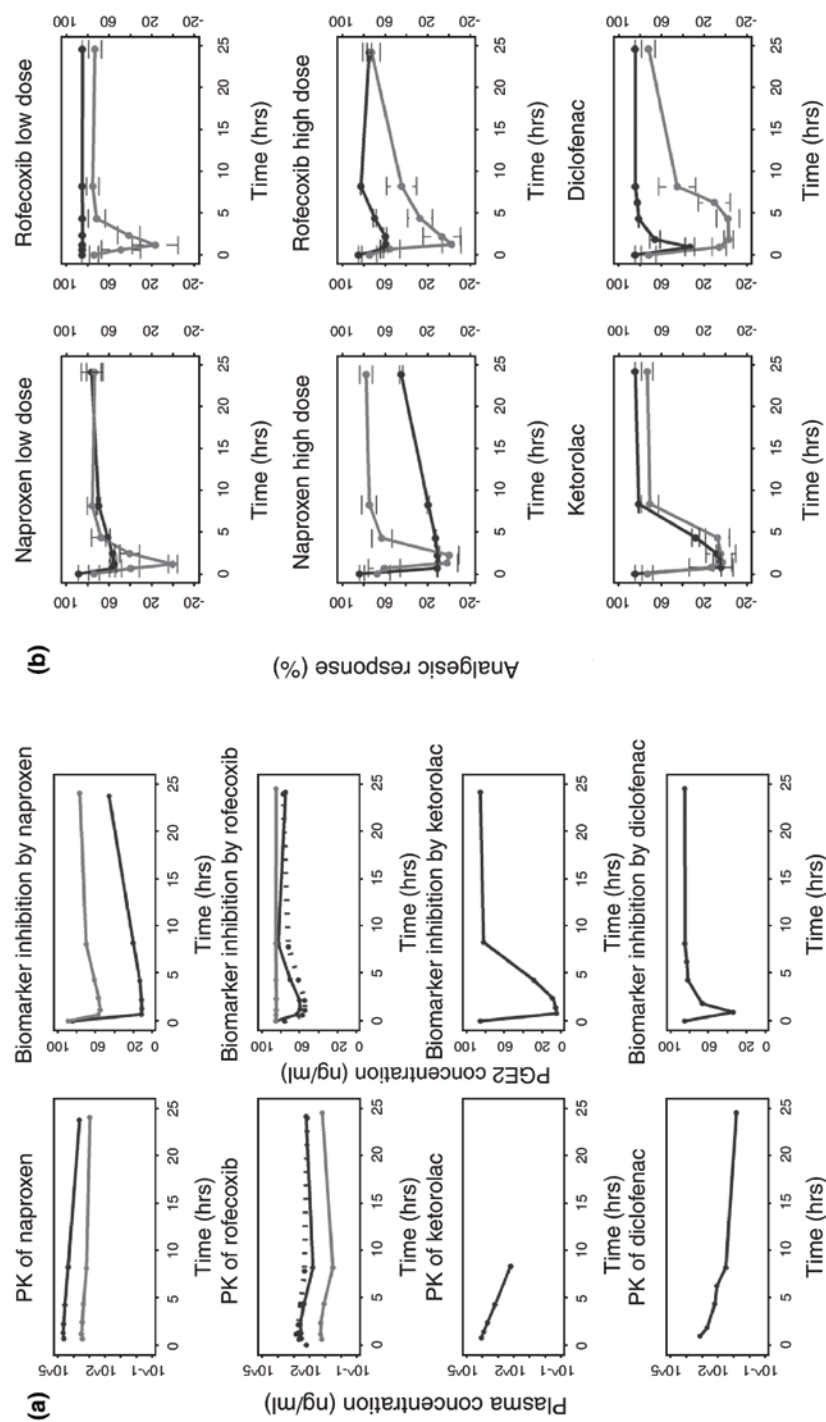


Figure 2.1: (Left panel) Simulated concentration time course and corresponding PGE₂ profiles following COX-inhibition by naproxen, rofecoxib, ketorolac and diclofenac. (Right panel) Time course of the predicted PGE₂ levels and corresponding analgesic response profiles in the FCA model of inflammatory pain. PKPD modelling of the anti-hyperalgesic effect shows that changes in paw pressure withdrawal threshold are nonlinearly correlated with PGE₂ inhibition. Such nonlinearity between pharmacological effects and behavioural measures must be considered when defining the dose rationale in humans. Data is presented as mean ± St. Dev. (Slightly modified with permission from [13].)

SCREENING AND DEVELOPMENT OF ANALGESIC DRUGS

From the above, it is evident that challenges exist not only in the identification of suitable targets but also in predicting *a priori* the efficacy of compounds in humans. Given the gaps in the understanding of the mechanisms underlying neuropathic pain disorders, drugs are tested pre-clinically without a hint of which target will yield a clinically relevant response[11]. This is further reinforced by the paradigm currently used for the screening of compounds, which relies on evoked-pain response associated with general positive symptoms such as allodynia and hyperalgesia. These models enable characterisation of the behavioural expression of pain processing, rather than specific features of the mechanisms of hypersensitisation[12, 13].

Advancements in this field require a clear distinction between the role of target, endpoints and drug properties, as primary causes of attrition. In this article we will explore the value of pharmacokinetic-pharmacodynamic (PKPD) modelling in conjunction with pre-clinical models of neuropathic pain as a tool for the translation of pre-clinical findings. This concept has been previously illustrated for the evaluation of the anti-inflammatory and anti-hyperalgesic effects of different COX-inhibitors [13, 14], (Figure 2.1).

Likewise, we envisage the use of model-based estimates of potency and efficacy as the basis for the dose rationale and improved prediction of efficacy in humans. Such an approach allows discrimination of drug-related factors from other causes of attrition in early drug development.

Animal Models and pain signalling and processing

In this review, the classification and underlying pathophysiology of pain are contextualised in terms of the various experimental models of evoked-pain (Figure 2.2) and common behavioural endpoints[15]. To this end, we refer to the mechanisms of pain signalling and processing, which have been described as a continuum with three phases, specifically from nociceptive (Phase I) to inflammatory (Phase 2) and finally neuropathic pain (Phase 3). A correlation between the pain phases and the various models and measures is given below as basis for further discussions on the choices of parameterisation for pharmacokinetic-pharmacodynamic models of pain.

The first phase within this continuum involves transient nociception and results from a noxious stimulus. Thus, at this stage there is minimal inflammatory response. Animal models of acute pain measure behavioural responses of naïve animals to noxious stimuli. Transmission of the stimulus occurs across A- δ and C fibres, which propagate fast and slow nociceptive response respectively [16]. Noxious heat is the most common stimulus applied in these models whereby the analgesic effects of drugs may be measured [17]

Phase 2 pain or inflammatory pain results from tissue damage and inflammation secondary

to a noxious stimulus. In this phase, there is input from the damaged fibres to the CNS. Tissue injury causes mediator release creating an '*inflammatory soup*', which up-regulates or activates nociceptive afferents. This barrage of information to the spinal cord triggers hyperresponsiveness of dorsal horn neurons [18]. A consequence of this hyperactivity is the development of symptoms such as hyperalgesia (an increased response to noxious stimuli) and allodynia (a painful sensation to non-noxious stimuli). Hyperalgesia and allodynia may be primary (i.e., close to the damaged area) or secondary (i.e., in tissues away from the damaged area). Secondary occurrence of these symptoms is indicative of central (spinal cord) sensitisation[16]. The neural pathways for transmission of this type of pain are similar to that for nociceptive pain. However, the spinal excitability is due to release of inflammatory neuropeptides from the activated C type primary afferents. Animal models that involve application of an inflammatory agent (formalin, capsaicin, mustard oil, carrageenan or CFA) elicit hyperalgesia and allodynia, thus mimicking phase 2 pain[16].

Phase 3 or neuropathic pain arises from lesions to or dysfunction of the nervous system, and thus all or part of the afferent input comes from damaged neurons. It manifests as spontaneous pain (stimulus-independent pain) as well as secondary hyperalgesia and allodynia [16, 18]. Animal models of NP in which phase 3 pain is elicited include partial or complete denervation of the sciatic nerve or of the spinal L5/6 nerves. In fact, partial denervation methods are widely used in NP research due to their resemblance to human NP. The Bennett, Seltzer and the Chung models are some of the common animal models used to describe this phenomenon[16].

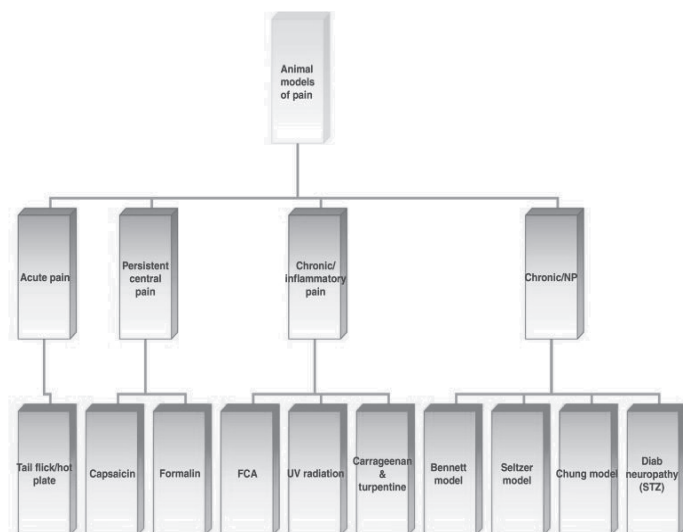


Figure 2.2: Overview of commonly used experimental pain models for the screening of novel molecules in drug discovery and early characterisation of the pharmacological properties in drug development.

Although NP may be due to different aetiologies, all causes lead to damage of the nociceptive pathway [19]. However, it is important to realise that the assessment of pain in animal experiments remains essentially an indirect measure. There is no way to characterise the quality (shooting, stabbing, lancing) or intensity of pain. With the exception of aversive behaviour to potentially noxious stimuli (which includes vocalisation, biting, licking, shaking of affected limb); all measurements are based on a nocifensive reflex [16]. These models are potential candidates for surrogacy of human evoked pain, but are inadequate models of spontaneous pain which is frequently observed in patients [7]. Other endpoints such as latency of hind-paw withdrawal or intensity of the pressure producing withdrawal are applied as measures of treatment effect in both acute and chronic pain states irrespective of underlying differences in pathophysiology [16]. The von Frey test is based on the assumption that a pain threshold can be reached, which reflects central hypersensitisation. However, in this test low threshold mechanoreceptors and nociceptors are activated, indicating the stimulus is non-specific[17] .

Specificity of pain response and endpoints in pre-clinical models

The endpoints used in experimental models of pain can be categorised into a) pain-related behaviour, such as paw licking or b) threshold response, such as the latency time to paw withdrawal[20].The behaviour considered most indicative of pain in animals include autotomy or self-attack (assessed by counting the number of wounds inflicted), hyperalgesia (a strong withdrawal response to a moderate heat stimulus) and allodynia (withdrawal in response to non-noxious tactile or cold stimuli)[21]. These measures of pain perception involve more than just nociceptive pathways. Their effectuation requires the contribution of various CNS structures, which may be indirectly or directly susceptible to primary or secondary effects of drugs and may differ considerably from their counterparts in humans. Standard evaluation methods such as the hot plate or tail flick tests assess the presence of pain-like behaviour, but provide little information on the nature or quality of ongoing pain. Furthermore, known differences exist in the biological substrate and natural course of disease associated with endpoints in different models of neuropathic pain (Figure 2.3). For example, dynamic, brush-evoked allodynia in humans is mediated by low threshold fibre input, whilst allodynia in rats is static in nature, mediated by high threshold fibre input [22]. The tail-flick method, capsaicin or formalin induced pain model, the Bennett or Seltzer neuropathic pain models do not have any human equivalent [23].

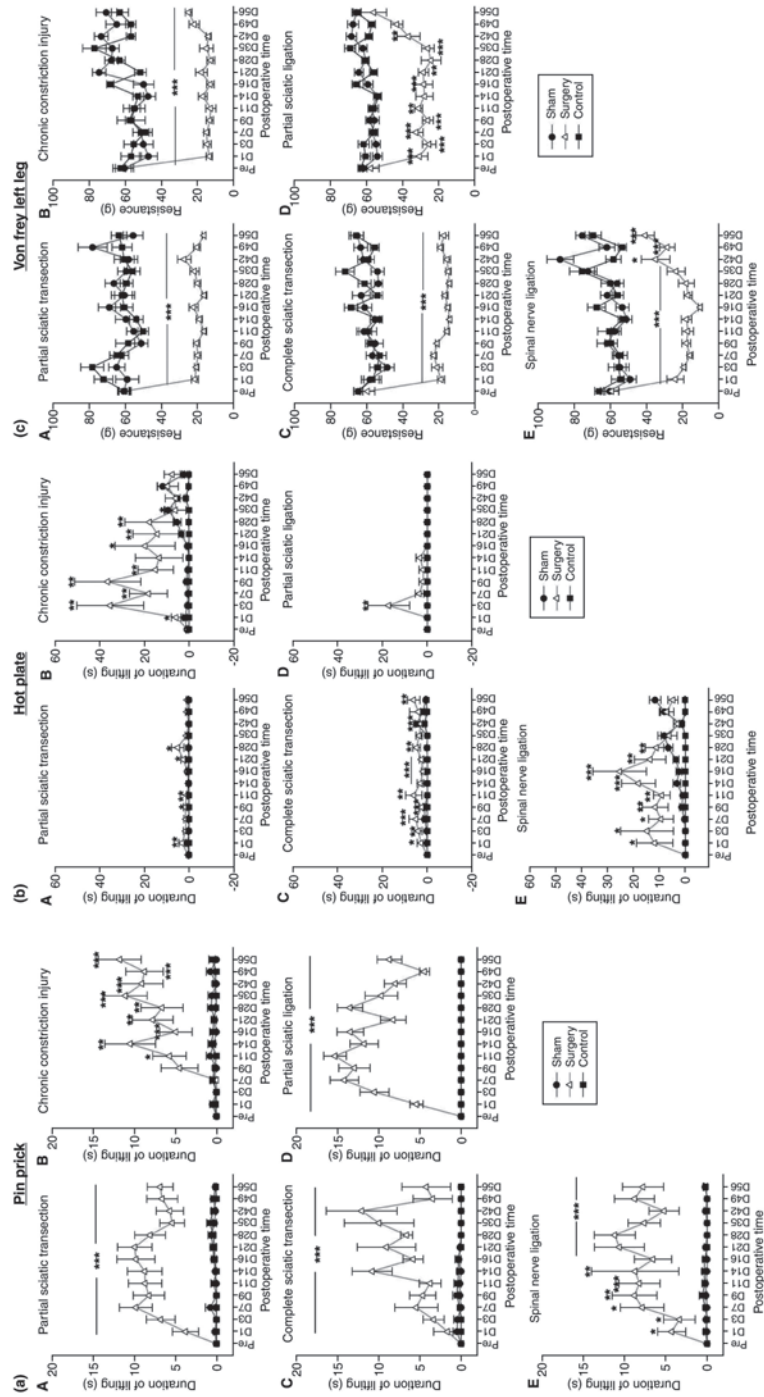


Figure 2.3: Behavioural measures of pain in five different models of nerve injury. The ability to discriminate drug effects does not solely depend upon the choice of experimental model, but also on the choice of the endpoint for pain response. As can be seen in the profiles above considerable differences exist in the duration of lifting of the left hind paw after stimulation with a pinprick (A), hotplate(B) and von Frey hair(C). These differences also vary over time, indicating the progression of an underlying disease process. Given are mean (S.E.M.) values of control, sham operated, and surgery groups before and at several time points up to 56 days after surgery in five different models of nerve injury. *Adapted with permission from*[24].

Similar considerations apply to the repertoire of behavioural changes that are indicative of spontaneous pain (e.g., increased weight bearing on the uninjured hind limb, guarding behaviour of the injured paw and licking of the injured paw coupled with 'gentle' biting and pulling of toe nails) [22]. In fact, endpoints classified as representative of spontaneous pain in humans have also been questioned as to the extent of their predictive validity. Yet, whilst pain-like behaviour in animal models of NP is common, the corresponding symptoms are relatively infrequent in patients[25].

Unfortunately, understanding of the correlation between the mechanisms of pain response (overt behaviour) and the underlying pathophysiology (pain signalling and processing) remains poor, especially if one considers the limited data on target site exposure or other downstream effects of drug action (Figure 2.4).

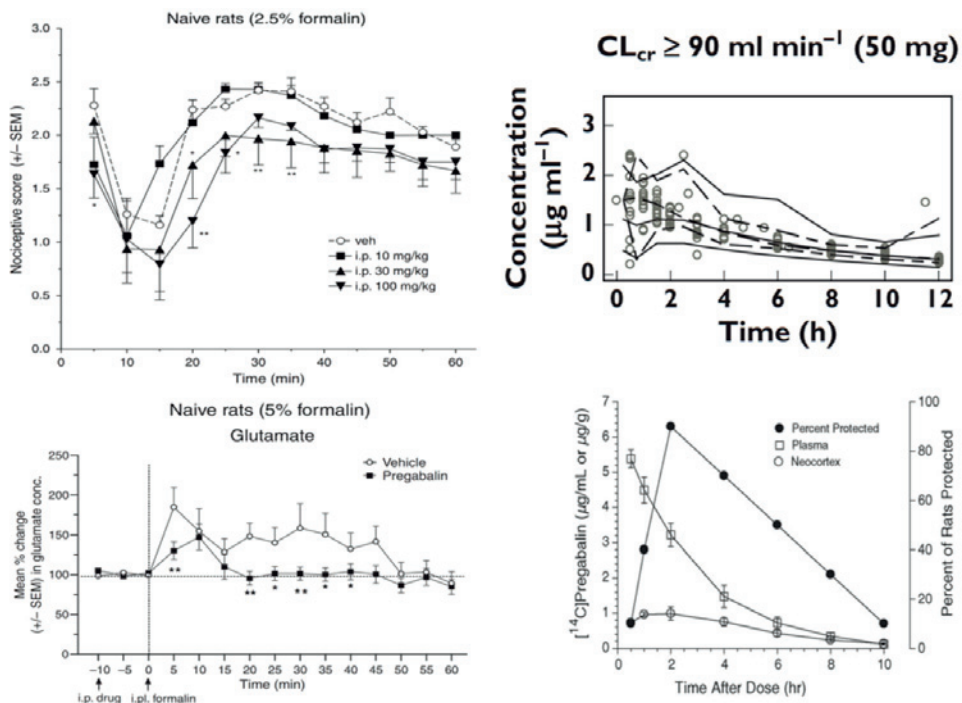


Figure 2.4: Despite evidence of peripheral hypersensitisation in the formalin-induced pain model, drug effects on nociceptive response are short lasting relative to the underlying pathophysiological changes, as indicated by the differences in the time course of pain behaviour (upper left panel) and concentrations of glutamate (lower left panel) in the spinal dorsal horn after administration of pregabalin[26]. Availability of pharmacokinetic and pharmacodynamic data is essential to explore such discrepancies in a quantitative manner and subsequently define a suitable dose range in humans. In this specific example, some discrepancy is observed between drug effects in animal models and humans. Response to pregabalin in rats occurs at levels above 4.0 μ g/ml, whilst therapeutic levels after administration of a 50 mg dose are below 2.0 μ g/ml. *Reprinted with permission from [26].*

Table 2.1: Proposed relationship between neuropathic pain mechanisms and clinical symptoms and signs and known targets in current therapeutic interventions.

| Compound/ Intervention | Targets | Neuronal processes/ Pathophysiology | Symptoms/ signs |
|--|---|---|---|
| Lidocaine, Carbamazepine, Lamotrigine | Na-channels | Peripheral nociceptor hyperexcitability Ectopic impulse generation, oscillations in DRG | Spontaneous pain |
| Capsaicin | TRPV1-receptor TRPM-receptor TRPA1-receptor ASTC-receptors | Peripheral nociceptor sensitisation Reduced activation threshold to: Heat Cold Mechanical stimuli | Heat hyperalgesia Cold hyperalgesia Static mechanical allodynia |
| Sympathetic blocks Opioids | α -receptor Presynaptic μ - receptors | noradrenaline Central dorsal horn hyperexcitability | |
| Gabapentin, Pregabalin, Ziconotide | Ca-channels Post-synaptic: NMDA-receptors NK-1 receptors Na-channels Intracellular cascade | Central sensitisation on spinal level Ongoing C-input induces increased synaptic transmission | Spontaneous pain |
| Ketamine | GABA-B receptors | Amplification of C-fibre input Intraspinal inhibitory interneurons (functional degeneration) | |
| Baclofen | Glycine-receptors | GABA-ergic Glycine-ergic | Dynamic and punctuate mechanical allodynia |
| Antidepressants Amitriptyline Venlafaxin Duloxetine | α 1, 5-HT-receptors | Changes of supraspinal descending modulation | |
| Clonidine | α 2-receptors | Inhibitory control (NA, 5-HT) | |

5-HT, 5-hydroxytryptamine (serotonin); ASIC, acid-sensing ion channel; GABA, γ -aminobutyric acid; NK1, neurokinin1; NMDA, N-methyl-D- aspartate; TCA, tricyclic antidepressants. *Adapted from* [4, 32].

This gap prevents the use of a mechanistic approach for extrapolation of drug effect on behaviour into accurate estimates of analgesia in patients. Furthermore, pain behaviour in humans is more varied and heterogeneous. In contrast to the pre-clinical measures, the representation of clinical characteristics of NP has been based on (i) spontaneous, ongoing pain, with clear reference to pain quality and sensory loss which includes varying degrees of hypoaesthesia, and (ii) evoked pain, manifested by hyperalgesia and allodynia (Table 2.1) [27]. Hence, a number of visual and numerical rating scales, such as the McGill pain questionnaire have been developed for the assessment of pain in clinical studies that capture the various dimensions of the disease [28]. These scales provide information about the intensity, duration and location of the pain [29].

Lastly, it should be noted that integral components of pain such as the cognitive-affective part may not be possible to reproduce in pre-clinical species. The differentiation between response features (e.g. pain relief and worsening) and mood disturbances in human pain cannot be replicated [22, 30]. This limitation is particularly important in chronic pain conditions, such as NP. Despite seeming pathophysiological similarities, none of the current models of neuropathy relate directly to clinical neuropathic pain conditions [23, 31].

Predictive value of pre-clinical models

Given the aforementioned differences across species, questions arise regarding the translational and predictive ability of pre-clinical models to describe the clinical phenomenon. Animal models are based exclusively on 'pain-like' behaviours or facilitated withdrawal reflexes, which in turn, are relatively infrequent in humans. In contrast, the commonest human complaints are tingling, paresthaesia and numbness rather than pain [25]. Therefore, the terms nociception and anti-nociception may be preferred in place of pain and analgesia [16]. It should also be unequivocal that animal models reproduce overt symptoms, i.e., they have face validity but are contextual in nature. For instance, paw withdrawal in response to a heat stimulus is observed both with inflammatory as well as neuropathic pain [33].

From a scientific perspective, the prerequisites for predictive value in experimental research include appropriate face and construct validity. An experimental model of disease or injury should be able to reproduce the symptoms observed in the clinical condition (face validity). At the same time, it is essential to mimic the pathophysiological changes which cause the overt symptoms (construct validity) [13, 34]. Commonly used animal models of neuropathic pain, such as the chronic constrictive injury (CCI) or sciatic nerve ligation (SNL), replicate elements of the pathology seen in clinical pain states, but have questionable construct validity with respect to specific pain syndromes, such as post herpetic neuralgia (PHTN) or diabetic neuralgia (DN) [24, 25]. In addition, homogenous strains are used which diverge from the heterogeneity of disease processes and progression in patients [35]. Such

an optimisation of experimental conditions may work against current efforts to predict treatment response in humans [12].

The failure in the development of NK1 antagonists as a putative mechanism for the treatment of NP illustrates the challenges in translational pharmacology research. The purported action of these compounds was blocking the actions of substance P, which has an important role in mammalian nociception. Lee *et al.* demonstrated that NK1 antagonists (intraplantar or intrathecal) attenuate NP symptoms in the SNT model. Based on their experiments, it was suggested that pain control methods targeting substance P and CGRP should be designed as new therapeutic strategies for peripheral NP [36]. However, in clinical studies, NK1 antagonists failed to reproduce the promise observed in animals [37]. In this example, the pathway selected had little relevance to the clinical disease, and the chosen endpoint was unable to differentiate this. Unfortunately, despite documented failures, some authors continue to defend the predictive validity of current animal models [22].

Target validation and new interventions

The absence of a mechanism based classification of pain syndromes consequently affects the selection of appropriate targets [38]. These hurdles are augmented by the lack of a process that ensures an integrated assessment of drug properties from target selection to proof-of-concept [11, 14]. In the subsequent sections of this paper, we discuss the advantages of establishing pharmacokinetic-pharmacodynamic relationships as the basis for the prediction of efficacy in humans. Our objective is to show how quantitative pharmacology techniques can assist drug discovery in distinguishing whether the high rate of false positive findings in early drug development is due the lack of construct validity of animal models or simply because of the poor sensitivity of evoked response endpoints, which consequently lead to inaccurate estimates of the dose required for the evaluation of efficacy in humans. PKPD relationships themselves will not eliminate the issues with construct validity or unsuitability of behavioural endpoints. We believe however that PKPD relationships can be extremely useful if used as a biomarker of pharmacology, in which properties like binding, target activation or blockade and subsequent downstream effects are characterised in a quantitative manner.

Translational Pharmacology

The high attrition rate in the development of compounds for neuropathic pain has prompted many investigators to revisit how pre-clinical findings correlate with efficacy data in patients [22, 25, 35, 39]. These reviews share a common view that evoked pain responses are inadequate measures of pain behaviour and do not represent pain processing in humans. They propose solutions ranging from modifications of current experimental models to the use of engineered mutant models [35, 40]. However, parameterisation of drug effect is

presented in terms of ED_{50} , without accounting for the differences in pharmacokinetics across species and the requirements for characterisation of a dose-response curve [41]. Finally, the duration of the effect and its relationship to target engagement is completely set aside [42].

Among the various publications, it is worth mentioning the attempt of Whiteside *et al.*, who explored the correlations between pre-clinical to clinical data by comparing drug exposure in rats at the minimum effective dose (MED) for different models of inflammatory and neuropathic pain with clinical exposure in patients at the maintenance dose levels. Their conclusion is that pre-clinical animal models are predictive of efficacious exposures in humans [41]. However, the authors seem to overlook the fact that efficacious exposure ranges were considerably higher in rats as compared to humans. They indicate that the MED in rats for a single dose is not representative of clinical exposure, nor of the effects observed after repeated dosing. Yet, total plasma concentrations are compared without taking into account interspecies differences in pharmacokinetics (i.e., drug distribution). For CNS drugs, the assumption that plasma concentrations reflect drug exposure at the site of action is often inappropriate. Another important aspect is the role of differences in metabolic rate, which lead to potential drug interactions if metabolites also have affinity for the target [43] (Table 2.1).

Overview of experimental pain models

As shown in Figure 2.2, despite the differences in the aetiology of pain in common experimental models, the screening for neuropathic pain compounds has also relied on the use of chronic inflammation models of pain [23]. In fact, the empiricism in the selection of compounds is further illustrated by the evaluation of anti-inflammatory drugs in neuropathic pain models.

In the subsequent sections, we will then explain which changes are required in the experimental design to allow appropriate characterisation of the concentration-effect curve for the screening of novel compound and translation of findings across species.

Inflammatory pain

Ultra Violet B model

Acute cutaneous overexposure to ultra-violet radiation (UVR) causes thermal and mechanical hyperalgesia in rats and humans. Using a UVB source, the plantar surface of the rat hind paws is irradiated to cause inflammatory reaction. This injury induces a significant dose-dependent reduction in both thermal and mechanical paw withdrawal thresholds, which peak 48 hrs after irradiation. The inflammation is caused by apoptosis of epidermal cells induced by DNA damage [44]. Algogenic chemicals are also released following UVR inflammation. The cutaneous hypersensitivity results in thermal hyperalgesia as well as

UVB mechanical allodynia. Spontaneous pain behaviour such as flinching, licking, excessive grooming or paw lifting are not seen with this model [45]. This model differs from the complete Freund's adjuvant (CFA) model in that it does not produce an increase in the spinal basal c-fos expression levels at the peak of sensory changes. There is little spontaneous activity in the primary nociceptors which in turn could sensitise the spinal nociceptive structure. This fact sets this model apart from the other algogenic models where central (spinal) sensitisation is observed. Hence the UVB model is sensitive to study effects of peripherally acting analgesics such as NSAIDs [45].

Neuropathic Pain

In neuropathic pain models, tissue injury is produced chemically or surgically, which leads to peripheral nociceptive sensitisation. These changes induce phenotypic alteration of sensory neurons and increased activation of spinal dorsal horn neurons. What follows is the development and maintenance of central sensitisation in the dorsal horn of the spinal cord [9, 18]. In persistent pain models, an algogenic substance such as capsaicin or formalin is introduced subcutaneously or intraperitoneally [46]. More specialised models envisage induction of nerve injury [47]. While they differ by locus and type of injury, all produce behavioural insensitivity as a result of the trauma [48].

Formalin-induced pain model (FIP)

The formalin test was developed for the screening of compounds with antinociceptive effects [46]. Recently it has been found to correlate with the CCI model, one of the best available models characterising neuropathic pain behaviour [49]. Two phases of nociceptive behaviour are observed following formalin injection. The first phase starts immediately after injection and lasts for 3-5 minutes. It is due to direct chemical stimulation of nociceptors and predominantly associated with activity in C fibres. Subsequently there is a quiescent phase of 10-15 minutes. The second phase starts 15-20 minutes after the formalin injection and lasts 20-40 minutes. Drugs effective in NP affect the onset and amplitude of the second phase (i.e. the quiescent phase is prolonged, whilst the maximum intensity of pain is decreased). The effect of formalin is believed to occur due to central sensitisation of dorsal horn neurones, as a result of the initial barrage of inputs from C fibre nociceptor afferents during the first phase. A role for higher brain regions in maintaining this pain state has also been hypothesised [50]. The frequency of paw licking behaviour (PLB) per time interval is measured for NP drugs as compared to placebo [46].

Chronic constrictive injury models

In contrast to complete transection of the sciatic nerve, which does not reflect neuropathic pain pathophysiology in humans [51], these models comprise either a loose ligature is

placed around the entire sciatic nerve (Benett model) or a tight ligature through half of the proximal sciatic nerve (Seltzer). Thermal and mechanical hypersensitivity is observed in both models but due to experimental complexities, the procedure is difficult to reproduce leading to variability in evoked responses [52, 53]. A variant to the CCI model was developed by Chung *et al*, wherein the L5/6 spinal nerves are ligated while leaving the L4 intact. In this way the intact dermatomes of the paw may be tested, though the L4 nerve is at risk of damage due to exposure [54].

Partial denervation models

These models were developed to further simplify technical feasibility as well as minimise variability associated with the degree of tissue damage. They enable the investigation of changes in injured primary sensory neurons and the neighbouring intact sensory neurons so that the relative contribution of both structures to the pathophysiology of neuropathic pain symptoms can be investigated. The spared nerve injury (SNI) model involves axotomy of the tibial and common peroneal nerves while sparing the sural nerve [47]. The tibial nerve transection (TNT) represents a further modification of the SNI model. Nerve injury models are considered representative of symptomatically induced pain, allowing the evaluation of three different components of neuropathic pain, namely mechanical allodynia, cold allodynia and spontaneous pain. The threshold to response (withdrawal of the injured paw) is the primary measure for the assessment of drug effects [25, 55].

PKPD MODELLING OF PAIN RESPONSE

Translational drug research requires the prediction in a strictly quantitative manner of the pharmacokinetic-pharmacodynamic (PKPD) properties of drugs [56]. Assessment of PKPD relationships enables better understanding of how changes in drug exposure correlate to the pharmacological effects and overall response to treatment. To that extent, experimental protocols must be designed and customised accordingly to ensure that appropriate pharmacokinetic and pharmacodynamic data are obtained. Among other things, one needs to consider which dose levels to use, how long to sample and whether systemic exposure reflects target site concentrations. These requirements contrast, however, with current practice for screening protocols, which are usually performed in a standardised '*one size fits all*' manner. As such these experiments are less informative, often precluding accurate estimation of the parameters of interest.

Experimental requirements

Given that behavioural models are primarily used for the purposes of screening and ranking of compounds, it is critical that potency estimates are accurate and expressed in terms of exposure (EC_{50}), rather than dose (ED_{50}). In this context, potency can be used as basis for

the scaling of PD estimates across species and/or endpoints. To that purpose, considerable changes to protocol design are required, which involve both modifications to the sampling procedures and the dosing rationale.

Experiments suitable for PKPD modelling must therefore keep in mind some basic requirements regarding the time course of response. For instance, a minimum number of samples per animal are necessary for reliable estimation of PD parameters (e.g., baseline, E_{\max} and EC_{50}). This can be achieved by the use of sparse sampling and treatment with different dose levels. In contrast, PK sampling should provide enough information to characterise the absorption, distribution and elimination of the administered drug. Although the ideal situation is to have PK and PD samples measured concurrently, this may not be feasible in behavioural models of pain. In this case, a PK experiment can be performed prior to the assessment of drug effects. The advantages of this approach have been elegantly demonstrated by Bender *et al.* [57, 58] for the analysis of drug effects in the CCI animal model. Another important point to consider is the observation window available for the evaluation of drug effects. Under non-stationary conditions, the disease process itself also needs to be characterised [26].

Optimisation of Experimental Design

In early drug development, screening of compounds ought to rely on accurate ranking of their pharmacokinetic (PK) and pharmacodynamic (PD) properties, yielding evidence of their pharmacokinetic-pharmacodynamic (PKPD) relationships [56]. However, the use of a model-based approach for the analysis of such experiments, while desirable, is often precluded by practical constraints and resources [14]. Suitable designs entail the use of repeated measurements that describe the time course of drug concentrations and the pharmacological effects of interest. Feasibility considerations often limit the collection of repeated samples in individual animal and thus compromise the design of the experiment. Given the requirement for sparse sampling, appropriate sampling times become critical [59]. Therefore, accurate and precise model parameters estimates depend greatly on the experimental design.

In optimal experimental design, D-optimality is by far the most used criterion in individual, and population modelling studies. Herein optimisation is carried out assuming there is no uncertainty (imprecision around the parameters of interest) i.e. there is no uncertainty distribution around this parameter. This assumption does not hold true in population studies though it has been considered the classic approach to designing an experiment optimally [60, 61]. This method may not be suitable for prospective evaluation of the compounds during screening experiments when little data is available and prior knowledge about the pharmacokinetic and pharmacodynamic properties are limited.

ED-optimality, represents an alternative for experimental data when the model parameters are expected to have distributions [62]. The use of ED-optimality assumes a prior distribution around the parameters of interest [63]. While optimal design has been extensively used for optimizing different types of continuous repeated measurements, with non-linear mixed-effects modelling, little work has been done with discrete data [64].

Data analysis requirements

Pharmacokinetic and pharmacodynamic data must be analysed in an integrated manner. Given the limitations to the number of samples that can be obtained per animal, nonlinear mixed effects modelling techniques are recommended for the assessment of concentration-effect relationships (see box 1). Population pharmacokinetic models can be developed to predict systemic or target drug exposure at the time at which PD measurements are performed. In addition, optimal design concepts can be applied that overcome operational limitations to the sampling scheme, ensuring maximisation of the information gathered in the experiment [62].

In contrast to the linear regression methods applied to most dose-response curves, which rely primarily on observed experimental variables, nonlinear mixed effects techniques rely on model parameterisation which facilitates the distinction between system and drug-specific properties. This subtle conceptual difference in data analysis gives a rational basis for ranking of compounds and allows preclinical findings to be translated to the clinical situation, assuming the model is applicable across species.

Box 1 - Nonlinear mixed effects modelling. Further details on quantitative pharmacological methods can be found in [65, 66].

In nonlinear mixed effects modelling, two types of parameters are estimated:

1) fixed effects, which are represented by parameters or factors usually explaining the correlation between the dependent and independent variables. These parameters define the structure of the model (e.g., a sigmoid curve) and their estimates reflect the typical value in the overall population.

2) random effects, which constitute the stochastic component of the mixed effects model. Random effect distributions can be identified for fixed effects with the objective of describing inter-individual and possibly inter-occasion variability. In addition, the residual error represents all the variability that cannot be described by the inter-individual and inter-occasion terms (e.g., measurement error)

PKPD models

Different approaches are available which account for the description of drug concentrations, drug effects and disease processes [67]. Direct models can be applied when plasma pharmacokinetics can be linked to the pharmacodynamic effects at any given time. Delays between response and pharmacokinetics in plasma due to slow biophase equilibration can be

described by effect compartment models [68, 69]. Indirect response models can be used to account for the pharmacodynamics of drugs that act by inhibition or stimulation endogenous mediators [70-72]. More complex transduction mechanisms can also be modelled by incorporation of a so-called transit compartment model [73]. Disease progression models require semi-mechanistic or mechanistic models to enable clear distinction between drug- and system-specific parameters [74, 75]. Various mathematical models can be implemented using different parameterisations for drug effect. Some of these aforementioned models are presented in box 2. Further details on ongoing efforts in the characterisation of PKPD relationships are also mentioned in [41]. Interestingly, these concepts have been used more often for the analysis of clinical data (Figure 2.5). Application of these concepts to preclinical models is scarce in the published literature. Recently, Bender *et al.* have modelled the effects of gabapentin in the CCI model (Figure 2.6). Using appropriate protocol design and advanced modelling techniques, the authors demonstrate the predictive performance of a model describing variability in treatment effect across different dose groups for two different endpoints (paw withdrawal threshold and static allodynia).

Box 2 : Pharmacodynamic models [66]

Direct linear model:

$$f(\text{baseline}_{ij}, \text{slope}_{ij}) = \text{baseline}_{ij} + \text{slope}_{ij} * X_{ijk}$$

Where baseline_{ij} and slope_{ij} are the parameters to be estimated and X_{ijk} is usually a measure of drug exposure (e.g., plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}

Direct Emax model:

$$f(\text{baseline}_{ij}, E_{\max ij}, EC_{50ij}) = \text{baseline}_{ij} + \frac{E_{\max ij} * X_{ijk}}{EC_{50ij} + X_{ijk}}$$

Where baseline_{ij} , $E_{\max ij}$ and EC_{50ij} are the parameters to be estimated and X_{ijk} is usually a measure of drug exposure (e.g., plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}

Sigmoid Emax model:

$$f(\text{baseline}_{ij}, E_{\max ij}, EC_{50ij}, \gamma_j) = \text{baseline}_{ij} + \frac{E_{\max ij} * X_{ijk}^{\gamma_j}}{EC_{50ij}^{\gamma_j} + X_{ijk}^{\gamma_j}}$$

Where baseline_{ij} , $E_{\max ij}$, EC_{50ij} and γ_j are the parameters to be estimate and X_{ijk} is usually a measure of drug exposure (e.g., plasma concentration, AUC) corresponding to the observed pharmacological effect y_{ijk}

The subscripts i, j and k indicate the individual subject i , at the time j and occasion or period k , respectively.

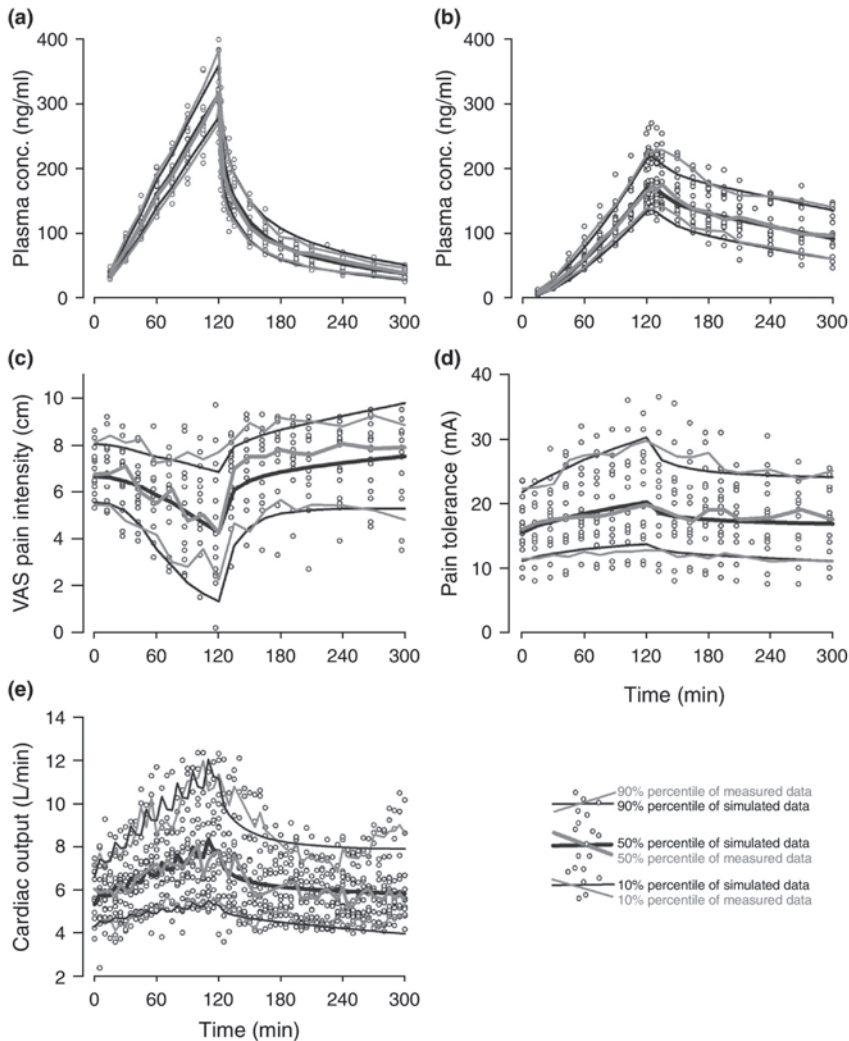


Figure 2.5: Predictive performance and accuracy of a pharmacokinetic-pharmacodynamic (PKPD) model describing the effects of S(1)-ketamine in healthy volunteers. Simulations were performed with the S(1)-ketamine parameters obtained from the study data. Despite between-subject variability, it is clear from the panels that the PKPD model describes treatment response. (A) S(1)-ketamine concentration; (B) S(1)-norketamine concentration; (C) heat pain intensity; (D) electrical pain tolerance; (E) cardiac output. The lines represent the 10th, 50th, and 90th percentiles for simulated responses (black lines) and measured responses (gray lines). The symbols are the actual observations in the healthy volunteers. VAS visual analogue. *Adapted with permission from [76].*

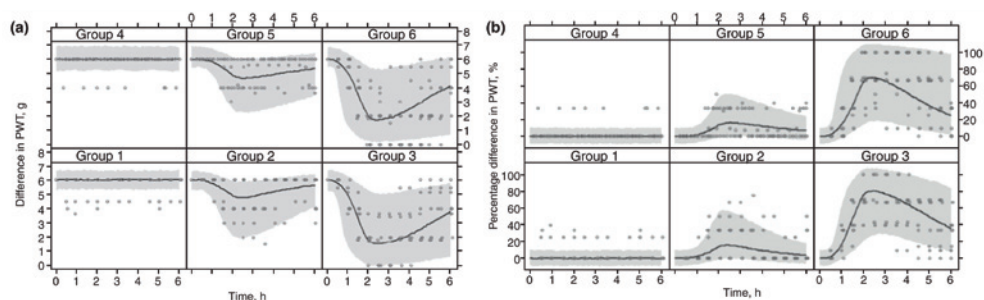


Figure 2.6: Time course of response for individual animals (circles) and the predicted population profiles (black lines) in the CCI model after administration of pregabalin alone or in combination with sildenafil. The drug effect on static allodynia was characterised by (A) the difference in PWT (i.e., the difference between the two paws divided by the difference at baseline). In both cases a continuous pharmacodynamic model was used to describe the concentration-effect relationship of pregabalin. The treatment groups included (1) saline, (2) pregabalin (4mg/kg/h), (3) pregabalin 10mg/kg/h+sildenafil, (4) sildenafil, (5) pregabalin 1.6mg/kg/h+sildenafil and (6) pregabalin 4mg/kg/h+sildenafil. Model predictions include the 90% prediction interval (shaded areas) [58]. Visual assessment of the random dispersion of the data within the predicted intervals provides evidence of the predictive performance of the model. *Reprinted with permission from [58].*

Scaling and dose rationale in humans

As indicated previously, one of main objectives of using a model-based approach is to identify parameters, which can be used subsequently to translate response from animal to humans. A direct application of the concept is the prediction of the efficacious exposure range and scaling of the dose across species. To this purpose, the concentration-effect relationships obtained in animals can be used to estimate the putative clinically effective dose range, taking into account the differences in protein binding across species. Given that differences in endpoints across species are not always correlated with each other in a fully mechanistic manner, derived parameters such as EC_{20} , EC_{80} , EC_{90} should also be considered. This concept has been elegantly illustrated by Huntjens *et al* in the evaluation of the anti-inflammatory pain by COX-2 inhibitors [13].

Given the complexity of pain signalling and processing, different results may be observed depending on which component or step is being quantified. We strongly believe that the answers regarding translational research lie in the use of experimental conditions which warrant construct validity [35, 78]. In fact, we defend a paradigm shift in the approach to interspecies scaling based on target/receptor occupancy. Receptor theory concepts can prove a useful tool for that purpose, enhancing the potential predictive value of a model-based approach [56]. Moreover, the use of target occupancy as a marker of pharmacology may elucidate situations in which a mismatch exists between target activation and effect, as in the case of poor sensitivity of the behavioural endpoint. The cold hyperalgesia model is one such model wherein lowering the temperature below the threshold for nociception does not always provide for a better differentiation between the injured and normal paws

of CFA rats [79]. In this context, a mismatch in the degree of target activation across species may explain differences in sensitivity in endpoints as well as in potency. Hence, it would be desirable and useful to integrate receptor binding, which is a measure of receptor activation or inactivation (in case of an antagonist), with downstream markers of pharmacology and overt behaviour. From an experimental perspective, the use of imaging techniques would support the identification of differences as well as correlations across species. It should be noted that incorporation of the binding kinetics in the evaluation of novel compounds also offers an opportunity for better understanding of secondary pharmacological effects and adverse events. On the other hand, one should consider secondary pharmacology and safety findings as a proxy for target engagement when evidence cannot be derived experimentally. This concept is illustrated by the use of NMDA antagonists (e.g. ketamine) and alpha-2 agonists (e.g. clonidine). Although tolerability in humans will ultimately determine dose selection, drug-induced adverse events are usually dose-dependent and specific markers of pharmacological action.

CHALLENGES AND LIMITATIONS

It is beyond doubt that numerous challenges must be overcome to translate preclinical findings into predictors of clinical efficacy. We have introduced the use of PKPD as a tool to bridge the translational gap but this approach itself has limitations. Animal models of pain are often technically laborious, making interindividual variability an intrinsic feature of these models [80]. Drug concentrations at the biophase are not constant over the course of treatment and can be altered by changes in the underlying pathophysiology or natural evolution of the experimental injury [81]. These variations may lead to biased estimates of parameters such as EC_{50} or E_{max} . Some aspects of disease are difficult to model; for instance NP symptoms wax and wane over time with intervals ranging from a day to as long as two or three months [82]. There are also feasibility limitations in terms of the number of samples that may be collected and of the overall duration of follow-up.

All these hurdles raise the question on whether R&D requires more extensive use of surrogate human models to ensure accurate prediction of clinical efficacy, forsaking preclinical experiments. Human models can reproduce many of the symptoms and sensory features in patients of NP [76]. By design however, these models lack construct validity, inducing acute plasticity i.e., phase 2 pain instead of long-lasting and irreversible modification of the nociceptive pathway, which is a hallmark of NP [83]. In other words, current human models mimic sensory symptoms reflective of both NP and nociceptive pain, yielding results which are relevant but not specific to NP [27]

DISCUSSION AND CONCLUSIONS

Empiricism has dominated target identification and screening of compounds for neuropathic pain. Moreover, the existing models of behavioural pain response have been developed under the assumption that face validity suffices to translate drug effects from animals to humans, ignoring the requirements for accurate characterisation of pharmacokinetic-pharmacodynamic relationships [84]. Four main factors can be identified which explain the high attrition rate in this therapeutic area. First, the lack of sufficient understanding of the mechanisms of disease in humans prevents the identification of suitable markers of pharmacological activity. Such (bio) markers could be used in lieu of behavioural measures, which often misrepresent clinical symptoms in NP patients. Second, summaries of treatment effect are limited to qualitative estimates of drug action, most of which cannot be translated directly to humans. Third, experiments continue to assess dose-response curves, ignoring differences in pharmacokinetics across species. Fourth, the duration of the effect and its relationship to target or biomarker engagement is completely set aside.

Our review shows that any claim regarding the translational value of a method requires comprehensive evaluation of the underlying PKPD relationship. Only then, is it possible to distinguish whether the lack of predictive value can be assigned to intrinsic limitations in experimental models (e.g., construct validity or poor sensitivity of the behavioural endpoints). We have also highlighted the relevance of the differences in the development of behavioural pain symptoms due to injury or noxious stimuli in these models, as compared to the onset and progression of symptoms observed in humans. While it takes a few weeks for the disease to be induced in an animal model, in patients this may take years to manifest [85]. A possible explanation for these discrepancies includes the differences in signalling mechanisms. Thus, one should accept that animal models may never be able to mimic all aspects of disease in humans and may be limited as a tool for qualitative distinction between active and inactive compounds.

In summary, the use of nonlinear mixed-effects modelling to characterise PKPD relationships in early drug development allows for a less empirical rationale for the selection of doses to be tested during first-time in human and proof of concept (POC)/Phase II studies. Model-derived parameters may be used to estimate the effective dose range in humans assuming that the free concentration of the drug at the biophase can be compared across species. Our examples also illustrate that the translational value of a model-based approach relies on appropriate parameterisation. An integrated approach is needed which takes into account target engagement and markers of pharmacology as basis for the dose rationale in clinical trials.

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CHAPTER 3

PKPD relationships and dose rationale in
analgesic drug development - Scope and intent
of investigations

The search for effective and safe compounds for the treatment of chronic pain remains a challenge for pharmaceutical research and development (R&D). Whilst the concept of an underlying dysfunction preceding overt symptoms and diagnosis is not unheard of in medical practice, the assessment of pain relief still prevails as the primary and often the only measure of efficacy in drug discovery and development. This paradigm has prevented the development of a more mechanistic approach for the selection and progression of candidate molecules for the treatment of chronic pain. In fact, one needs to realise that it is the dysfunction in signalling pathways that underpins the clinical symptoms of the post-traumatic events and chronic inflammation. Such a dysfunction causes progressive tissue damage, including changes of neuroplasticity and synaptic remodelling when neuronal tissue is involved. In addition, it should be noted that the delay between the onset of the disease and overt symptoms may be associated with irreversible tissue changes, which makes the timing of any therapeutic intervention a key factor for the success or failure of treatment.

Hence, the evaluation of the biological and pharmacological properties of a new chemical entity (NCE) should provide the basis for dose selection and subsequent clinical profiling in clinical trials. Despite the challenges mentioned above, experimental protocols currently used both pre-clinically and clinically, seem to add complexity to the problem. They rely primarily on the suppression of symptoms and on behavioural measures of pain to define efficacious doses. Furthermore, each experiment is performed independently of the others. Experimental evidence is not generated with the objective characterising concentration-effect relationships or to enable integration of the results from the preceding phase as the basis for predicting or extrapolating findings in a subsequent phase of the development process. The lack of data integration and the absence of suitable markers and measures of drug effect represent additional contributing factors to the poor decision making and inadequate go/no go criteria in chronic pain conditions. Undeniably, such an approach contrasts with the evolving understanding of disease and overlooks the role of quantitative tools in pharmacology and clinical pharmacology research.

In this thesis we will explore the advantages and challenges in the implementation of a model-based approach for the evaluation of efficacy of new chemical entities for chronic inflammatory and neuropathic pain conditions. More specifically, we will critically assess the requirements for data integration during the development process with the scope of facilitating the evidence synthesis and translation of findings across the different phases of development of a candidate molecule. Therefore, focus will be given to the relevance of pharmacokinetic-pharmacodynamic relationships and whenever possible to the use of suitable mechanism-based models. Our approach will highlight the extent to which the evidence obtained by empirical protocols disregards the tenets of pharmacology, making the discrimination of drug and system-specific parameters in a disease model very difficult. We will also show that the rationale for dose selection in humans must account for target

engagement in the population of interest. An important aspect of our work will be the use of mechanistic inference for the identification of key experimental requirements and decision criteria for the progression of a compound from early pre-clinical to late clinical development.

From a drug discovery perspective, we will evaluate how to optimally integrate data and explore model parameterisations that potentially facilitate the translation between species and experimental protocol conditions. Despite the extensive discussion among drug developers on the development and availability of potential biomarkers, very little attention has been paid to the need for quantitative information about drug effects in such a way that parameters, rather than experimental variables can be used to characterise the implications of such effects in the target patient population.

From a methodological perspective, we will challenge the current regulatory and clinical paradigm that imposes formal evidence of pain relief without further consideration of the underlying pharmacological activity. Our work will highlight how statistical methods such as optimal design can be used in conjunction with modelling and simulation for the evaluation of drug effects when noise in experimental measures and uncertainty in parameter estimation are high. Moreover, we will attempt to demonstrate the flaws in the assumption that empirical evidence from clinical trials suffices to support dose selection in a highly heterogeneous population. In this context, we will illustrate how differences in demographics as well as inclusion and exclusion criteria and protocol design which are applied to phases 2 and 3 trials can be evaluated *in silico* by clinical trial simulations.

Our working hypothesis is that a model-based approach is required for accurate characterisation of candidate molecules pre-clinically and subsequently for the selection of the dose to be used in humans throughout clinical development. We will evaluate model parameterisations which take into account the underlying pharmacological effects and demonstrate how critical they are for the dose rationale as well as for extrapolation across species and phases of development. Specifically, this thesis will evaluate existing data from compounds with known analgesic effects in neuropathic pain and explore the properties of an investigational molecule for chronic inflammatory pain to address the following questions:

1. Can modelling and simulation (M&S) facilitate the translation of experimental findings across different phases of drug discovery and development, improving the predictive value of experimental protocols?
2. Can historical data be integrated to support model parameter estimation and subsequently applied as priors during the evaluation of efficacy?
3. Which experimental and methodological requirements are critical for optimising the evaluation of novel compounds?

4. What is the benefit of characterising concentration-effect (PKPD) relationships as the basis for the dose rationale in humans?
5. Can clinical trial simulations be used for the optimisation of the dose selection and discriminate drug-induced effects from other confounders in the target patient population?

To ensure clarity and provide appropriate focus to the different questions and issues highlighted above, this thesis is split into three main sections. After a general introduction in which we revisit the pathophysiological basis of chronic inflammatory and neuropathic pain and describe the most common approaches currently used for the progression of an analgesic, we explore the technical requirements for model parameterisation and define the requirements for experimental design optimisation, with special focus on the use of binary measures of response during the screening and ranking of candidate molecules in neuropathic pain. Subsequently, focus is given to model parameterisations which enable the assessment of concentration-effect relationships as the basis for translation of the pharmacological properties of a molecule across species as well as for bridging across different phases of development. Lastly, an attempt is made to model clinical response in chronic inflammatory pain and establish correlations between symptom improvement and the underlying pharmacological effects using biomarkers. In addition to emphasising the role of biomarkers in drug development, our work shows how clinical trial simulations can be used as a design tool, enabling the evaluation of a variety of scenarios that disentangle the contribution of pharmacology from the confounding effects of placebo and disease dynamics. Our conclusions are presented taking into account the requirements for prospective evaluation of novel compounds where one or more of the aforementioned issues have to be dealt with.

1. GENERAL INTRODUCTION: TRANSLATIONAL PHARMACOLOGY OF DRUG EFFECTS IN CHRONIC PAIN

In **Chapter 1**, an overview of the ongoing research efforts in neuropathic and chronic inflammatory pain is presented. Focus is given to the pathophysiology of disease and to the notion that pain is the consequence of an underlying signalling dysfunction. In chronic conditions, such a dysfunction can lead to irreversible changes, which are common to the course of progressive diseases in general. We discuss how early diagnosis, timing of the intervention and reversibility of the underlying processes ultimately determine the success or failure of a treatment. The identification of effective targets and therapies must account for these factors. The limitations and flaws in the current paradigm for the screening and selection of compounds in drug development are then highlighted and used as a foundation

for the subsequent chapters in this thesis. An approach is proposed in which the treatment of pain is considered from a mechanistic perspective, rather than based on the suppression of symptoms, which are often non-specific manifestations of the multiple nociceptive and cognitive mechanisms. These principles are expanded in the discussion of the predictive value of animal models, which resides in our ability to understand which mechanisms are involved and which endpoints are measured, i.e., they must have construct rather than face validity. Lastly, we introduce important methodological concepts pertinent to the evaluation of biomarkers, which are essential for the prediction of efficacy if one takes into account the delay between the onset of the signalling dysfunction and the development of overt pain symptoms. In this context, we consider the requirements for experimental design, analysis and interpretation of results and the relevance of mathematical and statistical models as a tool to describe biological system and drug properties in a quantitative manner. Hierarchical or population models are proposed as the basis for identifying the different sources of variability at individual level. Using the appropriate parameterization, these models can then be used for inferences about treatment effects and disease progression.

The role of concentration-effect relationships and more broadly of modelling and simulation as the basis for translational research is advanced in **Chapter 2**, where various experimental models of evoked-pain and their inherent limitations are presented. Of interest is the lack of construct validity in most if not all experimental models used in preclinical research. This issue is further compounded by the use of endpoints, which usually describe pain-related behaviour or threshold response, without evidence of a clear correlation between target engagement and the underlying pharmacodynamic effects that can be observed after drug administration. Here we also draw attention to the fact that the measures of pain perception currently used in experimental models involve more than just nociceptive pathways. Their effectuation requires the contribution of various CNS structures and can therefore differ considerably from their counterparts in humans, explaining partly why so many compounds that appear to show pharmacological activity in animals ultimately fail in humans. This gap prevents the use of a mechanistic approach to translate and extrapolate drug effects on behaviour into accurate estimates of analgesia in patients. In fact, different from the pre-clinical measures, the representation of clinical characteristics of chronic pain in patients has been derived from spontaneous, ongoing pain, with clear reference to pain quality and sensory loss using a number of visual and numerical rating scales. These scales provide information about the intensity, duration and location of the pain.

Finally, using recent examples from the published literature, we emphasise the requirements for the assessment of concentration-effect relationships in pre-clinical species, including important changes in experimental procedures in standard screening protocols. Among other factors, we discuss the importance of expressing potency estimates in terms of exposure (EC_{50}), rather than dose (ED_{50}). Poor experimental design can lead to inaccuracies

in parameter estimation and consequently to biased selection and ranking of candidate molecules during screening. In this context, the use of optimality concepts is suggested as an opportunity prevent or address shortcomings in the data analysis due to inappropriate sampling procedures or poor dose rationale.

Based on a careful review of 1) the signalling dysfunction(s) underlying pain symptoms presented in the aforementioned paragraphs; 2) on the translational gaps in the experimental protocols currently used during drug screening for the selection of candidate molecules and 3) the empirical use of clinical scales during the evaluation of pain relief in clinical studies, specific issues have been identified in the current strategy for assessment of the putative therapeutic dose range in humans which will underpin the scope and intent of the investigations described in this thesis, as detailed here in **Chapter 3**. In the following paragraphs, we describe our endeavours to evaluate the role of historical data, protocol optimisation, model parameterisation and integration of biomarkers as the basis for statistical inference and prediction of the therapeutic dose in chronic pain.

2. MODEL BASED APPROACH FOR THE ANALYSIS OF BEHAVIOURAL PAIN RESPONSE IN PRECLINICAL EXPERIMENTS

As indicated previously, the lack of suitable markers of pharmacology and absence of an approach that enables better data integration for the selection of candidate molecules contributes to the attrition rate in chronic and neuropathic pain conditions. Whilst the assessment of concentration-response relationships across the different phases of development is a *sine qua non* condition to accurately account for the determinants of drug response and variability, the evidence of PKPD relationships for a compound in itself does not warrant accurate statistical inference from one experiment to another, in particular with regard to the translation or prediction of drug effects. PKPD relationships must have predictive validity or value for the target population, both in qualitative and quantitative terms. Such considerations are not formally embedded in the requirements for evidence generation, and consequently lead to the implementation of experimental protocols, which are often not fully informative or eventually even biased. In **Chapters 4 and 5** the fundamental questions to be addressed are which molecules show efficacy and how this effect can be reliably and quantifiably demonstrated. To this end, we explore how models and model parameters can be used prospectively in drug screening taking into account various experimental and technical limitations. Using theoretical concepts in optimal design we also explore the requirements for experimental protocol design. Our approach is based on the assumption of a binary outcome as benchmark or strategy for drug screening. In addition, we evaluate the possibility of dissecting system from drug-specific features, allowing the introduction of modularity, which is essential for the assessment of drug potency as parameter of interest during screening. Given the limited amount of data available, optimisation methods are

considered, which allow incorporation of uncertainty in parameter estimation. Hence, ED-optimality is applied in combination with a logistic regression model describing the relationship between drug exposure and response to evoked pain in the complete Freund's adjuvant (CFA) model in rats. Here the design variables selected for optimisation include the dose levels and sampling times required for the characterisation of the analgesic effects of two paradigm compounds, namely, gabapentin and pregabalin. Moreover, a general but robust parameterisation is used which enables integration of historical data into the analysis. Information regarding system-specific model parameters and relative *in vitro* potency data are evaluated as priors during optimisation. The approach allows a shift of the efforts usually required for model building into relatively simple parameter estimation procedures. Our aim is to decrease the uncertainty on EC_{50} estimates as well as on the corresponding inter individual variability. We anticipate that these procedures will also provide the basis for the validation of experimental models of pain in terms of sensitivity and specificity, i.e., enabling the characterisation of the false positive and false negative rates.

The advantages of model parameterisations based on drug- and system-specific properties is further explored in **Chapter 6**, where we illustrate the suitability of a semi-mechanistic model to describe the effects of gabapentin on formalin-induced pain. In the formalin-induced pain model, the observed behaviour in response to a painful stimulus, assessed as flinching frequency, is used as measure of efficacy. This behavioural measure is thought to reflect both the sensory and emotional aspects of pain, making it possibly one of the most predictive models among the available experimental models of pain. Even though differences are known to exist between the substrates underlying the pathophysiology of nociception in this pre-clinical model and in humans, it is imperative that model parameterisations are selected, which enable the assessment of correlations between species.

Except for a few publications, the vast majority of data arising from experimental models of pain are of limited value for quantitative and translational purposes, as measures of potency are in terms of dose or other empirical functions, which ignore systemic and target tissue exposure to the drug. We believe that any serious effort in translational research using experimental models of behavioural pain must take into account pharmacokinetic information as well as the time course of treatment response. Availability of such data provides insight into variability in drug disposition as well into the kinetics of drug action, i.e., the reversibility of the effects. Consequently, one can consider a range of ancillary approaches, such as allometric scaling to extrapolate PKPD relationships from animals to humans. Lastly, we explore the relevance of parameter estimates by comparing our findings with published data from other experimental models of pain as well as with clinical data in neuropathic pain patients. Among the possible explanations for the discrepancies across species, we emphasise the need to carefully assess the degree of target engagement and drug distribution to the site of action.

Yet, our endeavour to characterise the concentration-effect relationships for gabapentin using the inhibition of flinching behaviour as a measure of drug effect highlights the implications of the lack of biomarkers as an intermediate step for the translation of the pharmacological properties across different phases of development. Undoubtedly, there is a pressing need to obtain early signals of efficacy by means of biomarkers, which can be used not only as a “*scaling factor*” to translate drug effects from pre-clinical species to humans, but also as a tool for extrapolating drug effects across populations during drug development. The requirements and points-to-consider for the implementation of a biomarker guided approach are the presented in the next section of the thesis.

3. LOST IN TRANSLATIONS-FROM BIOMARKER RESPONSES TO CLINICAL END POINTS

As indicated in the previous paragraphs, the integration of biomarkers of pharmacology into drug development offers the possibility to eliminate part of the bias that arises from empirical evidence using nonspecific behavioural measures. In **Chapter 7**, we therefore make an attempt to show that opportunities exist for truly characterising the clinical pharmacological profile of novel molecules in humans when biomarkers are used as *predictors* of efficacy, enabling mechanistic insight into the exposure-response relationships and consequently better rationale for the therapeutic dose range. Moreover, the assessment of pharmacokinetic-pharmacodynamic relationships based on biomarkers can provide a stronger basis for personalised medicine, a concept which has mistakenly been linked to the tailoring of treatment based on the use of genetic information only.

Here we analyse data from a cyclo-oxygenase (COX) inhibitor to illustrate the concept of biomarker-guided dose selection and emphasise the importance of gaining insight into the clinical pharmacology of the compound as the basis for the dose rationale. The choice of the COX-2 system as a paradigm was dictated by the various reports arising from the withdrawal of different drugs from the market, for which the clinical pharmacology profile was clearly known to determine efficacy and safety across different therapeutic areas, such as rofecoxib and efalizumab. By integrating pharmacokinetic data from a first-time-in-human clinical trial with inflammatory mediators obtained in an *ex vivo* assay, namely prostaglandins E_2 (PGE_2) and thromboxane B_2 (TXB_2), we show how biomarkers can be used to guide dose selection in subsequent phases of drug development. With the help of simulation scenarios we then illustrate how biomarkers can be used to explore the need for dose adjustment in special populations.

Lastly, we expand the concept of biomarker-guided dose selection to Phase 2 clinical trials, in which efficacy is assessed as primary endpoint in a protocol, to gain further insight into the relationship between biomarkers and overt pain symptoms. By applying the mechanistic

classification proposed by Danhof *et al.*, it is possible to identify whether exposure-biomarker response relationships are drug- or disease-specific and consequently to establish whether they can be used as predictive and prognostic tools during the development and therapeutic use of novel medicines[1]. In **Chapters 8 and 9**, ACRn scores from a large clinical trial in rheumatoid arthritis patients are used to model the exposure-response relationships of a COX-2 inhibitor. Whilst the utility of the ACRn as an index of clinical improvement is beyond doubt, its correlation with the underlying pharmacological activity following administration of a COX-2 inhibitor has never been established.

An integrative approach is therefore proposed in which longitudinal data are used, i.e., information is derived about the time course of treatment response. This contrasts with current practice, in which efficacy is determined by comparing the differences in clinical response at completion of treatment in the active vs. placebo arms. In fact, this example also shows how biomarkers can be used to ensure that the appropriate level of pharmacological activity is achieved and maintained during the course of therapy. More specifically, our analysis is based on the assumption that the optimal benefit-risk balance is likely to be achieved when COX-2 inhibition is maintained above 80% but below 95%. From a conceptual standpoint, such an approach represents a shift from the empiricism which dominates the design of Phase 2b and 3 trials, allowing the implementation of a learn-and-confirm paradigm. Ultimately, a putative PKPD relationship can be derived, which forms the basis for the selection of the dose in efficacy trials.

4. CONCLUSIONS AND PERSPECTIVES

The final chapter of this thesis summarises the main findings and conclusions from the investigations presented throughout the various chapters. In **Chapter 10**, we advocate the need for data integration in support of experimental protocols that support the assessment of pharmacokinetic-pharmacodynamic relationships. We provide recommendations for the selection of candidate molecules and prediction of the therapeutic dose range based on the assumption that accurate inferences can be made about drug-specific properties, if data generation accounts for the distinction between drug- and system-specific characteristics. We emphasise that whilst a distinction can be made with regard to the focus on neuropathic pain, at the beginning of this thesis, and chronic inflammatory pain in the later chapters, most of the concepts underpinning this work are equally applicable to both conditions and more broadly to other chronic diseases. We concede that currently available models have limited value. Clearly, the issue in the evaluation of neuropathic pain conditions is whether existing or new experimental models may ever provide us the basis for translating drug

effects from animals to humans without evidence of common biological substrates. Yet, a model-based approach is essential to optimise the design and interpretation of preclinical experiments, making them more informative. It also contributes to mechanistic inferences, enabling systematic integration of data and information from a vast range of resources available to us. Apart from considering advancements in the field of imaging and proteomics as possible markers of disease progression, we offer insight into novel research protocols and how these can be used to reduce uncertainty about the potential clinical relevance of candidate molecules, enabling selection of the putative therapeutic dose range and transition from the pre-clinical phase to humans.

A slightly different scenario is presented with regard to the challenges one faces in the evaluation treatment effects in chronic inflammatory pain, for which biomarkers exist and imaging technology is already being used. We also reiterate the importance of revisiting current guidelines, which dismiss the role of pharmacological activity as the basis for dose selection and exclude the concept of learning and confirming elegantly stated by Sheiner *et al.* in their landmark paper[2]. Undoubtedly, the use of simulation scenarios will play an increasingly important role in the evaluation of the impact of heterogeneity in target population as well as of variability in pharmacokinetics, pharmacodynamics and response to intervention. Finally, we envisage that further advancements in the prediction of pain response can be obtained by expanding the concepts to multiple endpoints as well as by incorporating fully mechanistic models in the pharmacometric framework proposed in this thesis.

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SECTION II

MODEL BASED ANALYSIS OF BEHAVIOURAL PAIN RESPONSE

CHAPTER 4

Application of ED-optimality to screening experiments for analgesic compounds in an experimental model of neuropathic pain

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ABSTRACT

The high variability in the response to evoked pain prevents accurate ranking of compounds during the screening of drugs for inflammatory and neuropathic pain. In this study, we explore the feasibility of introducing optimality concepts to experimental protocols, enabling estimation of parameter and model uncertainty.

Pharmacokinetic and pharmacodynamic data from different experiments in rats were pooled and modelled using nonlinear mixed effects modelling. Pain data on gabapentin and placebo-treated animals were generated in the complete Freund's adjuvant (CFA) model of neuropathic pain. A logistic regression model was applied to optimise sampling times and dose levels to be used in an experimental protocol. Drug potency (EC_{50}) and inter individual variability (IIV) were considered the parameters of interest. Different experimental designs were tested and validated by SSE (stochastic simulation and estimation) taking into account relevant exposure ranges.

The pharmacokinetics of gabapentin was described by a two-compartment PK model with first order absorption ($V_2=0.118$ l, $V_3=0.253$ l, $Cl=0.159$ l/h, $K_a=0.26$ h⁻¹, $Q=1.22$ l/h). Drug potency (EC_{50}) for the anti-allodynic effects was estimated as 1400ng/ml. Protocol optimisation improved bias and precision of the EC_{50} improved by 6 and 11.9%, respectively, whilst interindividual variability (IIV) estimates showed improvement of 31.89 and 14.91%, respectively.

Variability in behavioural models of evoked pain response leads to uncertainty in drug potency estimates and consequently to inaccurate ranking of compounds during screening. As illustrated for gabapentin, ED-optimality concepts enable analysis of discrete data taking into account experimental constraints.

INTRODUCTION

Despite the number of compounds entering early clinical development, neuropathic pain is an area of high attrition rate, with most treatments failing at proof-of-concept in patients. This situation is critical if one considers that currently available drugs for neuropathic pain show less than 50% efficacy in the overall target population [1]. Among other factors, the lack of efficacy in humans has been assigned to the poor correlation between evoked pain in pre-clinical models of disease and the differences in aetiology in humans [2]. On the other hand, another important point which has remained less evident is the fact that experimental protocols in pain research are often based on empirical criteria[3] and little or no attention is given to basic concepts such as accuracy and precision. Poor experimental designs often lead to biased and inaccurate parameter estimates [4], which consequently may influence the selection of suitable candidate molecules for progression into humans.

In early drug development, screening of compounds ought to rely on accurate ranking of their pharmacokinetic (PK) and pharmacodynamic (PD) properties, yielding evidence of their pharmacokinetic-pharmacodynamic (PKPD) relationships [5]. However, the use of a model-based approach for the analysis of such experiments, while desirable, is often precluded by practical constraints and resources [6]. Suitable designs entail the use of repeated measurements that describe the time course of drug concentrations and the pharmacological effects of interest. Feasibility considerations often limit the collection of repeated samples in individual animal and thus compromise the design of the experiment. Given the requirement for sparse sampling, appropriate sampling times become critical [7]. Therefore, accurate and precise model parameters estimates depend greatly on the experimental design.

In the current investigation, we use the CFA model, a well-known experimental animal model of inflammatory pain [8, 9], as paradigm to explore the feasibility of introducing optimality concepts in the screening of analgesic compounds. Dichotomisation of response is proposed as the basis for generalisation of a model-based approach in this phase of development. In optimal experimental design, D-optimality is by far the most used criterion in individual, and population modelling studies. Herein optimisation is carried out assuming there is no uncertainty (imprecision around the parameters of interest) i.e. there is no uncertainty distribution around this parameter. This assumption is also a disadvantage since for D-optimality to be applied, the true parameter value should be determined based on prior knowledge or model fitting in a previous exercise [10]. Although D-optimality has been considered the classic approach to designing an experiment optimally [11, 12], this method may not be suitable for prospective evaluation of the compounds during screening experiments when little data is available and prior knowledge about the pharmacokinetic and pharmacodynamic properties are limited.

Here we apply ED-optimality, an approach which has been applied in different areas of clinical research when the model parameters have uncertainty distributions [4]. The use of ED-optimality assumes a prior distribution around the parameters of interest [13]. While optimal design has been extensively used for optimizing different types of continuous repeated measurements, with non-linear mixed-effects modelling, little work has been done with discrete data [14]. We aimed at defining optimal design requirements for screening experiments, assuming EC_{50} and inter-individual variability as the parameters of interest for optimisation.

We anticipate that improved parameter precision and accuracy will contribute to better ranking of compounds and enhanced ability in discriminating false positives from false negatives during the screening of compounds for neuropathic pain.

MATERIALS AND METHODS

Experimental Procedures

In the CFA model, central sensitisation (NP) is induced following injection of an algogen. Allodynia or pain with a non-noxious stimulus is then measured as threshold to affected paw withdrawal with increasing diameter of von Frey filaments [15]. The experimental protocol was performed according to a double-blind, randomized, placebo controlled study with 9 animals per cohort. Sprague-Dawley rats received single doses of 0, 10, 30, 100 mg/kg gabapentin orally. The study was approved by the Institutional ethics committee.

Pharmacodynamic measurements

The threshold to paw withdrawal to a normally non-noxious stimulus was measured as a marker of the anti-allodynic effect, whilst the change in threshold relative to baseline was selected as the PD endpoint. PD measurements were collected at hourly intervals for 4 h post-dose for each animal.

Pharmacokinetic experiments / measurements

PK data were obtained from 2 separate experiments in conscious rats. In the first, gabapentin was administered orally in the doses 0, 10, 100, 300 mg/kg in a formalin induced hypersensitivity experiment [16]. There were three rats per dose group, with each animal being sampled four times up to 6 hours post dose. In a second experiment, 63 animals received 50 mg/kg of gabapentin as an IV infusion. The rats were sampled 8 times up to 24 h post dose [17].

Data Analysis

Model parameterisation

The threshold for paw withdrawal was dichotomised as a binary response variable, with response being defined as changes in the threshold for paw withdrawal were >30% relative to baseline. The exposure-response relationship was modelled using a logistic regression (LR) model, using a parameterisation previously described by [18]:

$$p = \frac{e^{f(P, X)}}{1 + e^{f(P, X)}} \quad (1)$$

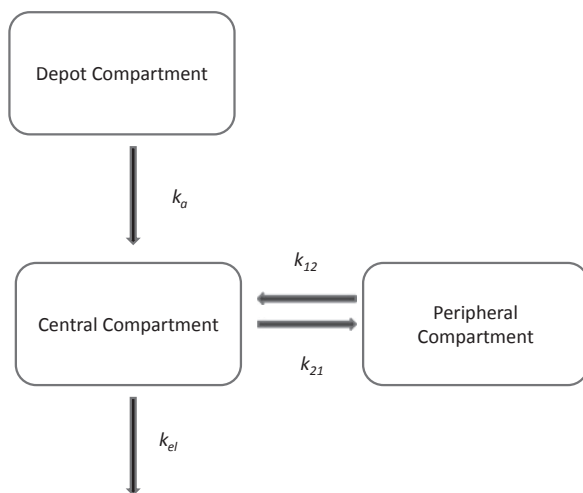
Where p is the probability of an event P , x are parameters and independent variables respectively. The odds of the event are therefore given by $p/1-p$ and its logit may be expressed as:

$$\text{logit}(p) = \ln \frac{p}{1-p} = f(P, X) \Leftrightarrow p = \frac{e^{f(P, X)}}{1 + e^{f(P, X)}} \quad (2)$$

An E_{\max} model was considered for the characterisation of the drug effect on the logit space. Random effects were denoted by η . This term represented both inter individual (IIV) as well as the random variability. Substituting for drug effects and p , the odds of an event may be represented by the following expression:

$$\frac{1}{1 + e^{\frac{Emax * CONC}{EC50 + CONC} + plac + \eta}} \quad (3)$$

Drug concentrations at time points corresponding to PD measures were simulated using a two-compartment pharmacokinetic model with dose-limited absorption (see Figure 4.1). The model was built using a two-step approach. First, IV data from a previous experiment was modelled to obtain parameter estimates. Subsequently, using these parameters, bioavailability estimates were obtained for oral data. Details of the PK analysis are presented in the appendix (supplemental material).



$$K_{el} = \frac{CL}{V_2}, K_{12} = \frac{Q}{V_2}, K_{21} = \frac{Q}{V_3}$$

Figure 4.1: Two-compartment model used to describe the pharmacokinetics of gabapentin.

k_a =absorption rate constant, k_{el} =elimination rate constant, CL =clearance, V_2 and V_3 =volume of distribution in the central and peripheral compartments, respectively, Q =intercompartmental clearance.

ED-Optimal Design

Since drug potency is the parameter of interest, we focused on optimizing the experimental design in order to yield precise estimates of EC_{50} as well as the corresponding IIV. A summary of the model parameters used for the optimisation are given in Table 4.1. Non-linear mixed effects modelling based on the maximum likelihood estimation method was used for optimisation purposes. Theoretical aspects on the optimisation strategy are described in the appendix (see supplemental material).

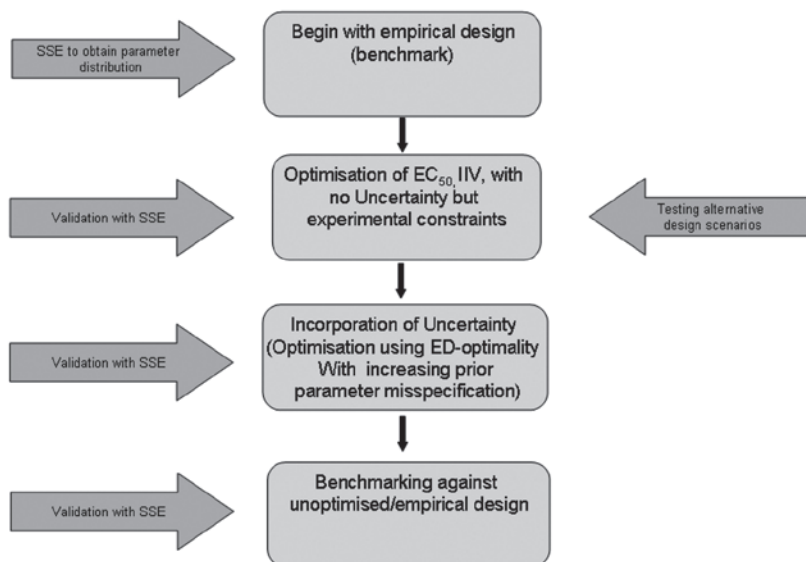
We assumed drug exposure to be a determinant of response and thus optimised for sampling times and dose levels. Given that the bioavailable fraction of gabapentin is dose-dependent, the doses were optimised taking into account such differences. Other experimental variables were kept constant keeping in mind experimental constraints. These included, sample size (9 per cohort), number of measurements /individual (5), no of dose groups (4) and a maximum dose of 300mg/kg. Any predicted sampling times which were identified to occur within 30 minutes interval were moved apart from each other to ensure at least 30 min difference between two consecutive sampling times. A diagram of the general optimisation process is outlined in Figure 4.2. The empirical design was the benchmark design and the parameters of interest were EC_{50} and IIV.

Table 4.1: PK and PKPD model parameter estimates used in the optimisation process. RSE is shown between parentheses.

| Model parameters | Values |
|--|-----------------------|
| Pharmacokinetics | |
| Central compartment volume (V_1) (l) | 0.118 (9.8) |
| Peripheral volume (V_2) (l) | 0.253 (4.2) |
| Clearance (Cl) (l h ⁻¹) | 0.159 (4.1) |
| Intercompartmental clearance(Q) (l h ⁻¹) | 1.22 (0.25) |
| Bioavailability fractions(F) | 1, 0.75, 0.22, 0.087* |
| Absorption Rate constant (K_a) (/h) | 0.26 (20) |
| Random error | 0.30 (3.3) |
| Pharmacodynamics | |
| E_{max} (%) | 97 (25) |
| Baseline/placebo effect (%) | 2.81 (40) |
| EC_{50} (ng ml ⁻¹) | 1400 (145) |
| IIV | 3.14 (74) |

* for doses 10, 30,100,300 mg/kg respectively

Optimization Strategy

**Figure 4.2:** Overview of the general Optimisation procedures used during the analysis.

IIV= inter individual variability, SSE=stochastic simulation and estimation, EC_{50} =drug potency.

Prior parameter misspecification and uncertainty was incorporated into the optimisation by ED-optimality. A lognormal distribution was assumed for the parameters of interest, with mean values fixed as the true estimate. The maximum prior parameter misspecification was

predetermined to be 50%. An additional scenario was simulated and optimized to account for the effects of linear pharmacokinetics, assuming no change in the bioavailability across dose levels. The standard deviation of the priors was chosen so as to take into account expected parameter uncertainty and was defined as a distribution rather than a point value. The validation of the optimised design was carried out using stochastic simulation and estimation (SSE).

Stochastic simulation & estimation (SSE)

SSE was used to test the robustness of the optimal designs. In brief, during optimisation, gabapentin concentrations were simulated using the PK the model. The combined PKPD model was then used to simulate 'optimal sampling scenarios'. The initial values of the PD parameters in these models were considered the 'true estimates' and specified as the upper, lower and middle points of the distribution. The simulated optimal sampling datasets were then fitted to a pharmacodynamic model using nonlinear mixed effects modelling to assess the parameter estimates yielded by the proposed design. This two-step process was performed 500 times for each optimal design scenario. Estimation was considered successful when a normal flag (minimization successful) was obtained. Values of the 500 first successful estimations were recorded and summarised for each SSE run, along with their standard errors (SEs). To prevent numerical problems causing failure of the design, we began by first applying the ED-optimal design criteria with point values for priors (i.e., D-optimality) then increased prior breadth, until they reached the relevant uncertainty.

Concordance between optimised parameters and true values

Agreement between estimated and values used for simulations ('true values') were assessed using the mean of the estimation, the mean prediction error (MPE) and root mean square error (RMSE), which reflect precision and bias in model parameters [19]. Their calculation is as follows:

$$RMSE = \sqrt{\frac{\text{mean}((Est_i - True)^2)}{True^2}} \quad (4)$$

$$MPE = \frac{\text{mean}(Est_i - True)}{True} \quad (5)$$

where Est_i is the i^{th} parameter estimate and $True$ is the simulation value (initial estimate) of the parameter. Matlab 7.9 (The Mathworks Inc., Natick, MA, 2008) and popED 2.11 (University of Uppsala, Sweden) were used for implementation of the optimization. PsN

3.12 (University of Uppsala, Sweden) and NONMEM 7.1 (ICON Development Solutions, Ellicott City, MD) were used for SSE. Data manipulation, graphical and statistical summaries were performed in R (www.r-project.org)

RESULTS

PK and PKPD model parameter estimation

An overview of the estimated PK and PKPD parameters are presented in Table 4.1. As can be seen from Figure 4.3(a), the simulated concentration profiles for all three doses of gabapentin are not significantly different from each other. When corrected for differences in bioavailability the observed exposures are equivalent to doses of 22.3, 22.5 and 27mg/kg respectively. Relative bioavailability was found to decrease nonlinearly from 100% at 10 mg/kg to 9% at 300 mg/kg. The resulting logistic model for the response data is shown in Figure 4.3(b).

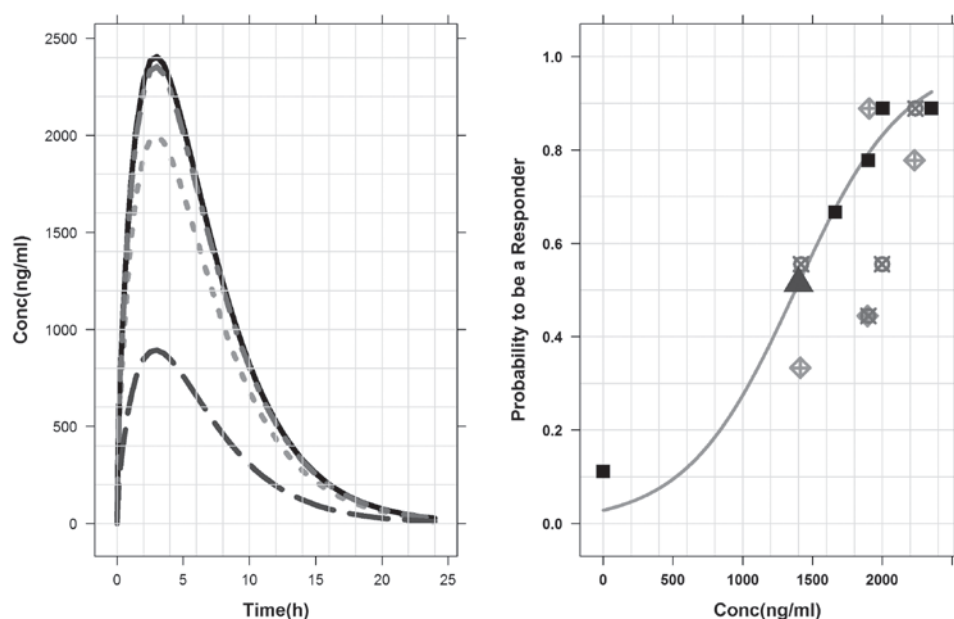


Figure 4.3: (a) Simulated plasma concentration vs. time profiles of gabapentin in rats after administration of 10(dashed line), 30(dotted line), 100(dash-dotted line), 300(solid line) mg/kg doses. (b) Logistic regression model showing the exposure-response curve (gabapentin concentration vs. probability of response). Symbols depict the proportion of observed responses after doses of 30(open diamond), 100(crossed circle), 300(filled square) mg/kg whereas the curve is the model-predicted probability.

Table 4.2: Priors used in the experimental protocol optimisation

| Parameter | True Value | ED-optimal Design (0% variance) | ED-optimal Design (10% variance) | | ED-optimal Design (50% variance) | |
|-------------------------------|------------|------------------------------------|-------------------------------------|--------------------|-------------------------------------|--------------------|
| | | Misspecification % | Mean | Misspecification % | Mean | Misspecification % |
| EC ₅₀ [#] | 1400 | 0 | 1400 | 10% | 1400 | 50% |
| IIV (η) | 3.14 | 0 | 3.14 | 10% | 3.14 | 50% |

[#] values were applied to the logit space

DESIGN OPTIMISATION

As shown in Table 4.2, uncertainty in parameter estimation was explored with prior distributions of 10% (initial runs) and 50% (final run). As it can be seen in Table 4.3, whilst the same sampling scheme is used irrespective of dose in a typical empirical protocol, optimal designs require a different scheme for each dose. The optimised design performed equally irrespective of the uncertainty (i.e., 10 or 50% variance in parameter distribution).

Table 4.3: Comparison of dose levels and sampling times for the empirical and optimised protocol designs. Dose levels are shown in mg/kg, sampling times are in hours (h).

| Design variables | Empirical design | ED-optimal (10% variance) | ED-optimal (50% variance) |
|------------------|------------------|------------------------------|------------------------------|
| Dose 1 | 0 | 0 | 0 |
| Sampling times | 0,1,2,3,4 | 0, 3.15, 4.72, 7.80, 9.93 | 0, 3.11, 4.23, 6.34, 10 |
| Dose 2 | 30 | 100 | 100 |
| Sampling times | 0,1,2,3,4 | 0, 1, 1.5, 2, 4.9 | 0, 1.45, 2.49, 3.54, 4.54 |
| Dose 3 | 100 | 150 | 150 |
| Sampling times | 0,1,2,3,4 | 0, 1.8, 2.80, 3.10, 4.10 | 0, 0.57, 1.7, 5.51, 6.01 |
| Dose 4 | 300 | 300 | 300 |
| Sampling times | 0,1,2,3,4 | 0, 0.62, 1.12, 7.23, 8.23 | 0, 0.69, 1.19, 1.69, 5.67 |

Table 4.4: Impact of nonlinear absorption on protocol design optimisation.

| Dose level (mg/kg) Sampling times (h) | Empirical design | Bioavailable dose level | ED-optimal (50% variance) |
|--|------------------|-------------------------|------------------------------|
| Dose 1 | 0 | 0 | 0 |
| | 0,1,2,3,4 | | 0, 2.74, 3.45, 5.78, 8.45 |
| Dose 2 | 30 | 29.5 | 100 |
| | 0,1,2,3,4 | | 6.15, 6.65, 7.65, 8.15 |
| Dose 3 | 100 | 41.7 | 150 |
| | 0,1,2,3,4 | | 0, 1.34, 4.79, 5.29, 5.79 |
| Dose 4 | 300 | 87.6 | 300 |
| | 0,1,2,3,4 | | 0, 0.50, 1, 1.5, 2 |

Findings with gabapentin show that bioavailability decreases with increasing dose levels. Dose levels are shown in mg/kg, sampling times are in hours (h)

Comparison of parameter estimates & respective standard errors

Optimal estimates were closer to the true estimates and parameter standard errors decreased by more than half when sampling was based on ED-optimality concepts. Figure 4.4 reveals that improvements can be achieved not only in parameter estimates but also in SEs after design optimisation as compared to empirical protocol designs. Furthermore, we show that with varying bioavailability (Table 4.4), the difference between the optimised and empirical protocols is more marked. As shown in Table 4.5, parameter precision increases and bias is lower.

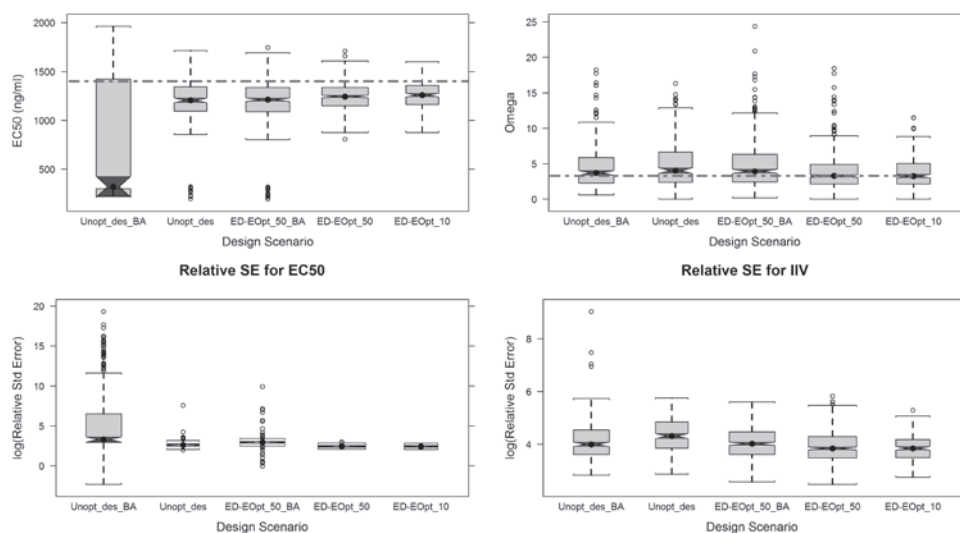


Figure 4.4: Comparison of model parameters (EC_{50} and IIV) and the corresponding estimates of bias and precision for various design scenarios described in Table 4.4. Dashed line indicates true estimate values.

Table 4.5: Comparison of parameter estimates for empirical and optimised experimental designs.

| Design type | EC_{50}^* median (range) | IIV (omega) mean (SD) |
|---|-------------------------------|--------------------------|
| Empirical | 1205.5 (200-1714) | 4.82 (3.30) |
| ED-optimality (variance 10%) | 1258 (876-1601) | 3.70 (2.13) |
| ED-optimality (variance 50%) | 1252 (806-1709) | 3.96 (2.30) |
| Empirical with varying F | 319 (223-1963) | 4.62 (3.25) |
| ED-optimality with varying F (variance 50%) | 1211(191.7-1754.90) | 4.78 (3.31) |
| True Value | 1400 | 3.13 |

unit of measurement ng /ml

Bias and precision of parameter estimates

Uncertainty was introduced in a stepwise manner. RMSE is indicative of precision whilst MPE reflects bias in parameter estimates. In Table 4.6, the RMSE & MPE obtained after empirical and optimal designs are compared with each other. The implications of optimised sampling times in experimental protocols is shown graphically in Figure 4.5. As indicated by the different symbols response is sampled at time points where drug concentrations are informative of the expected drug potency. The sampling points for the empirical designs describe a monotonic pattern while those for the optimal designs are different for each dose and distribute around the EC_{50} .

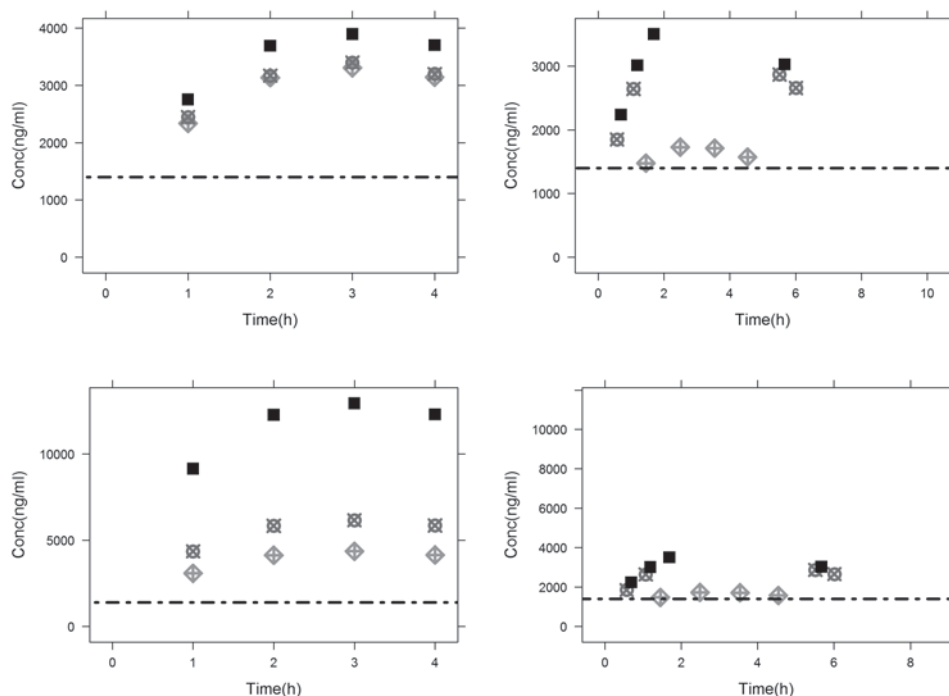


Figure 4.5: Selected sampling times relative to EC_{50} values (dashed line) based on a typical empirical protocol (left panels) and ED-optimal design (right panels). Based on theoretical principles, optimal sampling times should provide concentration values supporting the estimation of the parameter of interest. For gabapentin, our analysis show that variable bioavailability must be considered during optimisation to ensure accurate sampling times (lower panels). Symbols represent different dose levels namely; 30(open diamond), 100(crossed-circle), 300 (filled squares) mg/kg for all scenarios, except the top right panel where the optimal doses were, 100, 150 and 300mg/kg.

Table 4.6: Comparison of RMSE and MPE for the empirical and ED-optimal designs.

| Design Type | EC50 | | Omega | |
|--|-------|-------|-------|-------|
| | RMSE | MPE | RMSE | MPE |
| Empirical | 23.17 | 7.95 | 41.61 | 54.21 |
| ED-optimal(50% variance) | 11.27 | 2.37 | 26.70 | 22.32 |
| Empirical with varying F | 63.43 | 44.39 | 39.08 | 47.81 |
| ED-optimality with varying F (variance 50%) | 22.10 | 7.025 | 41.08 | 52.84 |

DISCUSSION AND CONCLUSIONS

The rat CFA model is typically classified as a model of inflammatory somatic pain. However the typical symptoms of allodynia and hyperalgesia are reproduced in this experimental model, which are considered indicative of central sensitization [20]. Hence this test is commonly employed as part of the battery of screening experiments when looking for analgesic/anti-neuropathic pain activity.

It is evident that challenges exist in the identification of suitable targets for the treatment of neuropathic pain [21]. Given the gaps in the understanding of the mechanisms underlying neuropathic pain disorders, drugs are tested pre-clinically without evidence as to which target will yield a clinically relevant response. However, this is further compounded by the paradigm currently used for the screening of compounds, which relies on evoked-pain response associated with general positive symptoms such as allodynia and hyperalgesia [22, 23]. Ongoing research strongly suggests that evidence of concentration-effect relationships is necessary for translational purposes and accurate ranking of compounds [24].

Although further advancement of the field will certainly depend on the identification of specific biomarkers of disease, the assessment of pharmacokinetic-pharmacodynamic relationships remains fundamental for characterising the properties of novel compounds and interpreting response across species. Unfortunately, screening procedures and protocols do not consider the implication of empirical designs, which can result in estimates of drug properties such as potency, which are often imprecise and extremely variable[25]. As a consequence, inaccurate ranking of compounds is likely to occur during the screening stage, which then progress into development. Our investigation illustrates the implications of optimality concepts to better design experimental protocols and obtain more precise and accurate parameter estimates.

There have been numerous attempts in the implementation of optimal designs for experimental protocols [26], but most designs were explored under the assumption of no parameter uncertainty [27], a condition which does not correspond to the screening phase of novel molecule candidates. Furthermore, application of optimality concepts imposes the availability of a model, which is unknown at the early stages of drug discovery and development, as is the case during the screening phase. These conceptual constraints are

further complicated by practical challenges during the experiments, such as the potential interference of blood sampling for pharmacokinetics between behavioural measurements and limited sampling frequency due to habituation and other possible effects on pharmacodynamics.

Here we have shown how the use of a binary response can overcome technical limitations associated with model building, by emphasising the assessment of drug- and system-specific parameters. The parameterisation of drug effects in terms of EC_{50} allows discrimination of drug properties, whereas baseline and maximum response E_{max} reflect experimental model characteristics and as such can be estimated in conjunction with historical data, which are incorporated as parameter priors. In addition, as shown in the different scenarios, ED-optimality also allows the inclusion of uncertainty in a formal manner.

Our analysis focused on the simultaneous optimisation of two design variables, namely dose and sampling times, in a similar way to what has been previously reported by Nyberg and collaborators [28]. In contrast to empirical designs, the results show that the use of optimality concepts yields sampling at time points around the expected parameter estimate (Figure 4.5), thereby maximizing the information obtained from the an experimental protocol. Even in the case parameter misspecification, ED-optimality appears to provide more informative data than designs based on ‘best guess’ estimates.

Since gabapentin exhibits carrier-mediated absorption, bioavailability was found to be nonlinear, decreasing with increasing doses [29]. This effect caused an unusual situation in which changes in the bioavailable fraction resulted in practically the same exposure across the different dose levels. In such circumstances, the ‘best guess’ estimates were not as biased as one would normally observe. To illustrate the effect of such nonlinearity we have therefore investigated an optimisation scenario in which bioavailability estimates decreased with increasing doses but yielding wide variation in plasma concentrations. The results reveal that empirical protocols perform much more poorly (as defined by the bias and precision of EC_{50}) than optimised designs.

Limitations

The optimisation procedures were constrained by the effect on nonlinear absorption and relatively sparse availability of oral pharmacokinetic data, which prevented accurate estimation of the pharmacokinetic parameters of interest. Therefore, drug concentrations from an IV experiment were used to support the estimation of clearance and volume of distribution. Whilst data were accurately fitted to the model, we did not attempt to describe the transporter-limited absorption of gabapentin in a mechanism-based manner. Instead, we applied a ‘curve linearization’ approach, under the assumption that bioavailability decreased linearly across the dose range.

In addition, it has been documented that optimal designs based on D-optimality have a tendency to cluster at parameter point estimates because model expectations is assumed to be the same for each individual. Apparently, this clustering effect may be minimised by the use of priors [30]. Regardless of the use of priors clustering was observed during the analysis, but this issue was resolved by imposing minimum interval between sampling times, as describe in the methods section. We have not performed a sensitivity analysis to explore the potential impact on the estimates of bias and precision obtained for the different optimisation scenarios. We anticipate however that the use of stepwise iterations during optimisation should minimise such issues, including failure due to numerical problems. Lastly, it should be noted that IIV estimates were higher with varying bioavailability. It is not clear if these findings may have been caused by inflated random residual variability in this specific scenario.

In conclusion, our study reveals that experimental requirements must be considered for the purposes of screening and ranking of compounds. Accurate estimation of drug potency (EC_{50}) entails modification to the protocol design, including specific changes to sampling procedures and dosing rationale, which cannot be guessed without applying ED-optimisation concepts. It is time for experimentalists to understand the implications of empirical protocols and make sure experiments are suitable for the evaluation of pharmacokinetic-pharmacodynamic properties of novel molecules.

APPENDIX 1: PK AND PKPD MODELLING DETAILS

The pharmacokinetics of gabapentin was described by macro-constants according to the following expression[31]:

$$C = \frac{K_a FD}{V_1} \left\{ \frac{(k_{21} \lambda_1) e^{-\lambda_1 t}}{(k_a - \lambda_1)(\lambda_2 - \lambda_1)} + \frac{(k_{21} \lambda_2) e^{-\lambda_2 t}}{(k_a - \lambda_2)(\lambda_1 - \lambda_2)} + \left(\frac{(k_{21} - K_a) e^{-K_a t}}{(\lambda_1 - k_a)(\lambda_2 - k_a)} \right) \right\} \quad (6)$$

Where k_a =absorption rate constant, V_1 =central volume of distribution, F =bioavailability of the administered dose, λ_1 and λ_2 correspond to the initial and terminal slopes representing bi-exponential decline respectively and K_{21} is a rate transfer microconstant between compartments 1 and 2.

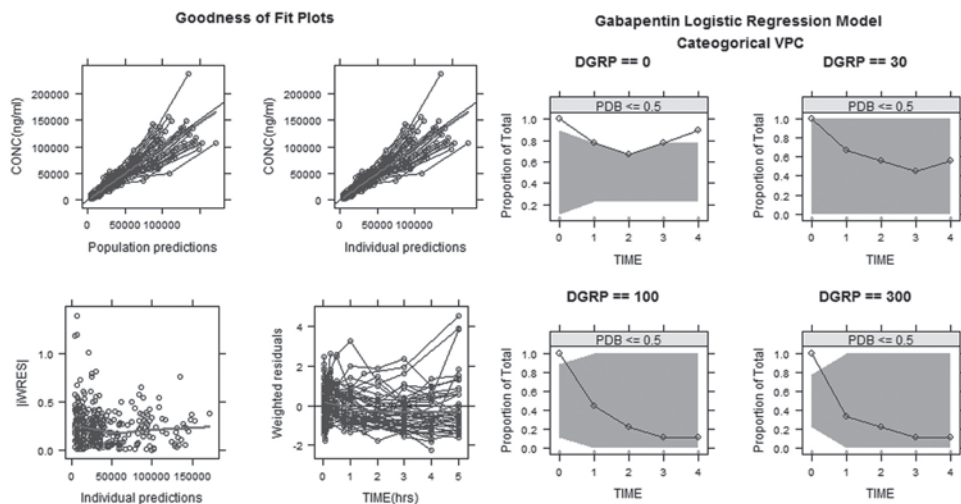


Figure 4.6: Goodness-of-fit plots for the pharmacokinetic (left panel) and PKPD (right panel) models. Lines represent the observed data, whereas the shaded area depicts the 90% confidence intervals

APPENDIX 2: - OPTIMISATION CONCEPTS FOR BINARY RESPONSE

In optimal design, the probability density $p(y_i|\theta)$ of the experimental observations y_i depends on a vector of likelihood parameters θ , where

$$\theta^T = [(\beta pop)^T, d^T] \quad (7)$$

The maximum likelihood estimate θ is the value that maximises the joint log-likelihood function:

$$L(\vartheta) = \sum_{i=1}^m \log p(y_i | \vartheta) \quad (8)$$

$$Cov\vartheta \geq (FIM)^{-1} \quad (9)$$

As a result of a smaller covariance matrix, the lower the FIM^{-1} the greater is the precision, where FIM is defined as:

$$FIM = E_y \left[\left(\frac{\partial}{\partial \theta} L(\theta) \right)^T \frac{\partial}{\partial \theta} L(\theta) \right] \quad (10)$$

By choosing the optimal design variables \hat{X} that minimize FIM^{-1} , one obtains the design variables that yield the smallest possible lower bound for the covariance matrix of the population parameter estimates [4].

Using the ED-optimality criterion, the parameters of interest are assigned a prior distribution and an expectation. A design X^D is said to be ED-optimal if it minimises the negative expected ($E\alpha$) determinant of the FIM with respect to the parameter priors.

$$jED(x) = E\alpha[det[F(\alpha, x)]] \quad (11)$$

$$Int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} p(\alpha).det[F(\alpha, x)]dx \quad (12)$$

$$x^{ED} = argmax_n [j^{ED}(x)] \quad (13)$$

Laplace approximation was used for calculation of the FIM. The optimization criteria used was D-/ED optimal (i.e., optimizing the determinant of FIM). The Latin hypercube (LH) sampling was used in the MC calculation of the likelihood to speed up and stabilise the likelihood calculation. The number of LH samples differed between the different models but was between 40-200 individual samples. The FIM was calculated both using the

expectation of the gradient product of the first derivative of the log-likelihood with respect to the parameters as well as the expectation of the negative 2nd order derivative of the log-likelihood with respect to the parameters.

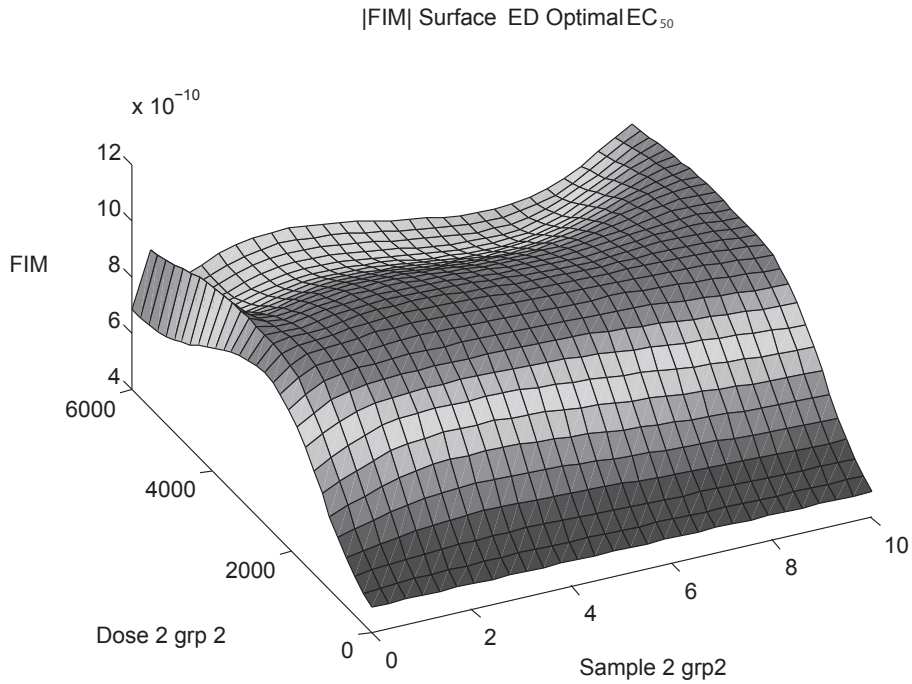


Figure 4.7: Fisher Information Matrix surface versus the highest dose (300mg/kg) and sample times combination for ED design with 50% variance in the expected parameter estimates. The dark red surface represents the optimal design for this dose and sampling time's combination.

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CHAPTER 5

Optimised protocol design for the screening of analgesic compounds in neuropathic pain

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ABSTRACT

We have previously shown how screening experiments for neuropathic pain can be optimised taking into account parameter and model uncertainty. Here we demonstrate how optimised protocols can be used to screen and rank candidate molecules. The concept is illustrated by pregabalin as a new chemical entity (NCE) and gabapentin as a reference compound.

ED-optimality was applied to a logistic regression (LR) model describing the relationship between drug exposure and response to evoked pain in the complete Freund's adjuvant (CFA) model in rats. Design variables for optimisation of the experimental protocol included dose levels and sampling times. Prior information from the reference compound was used in conjunction with relative *in vitro* potency as priors. Results from simulated scenarios were then combined with fitting of experimental data to estimate precision and bias of model parameters for the empirical and optimised designs.

The pharmacokinetics of pregabalin was described by a two-compartment model. The expected value of EC_{50} of pregabalin was 637.5ng/ml. Model-based analysis of the data yielded median (range) of EC_{50} values of 1125 (898-2412) ng/ml for the empirical protocol and 755 (189-756) ng/ml for the optimised design. In contrast to current practice, optimal design entails different sampling schedule across dose levels.

ED-optimised designs should become standard practice in the screening of candidate molecules. It ensures lower bias when estimating the drug potency, facilitating accurate ranking and selection of compounds for further development.

INTRODUCTION

Experimental protocols in pain research tend to be based on empirical criteria, in spite of the evidence for the advantages of a model-based approach for candidate selection and interspecies scaling[1]. In fact, the limitations of current screening methodologies for pain compounds have been extensively documented in literature [2]. In addition to the assumptions regarding the predictive value of behavioural measures of pain in animals, drug screening also implicitly rely on the accuracy of estimates obtained by standard experimental protocols. Unknowingly, such assumptions may lead to inaccurate assessment of compound efficacy and relative potency. Even when a more quantitative approach is applied to the analysis of the data, accurate estimation of compound potency may remain an elusive goal [3-5].

Although different pharmacological parameters may be considered as criteria during development (e.g., intrinsic activity, binding reversibility), it is usually potency differences that drives the selection of novel compounds during the screening process. Yet, the EC_{50} estimates of a compound are strongly dependent on the experimental conditions, which could affect their ranking and selection [6]. This issue is further compounded when pharmacokinetics is ignored and potency is expressed in terms of ED_{50} [7, 8]. Undoubtedly, poor accuracy in potency estimates can have repercussions on interspecies scaling and dose selection in humans, making the translation of preclinical findings a gamble rather than a scientific exercise. Therefore, differences in drug efficacy across pain models can be assigned to differences in potency and/or sensitivity only if estimates are deemed and proven to be sufficiently accurate [9].

Despite the potential implications of optimality concepts for experimental protocols in drug development, little effort has been made to demonstrate the impact of design optimisation on the evaluation of drug potency in experimental models of pain. In conjunction with an integrated PKPD approach, it would be possible to objectively rank compounds [6], but its application remains eschewed by experimenters because of the different requirements for the experimental protocol and skills involved in model building and validation [1]. In the current investigation, we apply ED-optimality in the screening of new chemical entity (NCE) using prior information from a reference compound (REF). Putative PKPD relationships are used for the prospective optimisation of the experimental protocol for the NCE. Optimisation of the parameters of interest (EC_{50} and interindividual variability) is performed under the assumption of parameter uncertainty. To this end, we define efficacy in terms of a binary response, using a logistic regression model which is parameterised in terms of system and drug-specific parameters. Such a parameterisation eliminates the need for repeated model building while allowing generalisation of a single structural model across compounds[10]. Furthermore, this approach allows the use of historical data during the estimation of system-specific parameters. The main requirement is the expected point estimates of the

parameters and its corresponding uncertainties. We anticipate considerable improvement in the selection of the doses required for screening and subsequent ranking of the compounds for further development.

MATERIALS AND METHODS

Experimental procedures

In vivo model

In the CFA model central sensitization (NP) is induced following injection of an algogen to the hind-paw. Allodynia or pain with a non-noxious stimulus is then measured as threshold to affected paw withdrawal using von Frey filaments [11]. The experimental protocol was performed according to a double-blind, randomized, placebo controlled study with nine animals per cohort. Sprague-Dawley rats received single doses of vehicle, 10, 30, or 100 mg/kg pregabalin orally. PD measurements were performed at hourly intervals from 0 to 4 hours post-dose for each animal. Drug concentrations at each the sampling times corresponding to PD measurements were simulated from a published pharmacokinetics model. Further details of the model are described below. The study was approved by the Institutional Ethics Committee.

In vitro data

Based on in vitro data, we have assumed that the potency ratio between the NCE and REF to be equal to 2 [12]. Using the median estimated EC_{50} values of 1275 ng/ml from 200 bootstrap runs for gabapentin from a previous investigation [13], the expected EC_{50} values for pregabalin were fixed to 637.5 ng/ml.

Pharmacokinetic and pharmacokinetic-pharmacodynamic modelling

Pharmacokinetic model:

The pharmacokinetics of pregabalin was characterised by a two-compartment model (Figure 5.1). Drug concentrations at the time points corresponding to pain measurements were simulated based on the parameters estimated previously [14].

PKPD modelling

The threshold for paw withdrawal was re-parameterised as a binary response variable, where response was defined when differences in the threshold for paw withdrawal were >30% relative to baseline. The exposure-response relationship was modelled using the logistic regression procedures as previously described[15]. We applied different parameterisations to ensure the most appropriate model was used, ensuring successful minimisation and plausible parameter values. The logit transformation assures that the probability, p_{ij} ,

remains between 0 and 1. Assuming that p_{ij} is the individual probability at time j of achieving response (success), the general structure for this model can be described using equation 1.

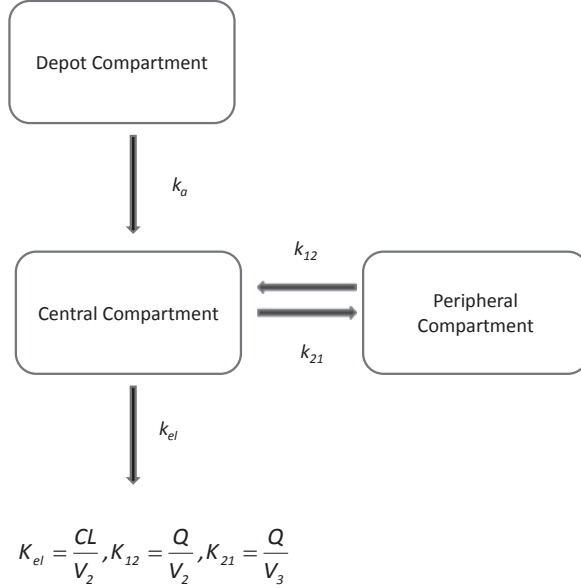


Figure 5.1: Two-compartment model used to describe the pharmacokinetics of pregabalin.

k_a =absorption rate constant, k_{el} =elimination rate constant, CL =clearance, V_2 and V_3 =volume of distribution in the central and peripheral compartments, respectively, Q =intercompartmental clearance.

$$p_{ij} = \frac{e^{f(P, X_{ij}, \eta_i)}}{1 + e^{f(P, X_{ij}, \eta_i)}} \quad (1)$$

Here f is a function of the typical value parameters (P), X_{ij} the individual independent (design) variables at time j , η_i the deviation from the typical individual. The term η_i is a normally distributed zero mean random variable which describes inter-individual (IIV) and random variability. This parameterisation has been discussed in detail in the companion paper [13]. All pharmacodynamic measurements were considered to be uncorrelated for the purpose of our analysis, i.e. no serial correlation was assumed.

A simplified model without logit transformation was also tested in which probability of an event is given directly by the Hill equation. In this expression, the placebo/baseline and E_{max} vary between 0 and 1. Thus $E_{max} = 1 - placebo$. In this case, the individual probability of a response at time j is:

$$p_{ij} = placebo + \frac{E_{max} \cdot C_{ij}}{EC_{50} + C_{ij}} = placebo + \frac{(1 - placebo) \cdot C_{ij}}{EC_{50} + C_{ij}} \quad (2)$$

where C_{ij} is the drug concentration at time j of the individual i . The random effects were applied to the EC_{50} as an additive error model.

As can be seen from equations 1 and 2, random effects associated with IIV are parameterised differently in the binary response models. In the case of gabapentin, IIV is assessed in the logit space, reflecting the total random variability (see companion paper), whereas for pregabalin, IIV is added to the EC_{50} , yielding estimates of variability specific this parameter.

Model Diagnostics

Model fitting was evaluated using simulation-based diagnostics, namely a categorical VPC and mirror plots [16]. Herein, using the final model, a number of simulated datasets are generated, following which the real observations are overlaid graphically on the simulated response (VPC) and agreement between the two is assessed. In case of mirror plots, a number of plots of the simulated response against the independent variable (Time or concentration) are made and compared with the observations against the independent variable. A total of 100 datasets were simulated. The threshold for defining a response (successful event) was ≥ 0.5 . Probabilities less than <0.5 were considered a failure in the VPC. Diagnostic plots were stratified per dose level.

Optimisation Strategy

Our approach assumes that baseline and maximum response (E_{max}) are system-specific parameters and can therefore be considered similar across compounds. On the other hand, EC_{50} and the IIV are considered drug-specific parameters and as such represent the parameters of interest, for which dose level and sampling times are optimised. The main assumptions for optimisation were:

- The logistic regression model can be generalised and is equally applicable to different compounds, i.e., the model can be applied to new compounds without recurring model building steps.
- IIV is expected to be of the same order of magnitude for different compounds, and determined primarily by experimental procedures
- Relative in vitro data provides reliable estimates of the putative relative in vivo potency.
- The bias due to empirical protocol design is comparable across compounds.

Experimental constraints in a typical screening experiment were accounted for by limiting sample size to 6 animals per dose group and maximum of 5 blood samples per animal for pharmacokinetic analysis. Given that experimental data for pregabalin were generated according to a typical (empirical) experimental protocol, this design was used as benchmark to compare the performance of the optimised design in terms of bias and precision. For

the sake of clarity, it should be noted that in a real-life scenario, experimental data for the prospective evaluation of new compounds will not be available a priori.

ED-optimality was used to optimise two model parameters, namely EC_{50} and the IIV. The design variables considered for optimisation were dose and sampling times [17]. Parameter uncertainty was assumed to be log normally distributed, with a 50% coefficient of variation (CV). For the purpose of optimisation, the true value of EC_{50} was set to 637.5 ng/ml (i.e., from *in vitro* data). The system specific parameters were derived from historical data on the paradigm compound (gabapentin) and fixed during optimisation to 97% (E_{max}), 2.81% (*baseline*) and 3.14% (IIV). Based on previous findings, placebo effect was considered to be minimal and assumed to be time-invariant during the sampling interval. Details of the optimisation algorithms and other theoretical concepts are described elsewhere (see companion paper [13, 18]). Optimisation was performed with default settings in PopED [19].

Validation

Validation procedures were implemented to assess the performance of the empirical and optimised designs. These included stochastic simulation and re-estimation (SSE) and bootstrap runs. SSE assumes that model is known and is used to compare designs [20, 21]. Parameter values of the 200 first successful simulations and estimations were recorded and summarised for each SSE run, along with their standard errors (SEs) based on the observed Fisher Information Matrix. On the other hand, the non-parametric bootstrap is a resampling method suitable for estimating a parameter distribution from which various measures of interest (mean, median, and standard error) may be calculated. The bootstrap makes no assumptions about the underlying parameter distributions and thus provides confidence intervals of the model parameter [22]. This technique consisted of repeatedly fitting the model to replicates of the (simulated) data set using the bootstrap option in PsN 3.2.12[23]. The estimates for EC_{50} and the corresponding interindividual variability obtained from 200 successful bootstrap runs were summarised as mean, 2.5 and 97.5 percentiles (denoting the 95% confidence interval) [24].

Comparison of Precision and Bias

Empirical and optimised designs were tested for precision and bias according to the method proposed by [25]. The root mean square error (RMSE) is given by:

$$RMSE = \sqrt{\frac{\text{mean}((Est_i - True)^2)}{True^2}} \quad (3)$$

where Est_i is the i^{th} parameter estimate and $True$ is the simulation value (initial estimate) of the parameter. The bias is given by:

$$MPE = \frac{\text{mean}(Est_i - \text{True})}{\text{True}} \quad (4)$$

The relative standard error (RSE) is given by:

$$RSE = \frac{SE(Est)}{\text{True}} \quad (5)$$

where SE is the standard error of the estimates.

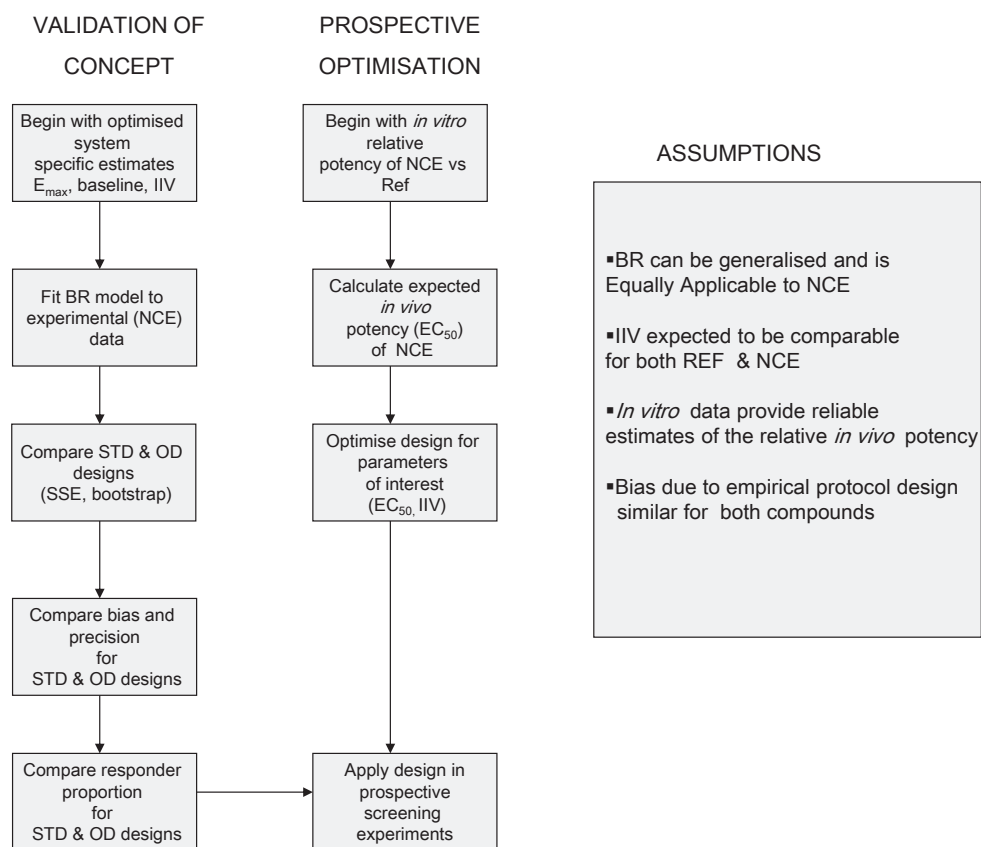


Figure 5.2: Concept diagram for prospective optimisation and validation steps. Each box in the diagram indicates the data flow required for the implementation and validation of the model-based approach as a screening tool. NCE=New chemical entity, BR=binary response, IIV=inter individual variability, SSE=stochastic simulation and estimation, STD =standard, OD=optimized design.

Simulation of Responses

As a last step in this exercise, binary responses were simulated for different time intervals according to optimised protocol design and compared with the corresponding responses obtained by the empirical design. The entire procedure is presented in a diagram in Figure 5.2.

RESULTS

Pharmacokinetics

The time course of pregabalin concentrations are shown in Figure 5.3 with the sampling time points overlaid for both the empirical as well as optimal designs. Parameter values are summarised in Table 5.1. These values were used to simulate typical concentration values to be used for the assessment of the concentration-effect relationship.

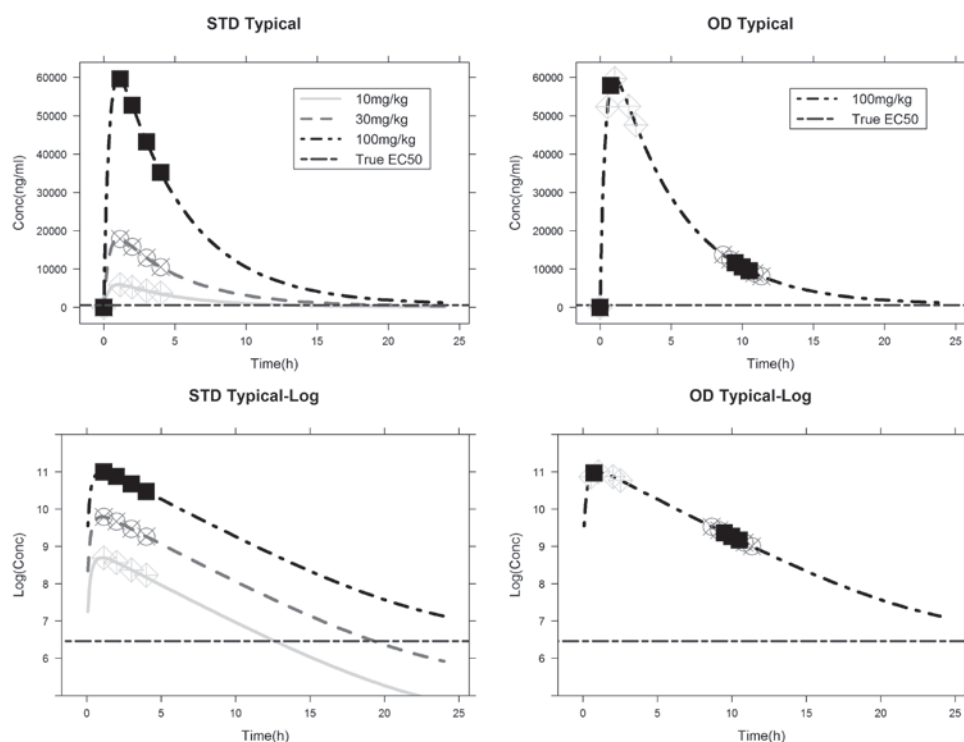


Figure 5.3: The time course of pregabalin concentrations (population profile) with sampling time points for both the empirical (STD, left panel) and optimised (OD, right panel) designs. In the STD design, different dose levels are used, namely 10(open diamond), 30(crossed circle), 100(filled square) mg/kg, while the OD design, relies on a single dose level of 100mg/kg. Dashed line indicates true estimate values for EC_{50} .

Table 5.1: PK Parameter estimates used for simulation.

| Parameters | Pharmacokinetic Parameter Value |
|--------------------------|---------------------------------|
| CL (L hr ⁻¹) | 0.036 |
| V ₂ (L) | 0.27 |
| Q (L hr ⁻¹) | 0.02 |
| V ₃ (L) | 3.3 |
| Ka (/h) | 2.66 |
| F | 1 |

Pharmacodynamics

An overview of the fitting of the response data to the logistic regression model is shown in Figure 5.4. Model performance is summarised in the diagnostics plots presented in Figure 5.5. The proportion of responders (number of events in which changes in pain threshold were >30% relative to baseline) is presented here for both observed and simulated data. The visual predictive check (VPC) shows that the model is more predictive for the active doses compared to placebo. In case of the placebo dose, observed response proportion varies from 10-30% while the corresponding figure for the simulated data is 10-15%. The final parameter estimates from the fit of experimental data are listed in Table 5.2.

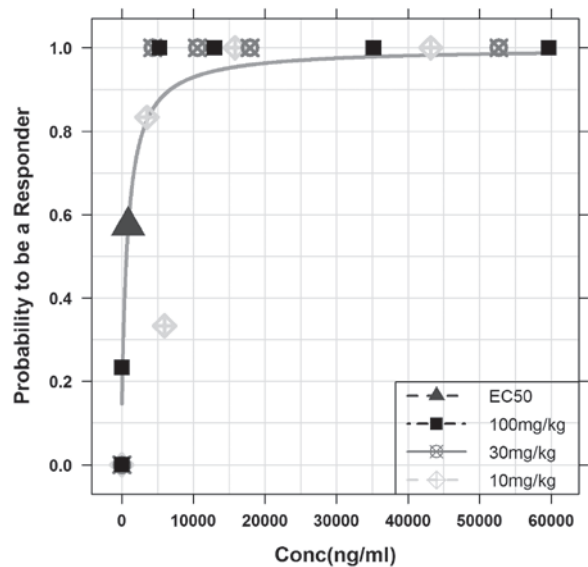


Figure 5.4: Probability of response to pregabalin (measured as change >30% in paw withdrawal threshold relative to baseline) after doses of 10 (open diamond), 30 (crossed circle), 100 (filled square) mg/kg, as determined by the logistic regression model.

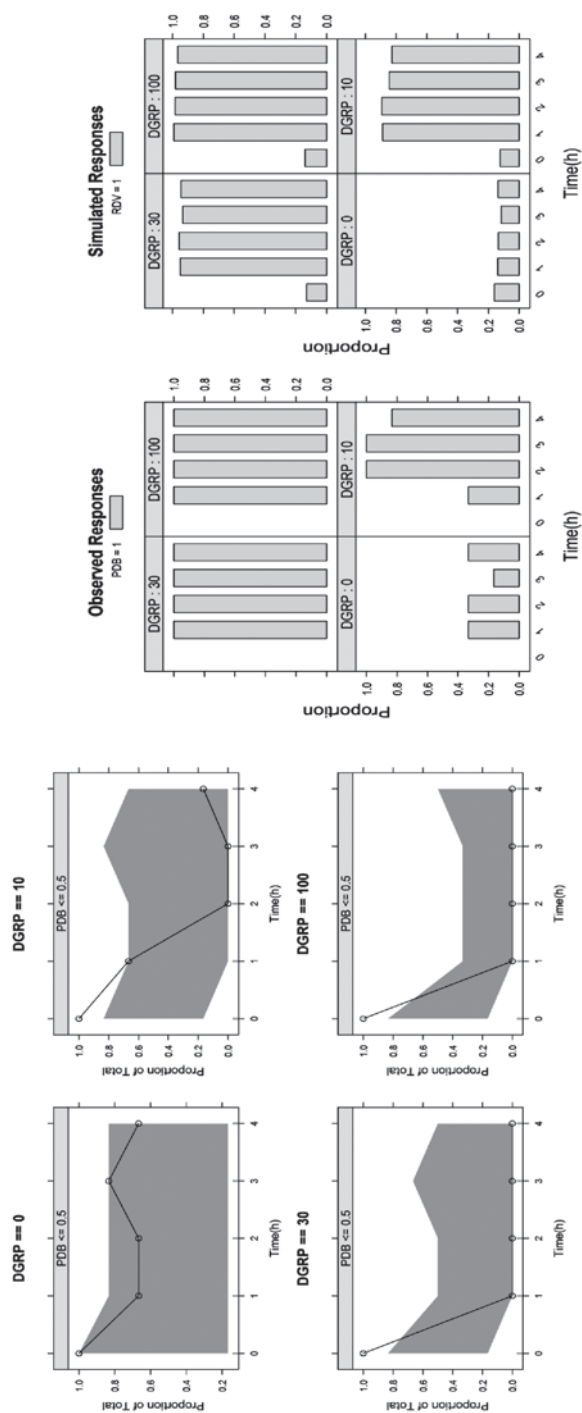


Figure 5.5: Model diagnostics. Visual predictive check (left panel) and mirror plots (right panel) showing observed and model-predicted response to pregabalin per dose (DGRP) level. PDB is the threshold probability of failure. A simulated response of <0.5 indicates failure. At lower doses, the probability of no response or failure is high, but decreases with increasing doses. The blue solid lines are the proportion of failure at each time point. Similar diagnostic plots can be obtained for success, i.e., probability of response >0.5. RDV=simulated proportion of responses. See results section for further explanation of the procedures.

Table 5.2: Pharmacodynamic parameter estimates from model fitting to the empirical protocol and priors used for the optimisation procedures with pregabalin.

| Parameter | Estimate (SD) | Priors based on paradigm compound |
|--------------------------|---------------|-----------------------------------|
| EC ₅₀ (ng/ml) | 897 (20) | 637.5 |
| Placebo(%) | 14.6 (28.0) | 3 |
| E _{max} (%) | 85.4 (28.0) | 97 |
| IIV | 3 (2.04) | 3.13 |

Design Comparison

An overview of the design variables for the original empirical protocol and the optimised design is provided in Table 5.3. Figure 5.6 shows the comparison of the parameter estimates (EC₅₀, IIV) for the empirical and optimised designs. The percentage runs which minimised successfully were 50% for the empirical design, whereas all runs achieved minimisation according to the optimised protocol design. For the optimised design, the median EC₅₀ value (range) was 755 (188-756) ng/ml, whereas considerably higher values were observed for the empirical design, namely 1125 (898-2411) ng/ml. Based on in vitro estimates, the EC₅₀ values were expected to be around 637.5ng/ml. IIV estimates for drug potency were 3.13 and 3.00 (2.39-4.77) for the optimised and empirical designs, respectively. These results are summarised in Table 5.2.

Table 5.3: Comparison of the design variables for the original *versus* the optimal design. Columns show the dose (mg/kg) and the sampling times (h).

| Design variables | Empirical design Dose (mg/kg) sampling times (h) | Optimised design |
|------------------|--|--------------------------------|
| Dose level 1 | 0 0,1,2,3,4 | 100 0,0.53,1.03,2.02,2.52 |
| Dose level 2 | 10 0,1,2,3,4 | 100 0,0.53,1.03,2.02,2.52 |
| Dose level 3 | 30 0,1,2,3,4 | 100 0,8.65,9.15,10.85,11.35 |
| Dose level 4 | 100 0,1,2,3,4 | 100 0,0.75,9.5,10,10.5 |

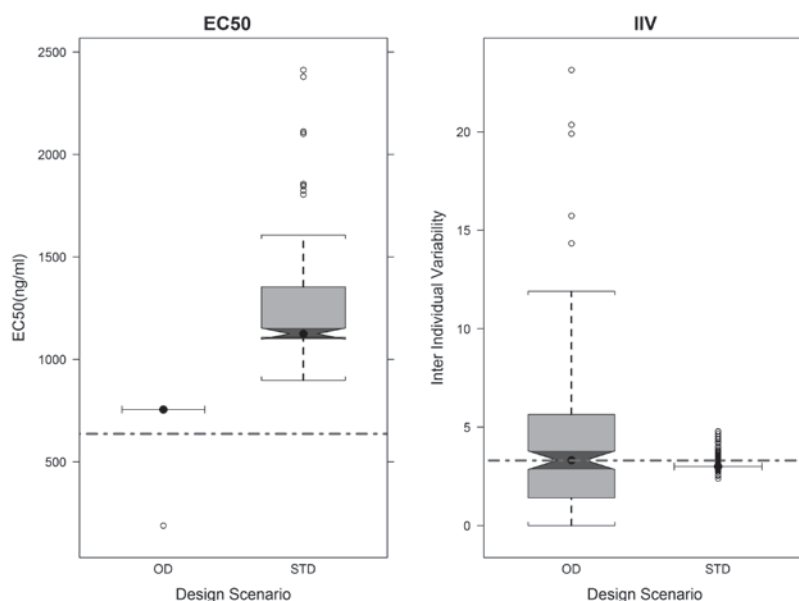


Figure 5.6: Comparison of the parameter estimates for the empirical (STD) and optimised (OD) protocol designs. Dashed lines represent expected theoretical values. 50% crashes were observed with the STD design.

The frequency distribution, median and range of the EC_{50} & IIV estimates obtained by bootstrapping are shown in Table 5.4.

Table 5.4: The RMSE, MPE, the RSE from stochastic simulation (SSE) and the bootstrap confidence intervals are summarised for the empirical protocol and for the optimised design.

| Design | Parameter | RMSE (% precision) | MPE (% bias) | % RSE (from SSE) | Median (range) (from bootstrap) |
|-----------|-----------|-----------------------|-----------------|---------------------|------------------------------------|
| Empirical | EC_{50} | 55.13 | 41.76 | 36.06 | 1147(897-2196) |
| | IIV | 41.43 | 41.32 | 3.01 | 3 (0.00-3.00) |
| Optimised | EC_{50} | 19.12 | 18.76 | 6.28 | 213(33-763) |
| | IIV | 58.03 | 49.12 | 129.27 | 0.28 (0.0003-1.51) |

In Figure 5.7 below, the proportion of responders per time interval for both designs is depicted. Simulated responses are shown along observed response for the optimised and empirical protocol designs, respectively. As can be seen in this figure, there were observations in the terminal part of the response vs. time curve for the optimised design, including samples up to 10 hours which were not recorded for the original design.

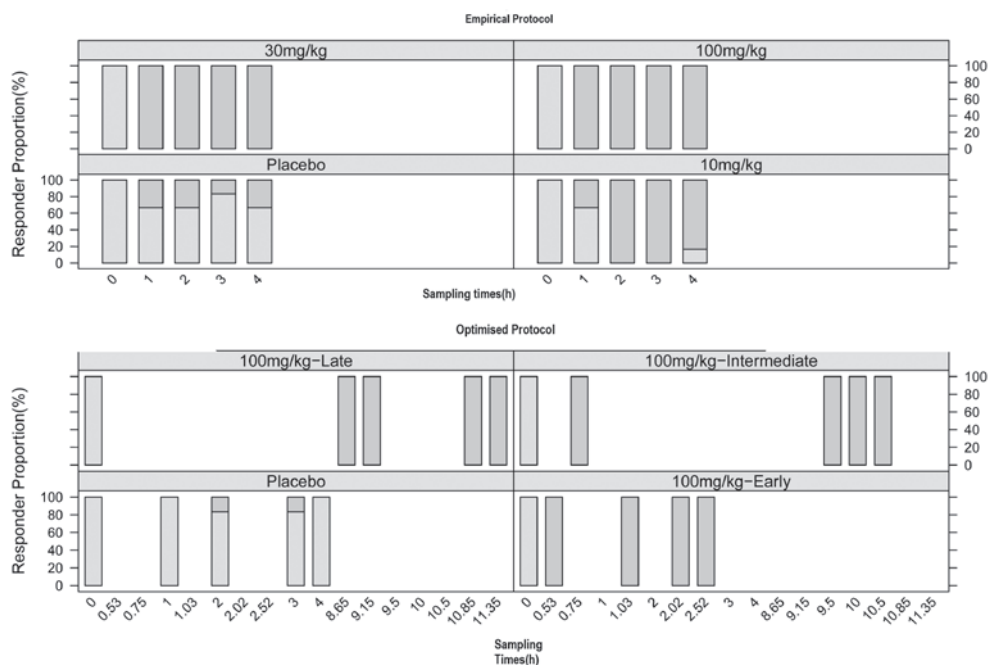


Figure 5.7: Sampling times and corresponding response rate for the empirical and optimised protocol designs. Response is shown in magenta (dark shade), whereas failure is depicted in light blue (light shade). Early, intermediate and late categories refer to pharmacokinetic sampling at different times after dosing.

DISCUSSION

Practical and scientific challenges appear to preclude the identification of suitable compounds for the treatment of neuropathic pain. Although further advancement of the field will certainly depend on the availability of specific biomarkers of disease, the assessment pharmacokinetic-pharmacodynamic relationships remains fundamental for characterising the properties of novel compounds in early drug development. Unfortunately, it has been shown that screening procedures and protocols do not consider the implication of empirical designs, which can result in estimates of drug properties such as potency, which are often imprecise and extremely variable [13].

Our results illustrate how optimality concepts can be applied to better design experimental protocols and obtain more precise and accurate parameter estimates, taking into account parameter uncertainty. Here we have shown how the use of a binary response can overcome technical limitations associated with model building, by emphasising the assessment of drug- and system-specific parameters. The parameterisation of drug effects in terms of EC_{50} allows discrimination of drug properties, whereas baseline and maximum response E_{max} reflect experimental model characteristics and as such can be estimated in conjunction

with historical data, which are incorporated as parameter priors. In fact, using published literature data, we have seen that pregabalin is known to be 2 to 3-fold more potent than gabapentin, both *in vitro* as well as *in vivo* [12, 26]. The assumption of potency values (EC_{50}) for pregabalin to be half that of the reference compound turned out to be correct, as indicated by model fitting of the experimental data. Both the hypothesised as well as the estimated value were found to lie within the same order of magnitude.

To further understand the implications of bias in parameter estimates, an overview is given of the maintenance doses and the corresponding exposures for a number of compounds used in neuropathic pain is depicted in Table 5.5 below. It can be seen that there is a discrepancy between the ranking of potency estimates for these compounds between species and between models [27].

Table 5.5: Comparison of human and rat maintenance doses (mg/kg) and exposures (AUC, ng/ml/h) for different compounds used in neuropathic pain [27].

| Compound | Maintenance dose in humans | Dose associated with the minimum efficacious exposure in rats |
|---------------|----------------------------|---|
| Amitriptyline | 2.1 (2526) | >100 (3540) |
| Milnacipran | 0.7 (8673) | 30 (939) |
| Duloxetine | 0.9 (8673) | 30 (584) |
| Lamotrigine | 2.9 (208200) | 10 (69754) |
| Carbamezapine | 17 (55780) | 100 (14120) |
| Gabapentin | 26 (146000) | 100 (125370) |
| Pregabalin | 4.28 | 10 |

We believe that some of these discrepancies may at least in part be attributable to uninformative designs. In fact, Gabrielsson *et al.* have shown that compounds with a potential to show response, often fail to do so in preclinical experiments since the design is sub-optimal or non-informative and lacks sufficient information on potency and efficacy [1]. In addition, it should be noted that optimised protocols appear to impose variation in sampling times across dose groups. This requirement can be justified by the need to take differences in exposure into account. Effectively, pharmacodynamic measurements should yield data across the whole exposure-response curve, and in particular around the putative drug potency. As can be seen in Figure 5.3, sample times for the optimised designs are distributed throughout the concentration time curve. We also showed that optimisation can be achieved even if experimental constraints exist. In the case of pregabalin, the sampling points had to be rationalised by ensuring intervals between samples of at least 15 min. Both the bootstrap and the SSE methods showed a wider distribution around the point estimate for the empirical design, as compared to the optimised design. It was also clear that bias was present in both designs, despite improvement achieved by optimisation

procedures. On the other hand, these results need to be interpreted carefully, as indicated by the higher estimates for IIV after optimisation. The empirical design was definitely more unstable, as evidenced by 50% of crashed runs both in the SSE and the bootstrap.

To compare design bias and precision, we presented RMSE, MPE and RSE. Kjellsson *et al.* have reported significant bias in parameters in modelling ordered categorical data, when most of the observations are found at the extreme of the possible outcomes[28]. This bias increases with increasing skewness of the response distribution and increasing IIV data. As a matter of fact, the frequency of rare events will be overestimated when simulation of the new data is performed using the biased parameters. Considering that the system specific parameter estimates as well as the IIV were derived from the prior information derived from gabapentin, there was a potentially unavoidable bias in the model, besides the evidence of physiological correlations between parameters[29]. Over and above this, our design was constrained by practical limitations. We also noted that the dose was not an important design variable for this experimental protocol, as long as the sampling scheme was sufficiently robust to yield required information around the parameters of interest.

Limitations

It may be argued that we have made too many assumptions in this prospective optimisation exercise. However, we clarify that this methodology is applicable only in a setting of biological plausibility i.e. if both the paradigm and the NCE act on the same target or are of the same class, as is the case here. In early development, the targets or mechanisms of action are often obscure and hence at this stage one needs to rely on functional assays which provide clues to the mechanism of action of the NCEs. We hypothesise that prospective optimisation can be attempted even if in vitro potency ratios are calculated on such prototype assays. Furthermore, relative potency estimates are expected to remain same irrespective of the bio phase.

The use of binary responses was essential for the implementation of a model-based approach during drug screening. At that stage of drug development resources are limited and expertise for model building limited. Often, statistical arguments arise regarding the potential loss in information when selecting binary response variables as opposed to continuous variables. We believe however that this limitation is offset by opportunity to implement a model-based approach and obtain more accurate answers regarding the selection of the most suitable compound to be advanced further in development.

We also acknowledge that given the absence of the true parameter values, our interpretations will be relative to the assumptions made initially, i.e., that the experimental data available can be used for validation purposes. However, despite some model misspecification, we showed that the experimental data from the typical empirical protocol could be fitted by

the same model applied to gabapentin. Yet, one should not overlook the fact that different parameterisations were used for pregabalin and gabapentin (i.e., logit and direct E_{\max} model). Thus, further evaluation would be required to explore the feasibility of applying the same simplified model to the paradigm compound. Lastly, we should remind the reader that we have made an assumption that the observations were uncorrelated [30]. We could not test the impact of possible correlations due to large noise and wide intervals between successive measurements.

In summary, we showed that despite some technical challenges, the application of optimisation concepts for the design of experimental protocols can still prove to be more informative than empirical designs. Moreover, in contrast to the ‘one size fits all’ approach, the implementation of optimisation procedures permits the use of historical data in a systematic, formalised manner. Such an integrated approach ensures that differences in pharmacokinetic properties are account for during the evaluation of the pharmacological effects.

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CHAPTER 6

Semi-mechanistic modelling of the analgesic effect of gabapentin in the formalin-induced rat model of experimental pain

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ABSTRACT

Purpose: The formalin-induced rat model of nociception involves moderate continuous pain. Formalin induced pain results in a typical repetitive flinching behaviour, and these data display a biphasic pattern characterised by peaks of pain. We described the time course of pain response and the analgesic effect of gabapentin using a semi mechanistic modelling approach.

Methods: Male Sprague-Dawley rats received gabapentin (10-100 mg/kg) or placebo 1 hour prior to the formalin injection. A reduction in the frequency of the 2nd peak of flinching was used as a behavioural measure of gabapentin mediated anti-nociception. The time course of the flinching response was modelled using a mono-exponential function to characterise the first peak and an indirect response model with a time variant synthesis rate for the second. PKPD modelling was performed using a population approach in NONMEM v.7.1.2.

Results: The time course of the biphasic behavioural pain response was adequately described by the selected model, which included separate expressions for each phase. Gabapentin was found to reversibly decrease, but not suppress the flinching frequency of the second response peak only. Mean IC₅₀ values (\pm RSE%) were 7510 (40.03) ng/ml.

Conclusions: A compartmental, semi-mechanistic model provides the basis for further understanding of the formalin-induced flinching response and consequently to better characterisation of drug properties, such as potency in individual animals. Despite high exposure levels, model predictions show that gabapentin does not completely suppress behavioural response in the formalin-induced pain model.

ABBREVIATIONS

PKPD= pharmacokinetics and pharmacodynamics, RSE=Relative Standard Error, GABA= gamma amino butyric acid, IIV= inter-individual variability, CV= coefficient of variation, MOFV= minimum objective function value, VPC= visual predictive check, CI= confidence interval, COX-2=cyclooxygenase-2, NMDA= N-methyl d-aspartate, NK1= neuroenkephalin 1, MED= median effective dose.

INTRODUCTION

Ideally, the evaluation of the efficacy of novel treatments for neuropathic pain should be based on pre-clinical models that mimic not only the symptoms of disease, but also consider the substrates underlying the pathophysiology of nociception in humans, i.e., show construct validity [1]. Nevertheless, most behavioural models of pain rely on withdrawal responses to evoked pain, which reflect sensory perception and consequently one's ability to discriminate its intensity, localisation and modality [2, 3]. As such, these measure ignore other features of human pain [4].

Regardless of the potential limitations mentioned above, the formalin induced pain (FIP) model is a well-accepted screening test. The method comprises moderate, continuous pain due to tissue injury following injection of formalin. In the FIP model, the observed behaviour in response to a painful stimulus, assessed as flinching frequency, is used as measure of efficacy [2, 5, 6]. This behavioural measure is thought to reflect both the sensory and emotional aspects of pain [7, 8]. From a mechanistic perspective, the presence of common elements of human pain behaviour in the FIP model makes it possibly one of the most predictive models among the available experimental models of acute pain. These properties have also made the FIP model an appealing tool for the screening of compounds showing potential central anti-nociceptive activity [6, 9]. In fact, various compounds have been found to affect flinching behaviour (e.g., indomethacin and Na⁺ channel blockers), as assessed by the inhibition of the second pain peak, which corresponds to the processes underlying peripheral and central sensitisation [2, 10].

In the current investigation, we evaluate the pharmacokinetic-pharmacodynamic properties of gabapentin in the FIP model. Gabapentin is believed to act via antagonism of voltage gated Ca⁺⁺ channels in afferent neurons, thereby indirectly affecting GABA activity [11]. It has been shown to affect the amplitude of the second pain peak, whilst leaving the other components of the pain response largely unaffected [12].

Despite the widespread use of gabapentin as a reference compound in preclinical models, no quantitative methods have been implemented so far that allow discrimination between drug and biological system properties, and consequently provide a more consistent ranking of candidate molecules. The availability of PKPD relationships would also serve as the basis for translating drug (analgesic) effects across species [1]. The use of PKPD modelling offers an opportunity to better understand the *in vivo* time course of pharmacological effects, providing further insight into the mechanisms of action [13, 14]. Nonetheless, these concepts have been underutilised in pre-clinical pain research [15]. This may be explained, at least partly, by the lack of pharmacokinetic information and the absence of the time course of treatment response [1].

The primary goal of this study was therefore to develop a semi-mechanistic model that allows the characterisation of the time course of formalin-induced pain and assess the effects of gabapentin on flinching behaviour. In addition to known experimental issues such as high variability in response, we show that the main challenges for the characterisation of PKPD relationships using experimental behavioural pain models are the lack of pharmacokinetic information and the absence of the time course of treatment response. Lastly, we explore the relevance of parameter estimates by comparing our findings with published data from other experimental models of pain as well as with clinical data in neuropathic pain patients.

MATERIALS AND METHODS

Experimental Design

Protocols and experimental procedures were reviewed and approved by the Home Office, UK, as required per project licence. The experiments were performed following approval by the Ethics Committee. Sprague-Dawley rats (Charles River, UK weight range 100-300 g) had metal bands attached to their right hind-paws and were placed in Perspex recording chambers and allowed to habituate for 15 min before administration of formalin. The animals were then injected with 50 µl of formalin, subcutaneously in the ventral surface of the right hind-paw at a 2.5% conc/vol. Following formalin administration, animals were returned to the Perspex recording chambers and the number of flinches was counted by the automatic teller for 1 hour. Four rats could be tested in parallel using this system. All animals were euthanized at the end of the experiment.

Gabapentin or vehicle was administered orally at doses of 0, 10, 30, 100 mg/kg approximately 1 hour prior to formalin administration. In 4 of the experiments, the animals were randomised to either the placebo or the 100 mg/kg dose group, while in the 5th there were 2 additional dose groups who received 10 or 30 mg/kg respectively. In each experiment, 8 animals were allocated to a particular dose level. Data from five different experiments were pooled together, making a total of 96 animals.

Pharmacodynamic measurements

The total frequency of flinches was recorded at 5-min intervals, from 5 to 60 min after administration of formalin.

Data Analysis

Pharmacokinetic simulations

Gabapentin concentrations were simulated using a previously published model based on two-compartment drug disposition and dose-limited absorption [16]. The model was built in a stepwise manner. First, intravenous data from a previous experiment was modelled to obtain disposition parameter estimates, namely clearance and volume of distribution. Subsequently, absorption parameters (bioavailability and input rate) estimates were obtained for oral data. More information on these experiments can be found in the appendix. This pharmacokinetic model is described by the following expression [16]

$$C = \frac{k_a F D}{V_1} \left\{ \frac{(k_{21} - \lambda_1) e^{-\lambda_1 t}}{(k_a - \lambda_1)(\lambda_2 - \lambda_1)} + \frac{(k_{21} - \lambda_2) e^{-\lambda_2 t}}{(k_a - \lambda_2)(\lambda_1 - \lambda_2)} + \left(\frac{(k_{21} - K_a) e^{-K_a t}}{(\lambda_1 - k_a)(\lambda_2 - k_a)} \right) \right\} \quad (1)$$

where k_a =absorption rate constant, V_1 =central volume of distribution, F =bioavailability of the administered dose, λ_1 and λ_2 correspond to the initial and terminal slopes representing bi-exponential decline respectively and k_{21} is a rate transfer microconstant between compartments 1 and 2. A summary of the PK model parameters is shown in Table 6.1.

An analytic solution to the 2 compartment model, implemented in NONMEM was used for the simulation and the derivation of the above expression from the estimated primary parameters (Volume, Clearance) and is elaborated in the appendix.

Exploratory data analysis

Before starting model building, we performed a graphical evaluation of trends in the experimental data, including the time course of gabapentin in plasma, the effect vs. time and the concentration vs. effect relationships. To ensure suitable model parameterisation and assess the existence of correlations in the data, pain response at any given point in time was also plotted against the preceding interval. Such correlations are of relevance for modelling purposes, as highly correlated data may lead to model misspecification. In fact, pain response (flinching frequency) at a given sampling time has been shown to correlate with preceding measurements [17, 18]. Given that the frequency of flinches /time interval was high >10, we decided to model the counts as continuous data.

Table 6.1: Pharmacokinetic parameter estimates used for deriving simulated concentrations at the time of measurement of the response.

| Parameter | Values |
|--------------------------------------|----------------------------|
| Pharmacokinetic | |
| Central compartment volume (V_1) | 0.118 (l) |
| Peripheral volume (V_2) | 0.253 (l) |
| Clearance (Cl) | 0.159 (l/h ⁻¹) |
| Intercompartmental clearance(Q) | 1.22 (l/h ⁻¹) |
| Bioavailability fractions(F) | 1,0.75,0.22 ^a |
| Absorption constant (Ka) | 0.26 (h ⁻¹) |

^a For doses 10, 30,100mg/kg respectively

PKPD model parameterisation

In the FIP model, there is a temporal delay between the appearance of gabapentin concentrations in plasma and the onset of pain response. Depending on the half-life of the compound, the analgesic is administered before the induction of hyperalgesia with formalin. Given that two pain peaks consistently occur after administration of formalin, this phenomenon was parameterised in terms of two independent pharmacodynamic (PD) compartments. The first peak (i.e., pain associated with the first phase) was described by the following exponential decay relationship:

$$\begin{aligned}\frac{dFO}{dt} &= -K_{df} * FO \\ PAIN_1 &= F_3 * FO\end{aligned}\tag{2}$$

where FO = formalin-induced stimulus, k_{df} = dissipation constant for formalin, F_3 =basal pain load in the first PD compartment, $PAIN_1$ = total pain in the first PD compartment

The first peak of pain is almost instantaneous following algogen administration, thus the parameter F_3 reflects pain at baseline, which wanes spontaneously soon thereafter.

The onset of the second peak of pain is after a quiescent phase and is considered to reflect the central hypersensitisation which ultimately manifests itself as a 2nd more prolonged phase of flinching. Similarly to the first peak, pain intensity increases to a maximum and then remits spontaneously. Given the lack of a direct correlation between the gabapentin concentrations in plasma and effect over time, an indirect model was deemed to be most appropriate to describe this ‘turnover’ of the pain response [19].

The onset of the second peak of pain is after a quiescent phase and is considered to reflect the central hypersensitisation ultimately manifesting as a 2nd more prolonged phase of flinching. After reaching peak intensity the pain remits spontaneously. An indirect model was deemed to be most appropriate to describe this ‘turnover’ of the pain response[19].

In these models the measured response (R) is assumed to result from factors controlling either the input or the dissipation of the response. The general expression to describe these models is given by the expression below:

$$\frac{dR}{dt} = k_{syn} - k_{deg} * R \quad (3)$$

where dR/dt is the rate of change in the response over time. k_{syn} represents the zero-order rate constant for the formation of the response and k_{deg} the first-order rate constant for loss of the response. We have replaced the response R in equation 3 with the term FL to make explicit reference to the time course of the flinching response triggered by the central sensitisation in the spinal cord following the first peak.

$$\frac{dFL}{dt} = k_{syn} - k_{deg} * FL \quad (4)$$

Given that the pain response wanes with time i.e., there is spontaneous recovery within 1 hour after injection of the algogen [2, 6], k_{syn} was treated as time-dependent variable. Depending on whether t , the time after formalin injection, was larger or smaller than $Tlag$ (i.e., the delay between the occurrence of the first and second peaks of pain), different estimates were considered for k_{syn} . Thus for $t \geq Tlag$, model parameterisation described the onset of the second phase of pain. If $t < Tlag$, $k_{syn} = 0$, which meant the second phase of pain had not yet begun. A modified gamma function was required to describe the time course of k_{syn} and equation 4 was thus transformed to an expression representing the natural change in pain frequency, described by the following expression:

$$K_{syn} = A * (\alpha^{(t-lag.t)}) * e^{(-\beta(t-lag.t))} \quad (5)$$

Where A (response unit h^{-2}), α (a dimensionless constant), β (h^{-1}) are the parameters of the gamma function describing the time course and intensity of the second phase of pain as assessed by the frequency of flinching.

As mentioned earlier, the time course of the disease is a result of the temporal change in the frequency of flinching represented by FL . At the start of the study, i.e., before onset of the 2nd peak, the frequency of flinching was assumed to be 0. Consequently, the generic equation 4 can finally be rewritten in terms of FL as follows:

$$\frac{dFL}{dt} = A * (\alpha^{(t-lag.t)}) * e^{(-\beta(t-lag.t))} - K_{deg} * FL \quad (6)$$

The model was applied to simultaneously fit both placebo and gabapentin data. It has been observed that drug response further decreases the frequency of flinches and thus

it is superimposed on the natural disease process. Gabapentin effects ($DEFF$) were best described by an inhibitory I_{max} function, which represents the reversible counteracting effects of gabapentin on the algogenic action of FL , i.e., the observed flinching behaviour:

$$DEFF = (1 - \frac{I_{max} * C_p}{IC_{50} + C_p}) \quad (7)$$

where I_{max} = maximum possible inhibition of pain, C_p = plasma concentration and IC_{50} = plasma concentration at which 50% of the inhibition occurs. As gabapentin only affects the second peak of pain, we assume gabapentin effects reflect a decrease in central sensitisation. It should be noted that indirect response models incorporate the Hill function directly in the turnover differential equation whereas we have chosen to parameterise gabapentin effect ($DEFF$) directly on the pain variable of the 2nd peak, rather than within the differential equation. This is because the analgesic does not alter the onset of the pain nor its eventual disappearance, but reversibly alters its peak intensity. In other words, the analgesic effect of gabapentin is a covariate on the behaviour or flinching response. A similar approach has been used previously to describe the effects of lumiracoxib on COX-2 inhibition [20]. The net pain observed is the product of the gabapentin effect ($DEFF$) and FL or the resulting PD compartment ($PAIN2$)

$$PAIN2 = (1 - \frac{I_{max} * C_p}{IC_{50} + C_p}) * FL \quad (8)$$

The total pain ($PAIN$) was described by the sum of the pain in the two model compartments:

$$PAIN = PAIN1 + PAIN2 \quad (9)$$

A schematic representation of this mechanistic PD model is presented in Figure 6.1.

Interindividual variability was modelled exponentially and applied serially to each parameter. Stochastic parameters were retained in all cases which showed significant improvements in the model, as defined by statistical criteria described below. Residual variability was best described by an additive error model.

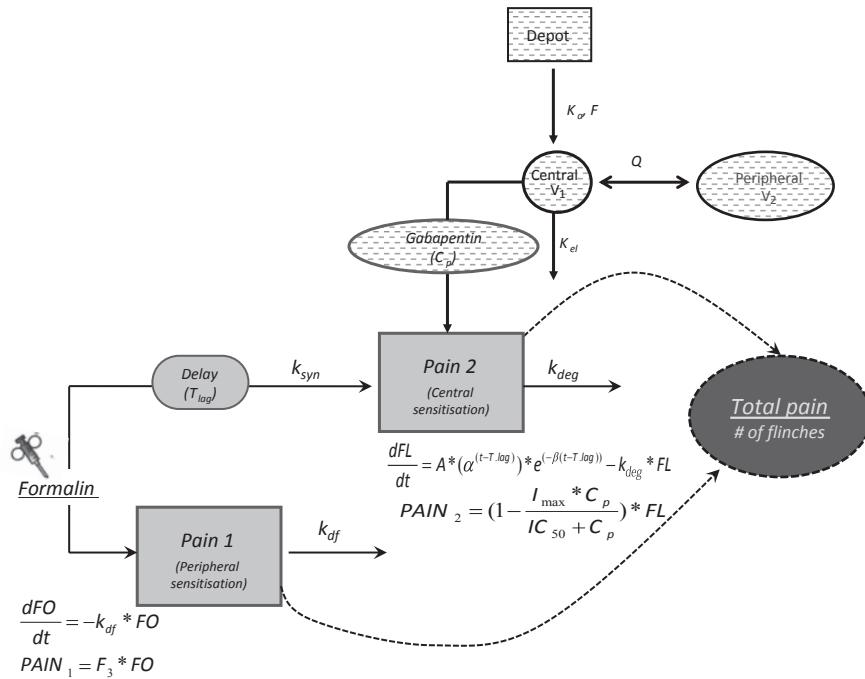


Figure 6.1: Schematic diagram of the pharmacokinetic-pharmacodynamic model. PK compartments are displayed with dashed horizontal (blue) hatching, while PD compartments are dark shaded (grey or black).

Model diagnostics and validation

Model selection was based on the visual examination of the goodness-of-fit plots using Xpose version 4.2.1[21], the precision of model parameter estimates is represented by the coefficient of variation [%], computed as the ratio between the standard error provided by NONMEM and the parameter estimate multiplied by 100, and the MOFV provided by NONMEM. The difference in the MOFV between two hierarchical models was considered statistically significant if the MOFV changed by 6.63 points which is equivalent to a p value of <0.01 for a χ^2 distribution. The final model was further evaluated based on visual and numerical predictive checks and bootstrap procedures [22, 23]. Using the final model, the 2.5th, 50th, and 97.5th percentiles from simulated pain response (n=500) were calculated and compared to the experimental data.

NONMEM 7.1.2 was used in conjunction with PsN 3.2.12 for all estimation and simulation procedures. Modelling was based on the first-order conditional estimation method with the INTERACTION option [24]. R statistical software (v 2.10) was used for data manipulation, statistical and graphical summaries [25].

Bootstrap

A nonparametric bootstrap with re-sampling was performed to estimate the confidence intervals for the parameters [23]. This technique consisted of repeatedly fitting the model to replicates of the data set using the bootstrap option in PsN 3.2.12. Parameter estimates for each of the replicate data sets were obtained. The results of successful runs from 500 bootstraps were obtained, and the median and 2.5th and 97.5th percentiles (denoting the 95% confidence interval) determined for estimated parameters.

RESULTS

Data analysis

Pharmacokinetic simulations

Gabapentin concentrations were obtained by simulation at each of the sampling times used for the pharmacodynamic measurements. A two-compartment model with first-order absorption obtained in a previous analysis was used for the purpose of this study. The PK model parameters are summarised in Table 6.1.

Exploratory Analysis

The typical concentration time profiles are shown in Figure 6.2 (left panel) with the corresponding time courses of the flinching frequency for the tested doses (right panel). It can be appreciated here that gabapentin only reduces the amplitude of the 2nd peak in a dose-dependent manner.

The disconnect between the two time courses are shown in the figure below with respect to the initiation of the experiment (formalin injection) and the PD observation window. From these it is clear that during the observation window, while the pain response begins and ends the drug is still in the distribution phase. Considerable variability in the response is also evident. From these plots, it is clear that during the experimental protocol, the pain response begins and ends while gabapentin is still predominantly in the absorption phase. Considerable variability in the response can also be seen between animals.

In Figure 6.4, the flinching frequency is depicted against time and gabapentin concentrations, stratified by dose level. From the two panels it can be seen that the concentration-effect relationship can be superimposed on the time course of response itself. The data suggests that gabapentin effects have limited effect on the time course of the second pain peak. Furthermore, this phenomenon is further confounded by high degree of correlation between consecutive measurements. Details are shown in the supplemental material (appendix, Figure 6.7).

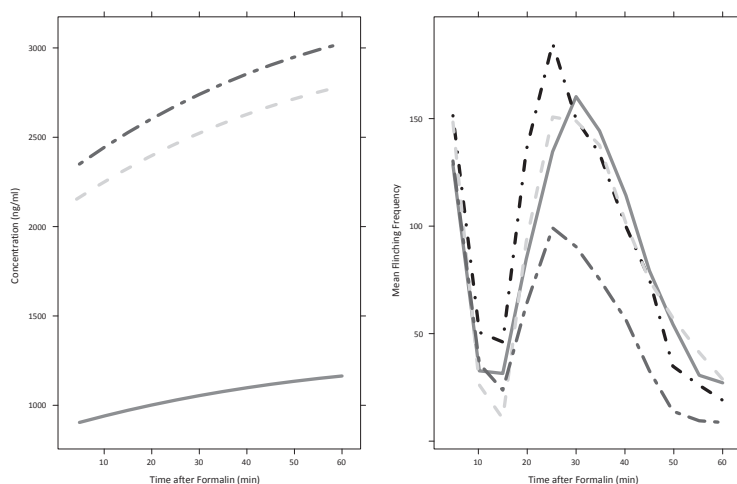


Figure 6.2: Population curves for simulated gabapentin concentrations in the plasma for doses 10, 30, 100 mg/kg (left panel) and the observed flinching behaviour in the formalin-induced model following placebo (dot-dashed), 10 (solid), 30 (dashed) and 100 (dash-dot) mg/kg curves (right panel).

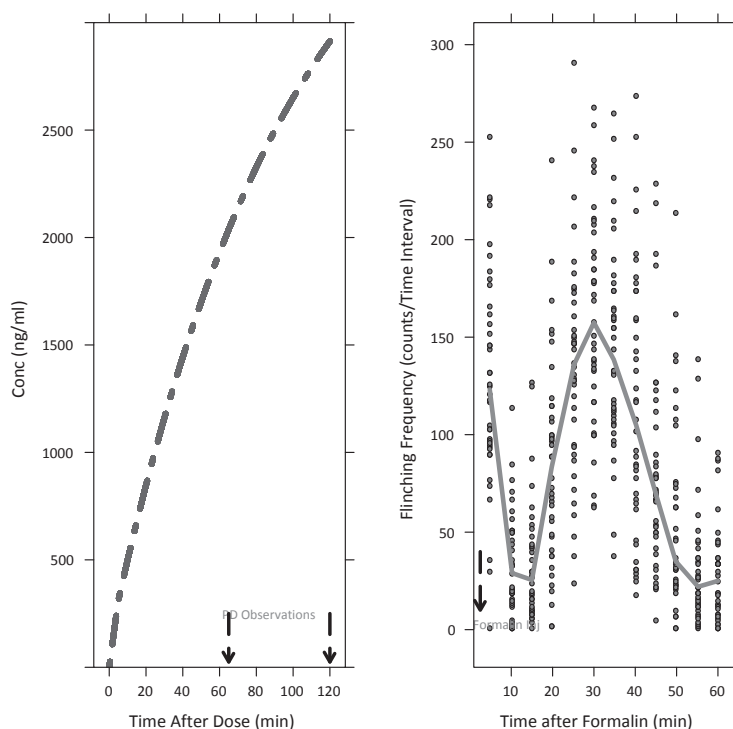


Figure 6.3: Disconnect between the (observed) onset of response (right panel) and the (simulated) time course of concentrations in plasma following a typical dose of 100 mg/kg gabapentin (left panel). Dots represent the individual observed flinching response; the solid line depicts the median response profile.

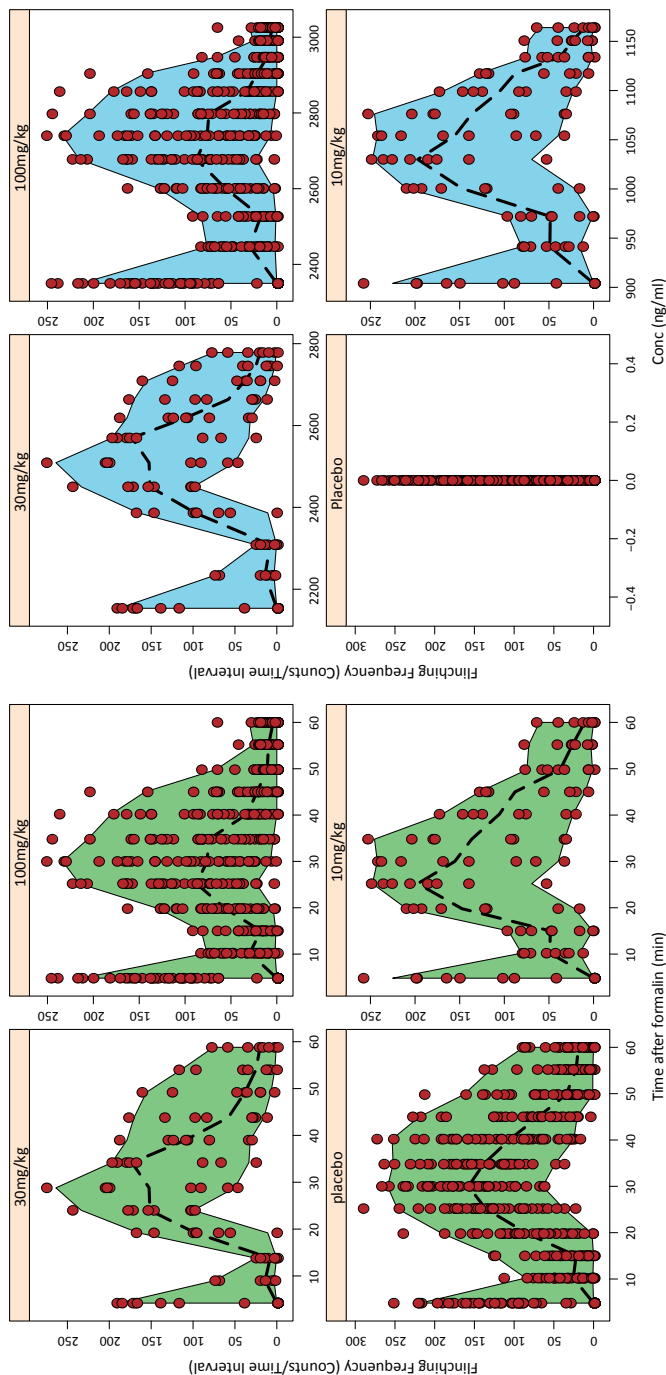


Figure 6.4 Flinching frequency versus time (left panel) and gabapentin concentrations (right panel), stratified by dose level.

PKPD modelling

The time course of the flinching behaviour as well as the inhibitory effects of gabapentin following drug administration were accurately characterised by the chosen indirect response model. The structural model described all three components of the pain response to formalin. The goodness of fit plots are presented in Figure 6.5. Sample individual fits are depicted in Figure 6.8 of the appendix. All structural model parameters were identifiable for the current dataset as evidenced from the RSEs (<40%) shown in Table 6.2 below.

Table 6.2: Parameter estimates from the final population PKPD model, including bootstrap estimates and confidence intervals.

| Parameter | Final PD Model | | | Bootstrap Estimates | | | |
|---|----------------|--------------|-----------|---------------------|--|-------|------------------------------|
| | Estimate (CV%) | IIV(CV%) | Shrinkage | Median of Estimate | 5 % 95 % Confidence Interval of Estimate | IIV | 5 % 95 % Confidence Interval |
| Degradation constant for formalin ($k_{df} h^{-1}$) | 12.3(5.39) | | | 249.30 | 12.33,1169.25 | | |
| Basal pain load in 1 st PD compartment (F_3 # counts) | 126(4.38) | 57.44(21.83) | 16.70 | 132.04 | 122.32,139.63 | 0.155 | 0.1,34.64 |
| *Delay between 1st and 2nd peaks ($T.lag h^{-1}$) | 0.29(0.20) | | | 0.242 | 0.0,0.30 | | |
| **parameter of gamma function ($A h^{-1}$) | 2720(6.65) | 66.33(17) | 14.08 | 2275.85 | 1099.145,2943.12 | 39.03 | 27.56,46.80 |
| Dimensionless gamma function parameter(α) | 2.29(239) | | | 24.84 | 1.33,159.012 | | |
| parameter of gamma function (βh^{-1}) | 8.37(30.82) | 67.08(78) | 15 | 9.29 | 5.43,12.17 | 25 | 7.21,44.84 |
| Degradation constant for waning of Pain 2($K_{deg} h^{-1}$) | 5.97(4.22) | | | 5.347 | 2.90,6.38 | | |
| ($EC_{50} ng ml^{-1}$) | 7510(40.33) | | | 6380.5 | 3961,15390 | | |
| Residual Error(additive) | | 29.09(7.8) | 9.40 | 35.34 | 33.07,43.77 | | |

IIV is presented as a percentage

* $T.lag$ is relative to the time after formalin injection

** While A , β are time-dependent parameters, α is a dimensionless constant

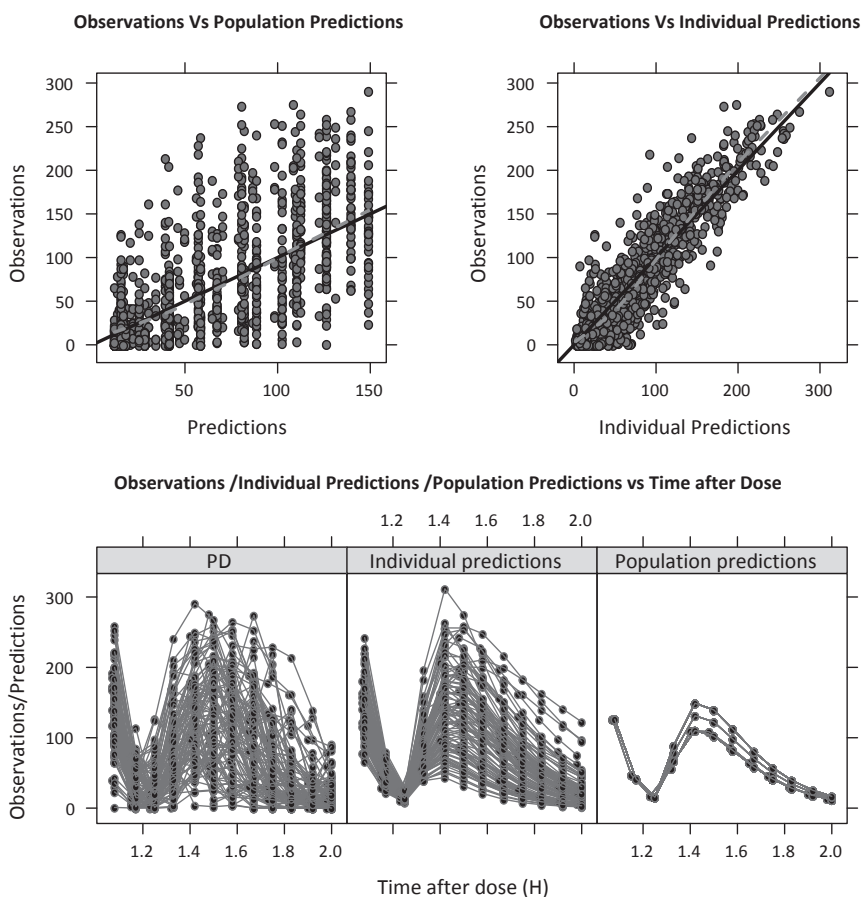


Figure 6.5: Goodness of fit Plots. The upper panels show the correlation between observed and population (left) or individual (right) predicted response. In the lower panels, the observed and predicted responses are depicted over time.

We have assumed the I_{max} to be 1, i.e., the maximum possible inhibition of pain. In practice, however, this is not the case, as the hypersensitisation attains different peak intensities in different subjects [18].

As can be seen from Figure 6.3 and Figure 6.4, there was considerable variability in the data. IIV was modelled exponentially and tested serially on all model parameters. The data supported the inclusion of IIV on the F_3 parameter of the first peak, β , and k_{deg} on the second peak, resulting in significant drops in the objective function value i.e., yielding statistically significant improvements in the model ($p < 0.01$).

Model validation

Model VPCs stratified by dose level are shown in Figure 6.6. The model is able to describe both the median trends in the data as well as the distribution i.e., the interquartile ranges. Since there was more data available for the placebo and 100mg/kg dose, the predictions for these dose levels are comparatively better than for the remaining dose levels. Approximately, less than 5% of the observations fall outside the prediction intervals. The model predicted 2nd peak response occurs slightly earlier than that of the real data.

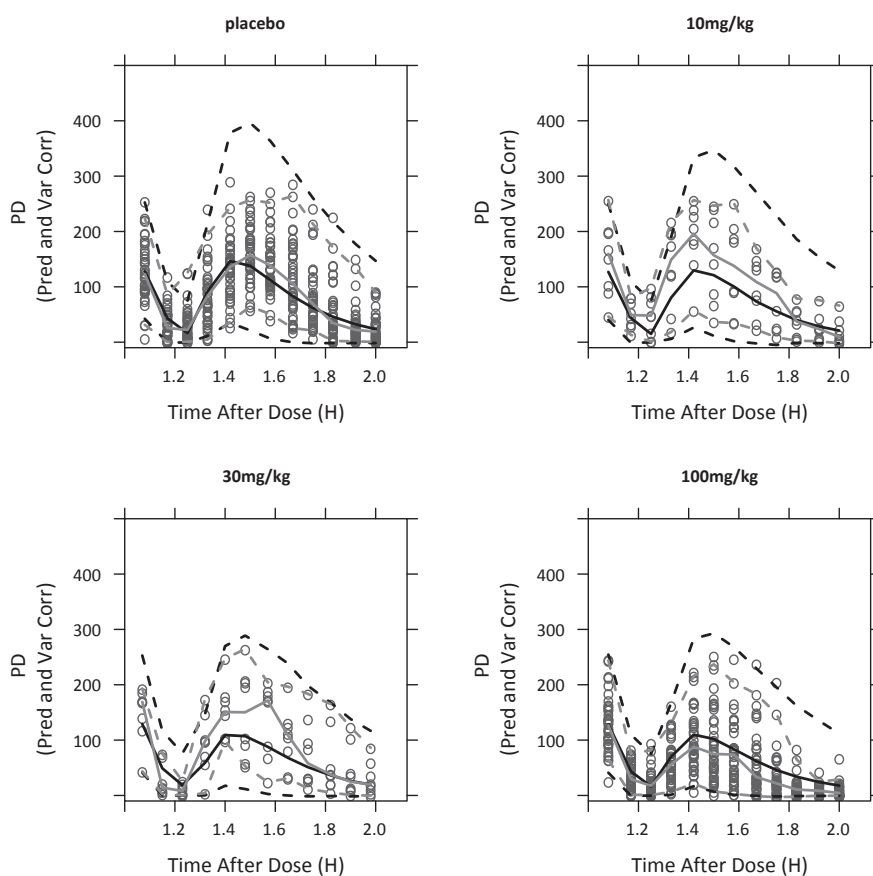


Figure 6.6: Visual Predictive Check for the final PKPD model stratified by dose. The results are based on 500 replicates. Open Circles are the raw data; the red and black lines denote the median of the observed and simulated data while the corresponding dashed lines represent the 2.5th and 97.5th percentile of the observed and simulated data respectively.

Table 6.3: Numeric Predictive Checks: Comparison of median of Observations at the maximum and minimum with corresponding simulations with 95% Prediction Intervals for Pain Phase 1 & 2 by Dose.

| Dose | Peak 1, Max PD (5min after formalin) | | Peak 1, Trough PD (10min after formalin) | | Peak 2, Max PD (30min after formalin) | | Peak 2, Trough PD (60min after formalin) | |
|----------|---|-------------------------------|---|-------------------------------|--|-------------------------------|---|-------------------------------|
| | Median Real data | Median SimIn data (95% CI) | Median Real data | Median SimIn data (95% CI) | Median Real data | Median SimIn data (95% CI) | Median Real data | Median SimIn data (95% CI) |
| 0mg/kg | 123.12 | 128.09 (109.49-148.49) | 22.14 | 16.48 (5.65-27.80) | 157.43 | 137.88 (107.44-173.76) | 19.09 | 23.46 (9.90-38.71) |
| 10mg/kg | 159.62 | 126.90 (90.41-170) | 48 | 14.90 (0.30-39.60) | 157.03 | 121.03 (71.33-192.02) | 9.73 | 21.62 (1.05-51.41) |
| 30mg/kg | 169.13 | 130 (91.60-174.90) | 8.17 | 18.50 (0.43-43.20) | 171.52 | 87.92 (44.11-143) | 19.70 | 19.64 (0.47-49.40) |
| 100mg/kg | 126.51 | 128.51 (110.60-150) | 17.50 | 16.20 (5.40-27.84) | 75.01 | 102.03 (80.02-127.32) | 4.40 | 17.75 (5.12-32.35) |

Numerical predictive checks are depicted in Table 6.3, where the median number of flinching counts for observed and simulated (95% CI) data is shown at four different points, with the objective of characterising the maximum and minimum values of the two pain phases. In general, predictions for the placebo and 100mg/kg doses are better as compared to the other two, except in the case of the trough response for peak 2 where the model seems to over predict the frequency of counts while under predicting gabapentin effects, for the top dose as compared to the other dose levels.

Bootstrap

Table 6.3 shows the results of final model parameter estimates along with the results from the bootstrap for 500 runs (median, 5 and 95% CI). Most model parameters were well estimated, with the exception of k_{df} , α and $Tlag$ as can be seen from the wide confidence intervals in the bootstrap. This suggests that not all parameters may be identifiable in subsets of the original dataset. However a sensitivity analysis showed the variation in these parameters did not have significant effects on the overall model fit. Similarly, the parameters describing IIV were not well estimated in the bootstrap. We experienced a high minimisation failure rate in the bootstrap (~70%), which has caused a possible underestimation of IIV during bootstrapping. Therefore, all final IIV estimates are based on objective function criteria used during the initial fitting procedures.

DISCUSSION

Despite its wide use in the screening of compounds for neuropathic pain, till recently no attempts had been made to characterise PKPD relationships in the FIP model, with the exception of the recent work of Velez de Mendizabal *et al.* on lumiracoxib [20]. Though the subject of both investigations was the same (the FIP model) the applications were different. We are interested in describing exposure response relationships of gabapentin while they have studied lumiracoxib topical versus intrathecal drug interactions.

Consequently, quantitative approaches in either case were different. Thus while we have explored exposure~response relationships, Velez de Mendizabal *et al.* have used a KPD (kinetic pharmaco-dynamic) model. In contrast to previous investigations using the FIP model, the semi-mechanistic approach proposed here fulfils two important conceptual requirements for the purposes of compound screening, i.e., it describes the time course of the disease and the gabapentin effect in an independent manner. In addition, our choice of parameterisation took into account the possibility and importance of generating evidence of PKPD properties that can be easily used to translate treatment effects across species. Therefore, model parameterisation has not relied on typical measures such as cumulative response, which despite being technically less demanding has important drawbacks. For instance, if data were to be modelled using cumulative flinching counts, gabapentin potency

would be expressed in terms of the time required to halve the maximum response. Such a parameter would have little physiological meaning even though many consider it suitable for ranking of compounds. Moreover, the use of such cumulative measures of response would not warrant a unique PKPD relationship (see Figure 6.4). It became clear during our exploratory analysis that the flinching behaviour induced by formalin produces a unique fingerprint which prevails over any attempt to characterise the underlying exposure-response relationship using direct response models [26].

By contrast, our approach explores drug(compound) and disease properties using independent parameters. From a pathophysiological perspective, the first peak is caused by peripheral sensitisation, whilst the second may reflect central hypersensitisation. The same phenomenon appears to be reproduced in other species such as mice, gerbils, cats, monkeys [6], suggesting the opportunity for wide use of the concepts presented here. Gabapentin effect was therefore parameterised in terms of an indirect response model, which describes the changes in flinching behaviour in terms of a declining exponential expression. The formation rate of such response (k_{syn}) was further characterised by a gamma function, which indicates the time varying course of formalin-induced symptoms, and consequently modifying the classical indirect response model of Dayaneka *et al.* [19]. This function has been previously described for endpoints where spontaneous recovery from inflammation can be expected [14]. Historically, negative power functions of time have been applied to describe clearance curves in PK studies and tracer kinetics in general, with a view to replacing multicompartment analysis. Though non-physiological, they require considerably fewer parameters and yielded more accurate predictions[27]. We have modified the traditional gamma function by parameterising the variable T_{lag} as the exponent of the dimensionless constant α . This led to better fits and lesser numerical difficulties with the minimisation routine. The time to onset of the 2nd peak was about 20 min in our analysis which is in agreement with the observed data and also literature estimates of 10-20 min post formalin[2]. However, there was considerable variability in this parameter as can be seen from the median effect vs. time curves in Figure 6.2. It should also be noted that the I_{max} was defined as the maximal change in pain intensity i.e., return to a baseline state, and therefore set to a theoretical maximum of 1. Fixing of the parameter to a single maximum value was applied even though the disease process and treatment response was not expected to be same in all subjects. The approach has been previously applied by Maas *et al.* to describe migraine pain [18].

Indirect response models incorporate the Hill function directly in the turnover differential equation whereas we parameterise the drug effect ($DEFF$) directly on the pain variable of the 2nd peak and not within the differential equation. This is because the drug does not alter the onset of the pain nor its eventual disappearance but reversibly alters its peak intensity. In other words, the drug effect is a covariate on the behaviour or flinching response. Therefore application of the drug effect to either k_{in} or k_{out} would have been non-mechanistic. This

effect can be visualised in Figure 6.2 where the typical profile for the 100 mg/kg dose exhibits a smaller 2nd peak compared to the placebo time course. Velez de Mendizabal *et al* employed a similar approach to describe the effects of a COX₂ inhibitor lumiracoxib on COX₂ [20]

Focus should also be given to the observed high between-subject variability in the FIP model, a phenomenon that is well known in clinical pain conditions [28]. Although most investigations consider such variability a purely stochastic process which cannot be assigned to any specific source or mechanism, we have tried to estimate between-subject variability for all relevant model parameters, such as I_{max} or IC_{50} . Unfortunately, this was not always supported by the data. Yet, it is reasonable to assume that individual differences in gabapentin potency do exist and occur due to the time varying effects of formalin, which can affect both maximum frequency of flinching behaviour as well as modulate gabapentin effects on central hypersensitisation. On the other hand, IIV could be identified for parameters associated with the induction of formalin-induced pain. The basal load of pain (F_3) differed among subjects and an η on this parameter improved the fit. The waning of the pain phenomenon (k_{deg}) was also found to differ among individuals and fitting showed significant improvements when IIV was applied.

Diagnostic and validation plots, such as the VPCs show the model has adequate predictive properties. Ideally, in such circumstances, the next step would be to fit the model to external datasets. Regretfully, we have not been able to identify such data.

Limitations

A potential drawback in our approach is that the IC_{50} estimates appear to be beyond the range of observed gabapentin concentrations. This situation is caused by the use of a theoretical maximum (I_{max}), which was not reached by gabapentin. Had this been the case, the second peak would have been suppressed completely. However, all concentrations tested were in the linear part of the curve. On the other hand, it is well documented that gabapentin produces partial symptomatic relief in neuropathic pain, rather than showing any disease modifying effects. It is therefore plausible to infer that incomplete suppression of the second peak reflects actual clinical effects of gabapentin [29, 30]. Yet, we consider the ability to discriminate between compounds that cause total pain suppression and partial relief highly desirable and do not anticipate any bias in the way compounds can be ranked on the basis of their potencies.

In a situation where $C_p \ll IC_{50}$, the DEFF in equation 7 would reduce to the following expression:

$$DEFF = \left(1 - \frac{1}{IC_{50}}\right)$$

It could be argued that the IC_{50} would then be a linear coefficient rather than a true measure of potency and consequently the estimate of the IC_{50} would not be robust.

It is important to mention that we assume that an ideal or an efficacious drug would completely suppress flinching and by fixing I_{max} we assume maximal response is possible in this biological system. The IC_{50} then becomes a relative parameter, conditioned on an I_{max} of 1. If this is the case then this parameter can be reasonably used to compare potencies across compounds. Modelled parameters were not always identifiable in the bootstrap as can be seen in Table 6.2. This suggests that a rich dataset may be required to fit this model and here is where the advantages of a model based approach come in. Existing data may be used in combination with future data whereby the new data are used only to estimate compound specific parameters and existing data support estimation of system specific parameters.

We also acknowledge that the gamma function may have little physiologic basis, and thus future improvements to the model could be aimed at replacing this function with a more physiologic alternative. Such an alternative parameterisation may however require the availability of rich datasets. We anticipate that historical data may be used in combination with newly generated experimental data whereby only analgesic-specific parameters need to be estimated.

Complex pathophysiological processes underlie the generation of second peak, such as the release of various excitatory neurotransmitters acting through NMDA and NK1 receptors which then initiate a cascade leading to central sensitisation [31]. We have parameterized these underlying processes collectively as FL , under the assumption that differences in the individual time course of neurotransmitters was not statistically different. This choice was made to ensure description of the observed phenomenon rather than the pathophysiology of the pain response.

We also highlight a few shortcomings of the experimental design in our study, which was performed according to standard experimental procedures. The time of dosing of gabapentin should have been planned taking into consideration potential differences in pharmacokinetic properties. If gabapentin had been administered earlier, the return to baseline of the flinching events might have coincided with its elimination phase. Secondly, no baseline behaviour was recorded i.e., flinching counts between the administration of gabapentin and that of formalin ($T=0$). As explained previously, pain burden at baseline also showed differences between animals (η on F_3).

Comparison with other pre-clinical and clinical findings

We have attempted to compare our results with other published pre-clinical and clinical data on gabapentin. Table 6.4 gives an overview of the EC_{50} and ED_{50} values reported for different pain models. Except for one pre-clinical experiment[32] and one clinical study [33] no other publications have applied modelling to analyse or interpret the data. Most authors used

ED₅₀ and MED as measures of potency with no mention of concentrations, rendering direct comparisons rather difficult, if not impossible [12, 34-37]. Noteworthy is the wide variability observed in the findings of different authors. There were other important differences such as the ceiling effect being observed by Iyengar *et al.* at a relatively low dose of 50 mg/kg while others reported peak effects between 100-300 mg/kg [31, 37].

Among those studies where direct comparison with our work was possible, Todorovic reported an EC₅₀ of 467 nM as compared to 43 nM reported here. More consistent results for clinical EC₅₀s were reported by Lockwood *et al.* (31.28 nM), whilst Whiteside *et al.* provide estimates for clinical MED values of 69.72 nM [38, 39]. Notably, Whiteside's work is the only effort at inter-species correlations, amongst the publications we reviewed, albeit not based on modelling concepts.

CONCLUSIONS

In summary, differences in analgesic potency exist in pre-clinical models, which cannot be interpreted simply in terms of precision. A comprehensive evaluation is missing of the differences and similarities in the underlying mechanisms affected by evoked pain in the various models currently available for pre-clinical evaluation of neuropathic pain.

Clearly, the challenges for the identification of suitable compounds for the treatment of neuropathic pain will not be overcome until adequate biomarkers of pharmacology are identified [40, 41]. Yet, irrespective of such differences in pathophysiology, approaches are required that facilitate the translation of pre-clinical findings and provide the basis for the characterisation of analgesic-specific properties. A parametric, model-based approach is essential to ensure distinction between disease processes and analgesic effects.

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Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies.

| No. | Author | Experimental Model | Study Protocol | Main findings | Comparison with our work/ other remarks |
|--|---|---|---|---|---|
| Comparison with Preclinical experiments | | | | | |
| 1. | Shannon (Shannon <i>et al.</i> , 2005) | FIP in rats | Comparison of anticonvulsants with different mechanisms of action in the formalin test of persistent pain (rats, mice). Gabapentin doses tested were 30-300mg/kg (IP) | In rats efficacy was seen across the dose range from 30-300mg/kg Minimal effective dose (MED) in rats was 30mg/kg for the 2 nd peak of pain. The MED for locomotor activity in mice was 100mg/kg | Drug effects on the 2 nd peak between 30-100mg/kg |
| 2. | Iyengar (Iyengar <i>et al.</i> , 2004) | FIP in rats | A comparison of the effects of analgesic agents such as uptake inhibitors, tricyclic antidepressants, anticonvulsants on attenuation of formalin induced late phase paw-licking behaviour. Gabapentin was administered in doses of 10/30/50mg/kg IP | Gabapentin attenuated paw licking behaviour in the doses administered. A plateauing of effects was observed beyond 50mg/kg | We observed analgesic effects at 100mg/kg as well |
| 3. | Hama <i>et al</i> (Hama and Sagen, 2007) | Rat model of acute NP resulting from experimental spinal compression injury | A placebo controlled 12 week study. A number of compounds among which opioid analgesics, antidepressants, anticonvulsants were tested. Gabapentin was administered at doses of 10/30/100mg/kg IP | Gabapentin dose-dependently reversed mechanical hypersensitivity. The A ₅₀ (antinociceptive) dose was 26(16-42) mg/kg. The peak efficacy was observed 90 min after injection. | Estimated EC ₅₀ was higher than the administered dose range of 10-100mg/kg |
| 4. | Whiteside <i>et al</i> (Whiteside <i>et al.</i> , 2004) | Spinal nerve ligation rat model, clinical data | Comparison of human C _{max} at MED at daily maintenance dose (1800mg) to rat MED based on published literature | The concentrations at the rat MED (100mg/kg) was 191.54 nm compared to 69.72 nm at the human maintenance dose of 26 mg/kg | We estimated the EC ₅₀ at 43nm with a CV of 40% |
| 5. | Yoon MA (Yoon and Yaksh, 1999) | FIP in rats | The antihyperalgesic effects of gabapentin (10, 30, 100, 300mg/kg) IP alone and in combination with ibuprofen (3, 10, 30mg/kg) IP were tested. An isobolographic analysis was used to study the nature of the interaction. | The ED ₅₀ for gabapentin was 88mg/kg (51-141mg/kg, 95% CI) while that for ibuprofen was 19mg/kg (7-50, 95% CI) | Our EC ₅₀ was >100mg/kg |

Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies. (Continued)

| No. | Author | Experimental Model | Study Protocol | Main findings | Comparison with our work/ other remarks |
|--|---|---|--|---|--|
| <i>Comparison with Preclinical experiments</i> | | | | | |
| 6. | Hurley RW(Hurley et al., 2002) | Rat model of carrageenan induced inflammation | Gabapentin ,pregabalin or naproxen administered alone or in combination as oral gavage was administered to rats. An isobolographic analysis was used to study the nature of the interaction. Gabapentin was administered at doses ranging from 3-300mg/kg | The ED ₅₀ for gabapentin was 19.2mg/kg(5.5-43.1mg/kg, 95% CI) while that for pregabalin was 6mg/kg (2.3–10, 95% CI) and for naproxen this was 0.48(0.05-1.38mg/kg) | In this animal model the ED ₅₀ for gabapentin was lower indicating higher potency as compared to that in our work |
| 7. | Whiteside et al(Whiteside et al., 2004) | Rat model of incision pain | A number of analgesic drugs such as gabapentin, indomethacin and morphine were compared. Gabapentin doses were 10,30,100mg/kg | The MED for mechanical hyperalgesia was 30mg/kg, ED ₅₀ 11.3mg/kg For tactile allodynia the MED was 11mg/kg and ED ₅₀ 3.4mg/kg | In this animal model the ED ₅₀ for gabapentin was lower indicating higher potency as compared to that in our work |
| 8. | Todorovic et al(Todorovic et al., 2003) | The radiant heat rat model of NP. | Anticonvulsants were injected intradermally into peripheral receptive fields of sensory neurons in the hind paws of adult rats, and paw withdrawal latency measured. Gabapentin (5-170µg), phenytoin (0.1-3 µg) , carbamazepine (0.1-2 µg), ethosuximide (140-1400 µg) were evaluated. Dose–response data were fit to the function $PI([-DRUG])=PI_{max}/(1+(ED_{50}/[DRUG])^n)$, where PI_{max} is the maximal percentage increase in PWLs caused by a drug in the injected vs. non-injected paw 10 min following injection, and n is the apparent Hill coefficient indicating the slope of the curve. | The ED ₅₀ was 80 µg/100ml or 4.67 nm | The 10 fold difference from our findings may be, in part, explained, in part, by different routes of administration in this study apart from a different experimental model and study setup. |

Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies. (Continued)

| No. | Author | Experimental Model | Study Protocol | Main findings | Comparison with our work/ other remarks |
|---|---|--|--|---|--|
| <i>Comparison with clinical/Translational experiments</i> | | | | | |
| 9. | Whiteside <i>et al</i> (Whiteside <i>et al.</i> , 2004) | Spinal nerve ligation rat model, clinical data | Comparison of human C_{max} at MED at daily maintenance dose (1800mg) to rat MED based on published literature | The rat MED was 191.54nm compared to 69.72nm for humans | We estimated the EC_{50} at 43nm with a CV of 40% |
| 10. | Lockwood <i>et al</i> (Lockwood <i>et al.</i> , 2003) | A phase 3 study on patients with NP | The study was placebo controlled, double blinded. Patients were randomized to placebo or gabapentin treatments. A PKPD model (E_{max}) was fitted to data from patients with NP. The PKPD model was further used to simulate the MED for an investigational compound (pregabalin) based on in vitro potency information. | The EC_{50} estimated as 31.28nm (16% CV). | Correlates with EC_{50} estimated by us, however needs further confirmation. |

APPENDIX

Pharmacokinetic concentrations

Gabapentin concentrations were obtained by simulation time points corresponding to those when pharmacodynamic measurements were recorded. A 2 compartment PK model with first order absorption was used for the simulations[16]. This model was based on data from two different experiments in Sprague-Dawley rats. In the first experiment, gabapentin was administered orally to conscious rats at doses of 0, 10, 100, 300 mg/kg in a formalin-induced hypersensitivity experiment similar to the current one, both of which, in turn, are based on standard published experimental protocols.[2, 6]. Experimental groups consisted of three rats per dose level, with each animal contributing with four samples over a period of up to 6 h post-dose. The second experiment consisted of animals used in a microdialysis protocol receiving intravenous doses of 50mg/kg gabapentin (n=63). Each animal contributed with eight samples over a period of up to 24 h post-dose [42].

Published bioanalysis of gabapentin

Blood samples (100µl) were taken at the pre-defined time points up to 5 hours post-dose, namely 0, 2, 5, 15, 30, 60, 120, 180, 240 and 300 min. Plasma samples (50µl) were obtained by centrifugation at 4°C for 10 min and stored at -80°C until analysis. Gabapentin concentration in plasma was subsequently analysed by HPLC using pre-column derivatisation. Gabapentin and the internal standard 1-(aminomethyl) cycloheptanecarboxylic acid were allowed to react with 2,4,6-trinitrobenzenesulfonic acid to form trinitrophenyl derivatives, which were then extracted with toluene, evaporated to dryness and reconstituted before injection. Analytes were resolved on a C₁₈ reverse phase column using isocratic conditions. Mobile phase consisted of 58% acetonitrile in water containing 0.5% acetic acid. Ultraviolet absorbance was monitored at 355nm. Quantification of the drug levels was based on the peak-height ratio. The lower limit of detection for gabapentin was typically 0.02µg ml⁻¹ [43, 44]

Analytic Solution for the 2 compartment PK model-used for the PK simulations:

$$k_{el} = \frac{Cl}{V_1}, k_{12} = \frac{Q}{V_1}, k_{21} = \frac{Q}{V_2}$$

Where k_{el} =elimination rate constant (from the plasma compartment), k_{12} =micro-rate constant for transfer of gabapentin from the central to peripheral compartment, k_{21} =micro-rate constant for transfer of gabapentin from the peripheral to central compartment, V_1 =central volume of distribution, V_2 =peripheral volume of distribution, Cl =clearance from plasma, Q =intercompartmental clearance.

From the above the coefficient A may be calculated

$$A = k_{12} + k_{21} + k_{el}$$

From A , the two macro-constants or λ_1 and λ_2 (corresponding to the initial and terminal slopes representing bi-exponential decline respectively) may be further derived as follows

$$\lambda_1 = \frac{A + \sqrt{A^2 - 4k_{21}k_{el}}}{2}$$

$$\lambda_2 = \frac{A - \sqrt{A^2 - 4k_{21}k_{el}}}{2}$$

From these above the expression in equation 1 of the main text– for plasma concentrations is then derived.

$$C = \frac{k_o FD}{V_1} \left\{ \frac{(k_{21}\lambda_1)e^{-\lambda_1 t}}{(k_o - \lambda_1)(\lambda_2 - \lambda_1)} + \frac{(k_{21}\lambda_2)e^{-\lambda_2 t}}{(k_o - \lambda_2)(\lambda_1 - \lambda_2)} + \frac{(k_{21}k_o)e^{-k_o t}}{(\lambda_1 - k_o)(\lambda_2 - k_o)} \right\}$$

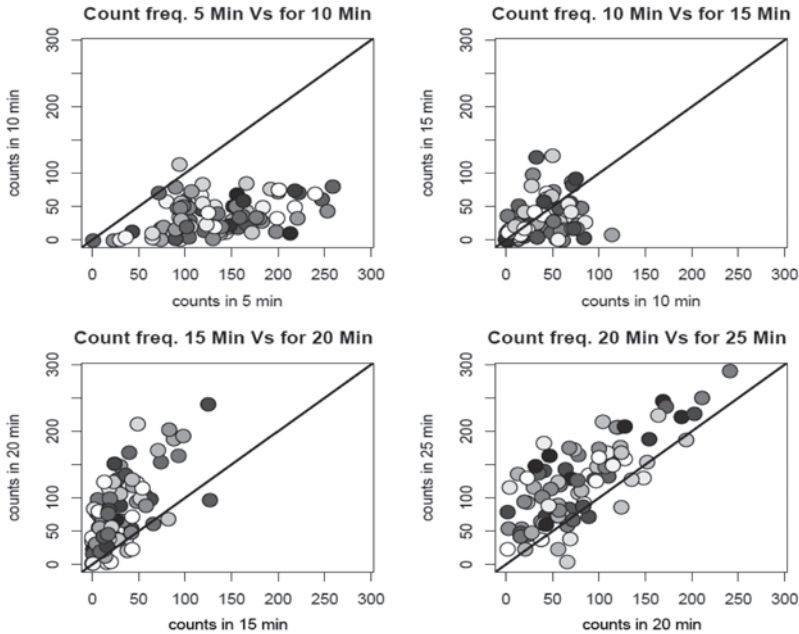


Figure 6.7: Correlations between observations at successive observation intervals. In the 4 panels, flinching counts at a particular interval are plotted against the corresponding counts in the next interval. Due to correlations between successive observations, the flinching patterns show trends, towards decreasing frequency in the upper panels and increasing in the lower panels.

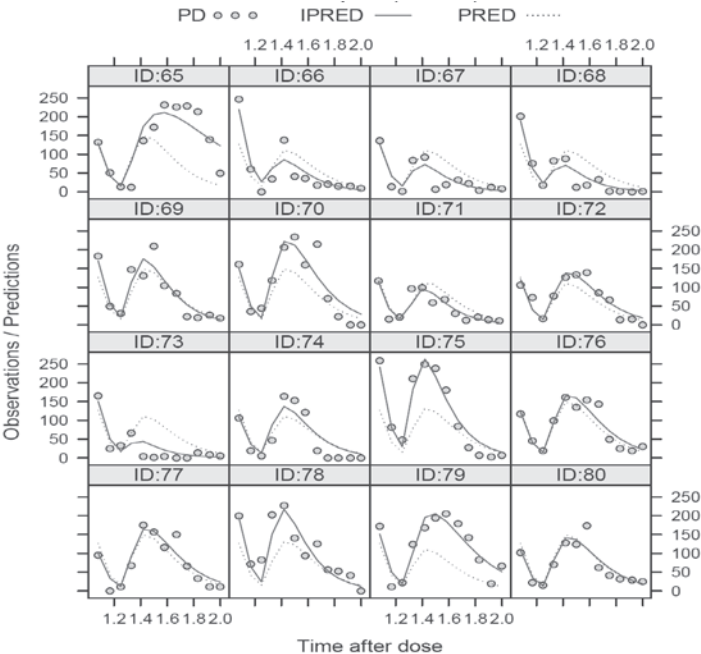


Figure 6.8: Example of randomly selected observed individual profiles (shaded circles) with the corresponding individual (IPRED, solid line) and population predicted (PRED, dotted line) response.

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SECTION III

**LOST IN TRANSLATION-FROM BIOMARKER TO
CLINICAL ENDPOINT**

CHAPTER 7

Biomarker exposure-response relationships as the basis for rational dose selection: lessons from an ex-vivo model of inflammatory pain

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ABSTRACT

Lack of efficacy is one of the major causes of attrition in early clinical development. This is of particular concern in areas of high unmet medical need such as chronic inflammatory pain, where measures of efficacy cannot be quantified directly in healthy subjects. The evaluation and selection of an effective dose range for COX-inhibitors has been a matter of debate over the last decade. Yet, a systematic approach has not been fully implemented that enables the use of pharmacodynamics as a biomarker in a mechanistic manner to support the development of anti-inflammatory compounds. Here we apply PKPD modelling and simulation to assess the pharmacodynamic effects of a selective COX inhibitor across various clinically relevant scenarios and use biomarker response rather than drug exposure as the basis for dose selection in subsequent efficacy trials. Thereby, we are able to personalise and optimise the effective dose range in different patient sub-populations.

INTRODUCTION

A landmark study on attrition rates in drug development revealed that the major dropouts occur in Phase 2 and 3 [1]. In some therapeutic indications, such as chronic pain, such challenges are also observed during proof-of-concept (POC) studies. Potential explanations for these findings are species differences in target pharmacology or tissue distribution, poor tolerability due to inaccuracy in predicting the therapeutic index, insufficient target engagement, timing of the intervention relative to the onset of disease and trial design factors[2]. Whilst all the aforementioned factors can play a role in the results of a trial, target pharmacology and target (tissue) distribution have been considered a matter for basic rather than clinical pharmacology. Yet, they are essential for the characterisation of efficacy and safety and as such underpin the rationale for dose selection. In fact, as illustrated by Morgan *et al.*, development programs that have a positive readout at clinical POC also clearly express the pharmacology of the compound in humans [3]. Nevertheless, despite the increasing appreciation of the role of pharmacodynamic markers in clinical development, Phase 1 trials, including first-time-in-humans studies are typically designed to evaluate, systemic pharmacokinetics and tolerability[4]. These studies may be complemented by data from experimental models of pain, but measures of pain such as global pain scores are not integrated to pharmacokinetics or pharmacodynamics, and consequently, doses are selected without quantitative evidence about the extent and rate of target engagement [2]. These findings then become the reference for designing dose ranging studies in Phase 2 and defining the therapeutic dose(s) in Phase 3.

Undoubtedly, there is a pressing need to obtain early signals of efficacy and safety to prevent high attrition at late stages of development. This prerequisite is key for areas of high unmet medical need such as chronic pain and other immunoinflammatory conditions [5]. In these conditions inadequate decisions regarding dose selection during Phase 2a can propagate undetected into late development and have a disastrous impact on the life cycle of a novel molecule. Despite our evolving understanding of pharmacodynamics (PD), the evaluation of what constitutes a clinically relevant dose still relies primarily on empirical evidence, without any quantitative consideration of the underlying pharmacology or target engagement (e.g., receptor occupancy levels) in the patient population[3]. Currently, opportunities exist for truly characterizing the clinical pharmacological profile of novel molecules in humans, enabling mechanistic insight into the exposure-response relationships and consequently better rationale for the therapeutic dose range. Integration of biomarkers of pharmacology into drug development therefore becomes an opportunity to allow the implementation of the aforementioned concepts, eliminating part if not all the unobserved bias that arises from empirical evidence. Moreover, the assessment of pharmacokinetic-pharmacodynamic relationships based on biomarkers of pharmacology can provide a stronger basis for personalised medicine, which is often restricted to tailoring of treatment based on the

use of genetic information only[6]. By applying the mechanistic classification proposed by Danhof *et al.* [7], it is also possible to identify whether such relationships are drug or disease-specific and consequently to establish whether they can be used as predictive and prognostic tools during the development and therapeutic use of the drug.

In the current investigation we use data from a cyclo-oxygenase (COX) inhibitor to illustrate the concept of biomarker driven dose selection and emphasise the importance of gaining insight into the clinical pharmacology of the compound as the basis for the dose rationale and other relevant labelling information. The choice of the COX-2 system as a paradigm was dictated by the various reports arising from the withdrawal of different drugs from the market, for which the clinical pharmacology profile was known to determine efficacy and safety across different therapeutic areas, such as rofecoxib (2004), rimonabant (2008) and efalizumab (2009) [8-10]. Although complex interactions in mechanisms underlie the pathophysiology of chronic inflammatory conditions, the role of the COX-2 enzyme in the production of inflammatory mediators such as thromboxane B₂ (TXB₂) and prostaglandins (PG) has been clearly elucidated [11]. Selective COX-2 inhibitors are known to primarily inhibit PG synthesis [12].

Based on the aforementioned classification [7], PGE₂ and TXB₂ rank as biomarkers that reflect target engagement. Notably, the therapeutic dose range for chronic inflammatory pain for most non-selective and many of the selective COX-inhibitors has been defined according to empirical evidence of pain relief and analgesia after administration of discrete dose levels in clinical trials, regardless of the underlying pharmacology[13, 14]. It has been demonstrated, however, that pain relief appears to occur at PGE₂ inhibition levels of around 80%, i.e., complete suppression of COX-2 activity is not required to translate pharmacology into clinical improvement [15]. From these findings it can also be inferred that analgesia will also be observed at still higher levels of COX-2 inhibition, but such levels will lead to long term disruption of the normal physiological and homeostatic functions of the prostacyclin system, including tissue repair [16].

We use data from GW406381, an investigational and potent COX-2 inhibitor with demonstrated pre-clinical anti-inflammatory and analgesic activity [17, 18], to show that the study of such a mechanistic biomarker should be at the cornerstone of analgesic and anti-inflammatory drug development. The compound's pharmacokinetics as well as its effects on PGE₂ and TXB₂ were evaluated in an ascending dose study in healthy subjects, allowing the use of a biomarker-driven approach to select the doses for a Phase 2 study [19]. With the help of simulation scenarios we illustrate how biomarkers can be harnessed to explore the need for treatment personalisation (e.g., hepatic impairment) and quantitatively evaluate the rationale for the dosing regimen (e.g., optimised benefit-risk ratio).

METHODS

Clinical studies

Data from a human pharmacology study in healthy male subjects from the GSK (GlaxoSmithKline) clinical trial repository was used for the purposes of our analysis. This was a randomized, placebo controlled, double blind dose escalation parallel group study aimed at the evaluation of safety, tolerability, pharmacokinetics and pharmacodynamics of GW406381. Treatment consisted of a single dose followed by a 10-day repeated dosing phase (n=9 for the active and n=3 for the placebo arm). Data from placebo, 35 and 70 mg dose arms after the single dose phase and 35 mg dose arm after repeated dosing were used in our analysis. The study was conducted according to the principles of good clinical practice (GCP) and the declaration of Helsinki pertaining to research on human subjects [20, 21]. All subjects provided their written informed consent for participation and the study was approved by the Institutional ethics committee. Further information on subject demographics and the study protocol is provided in Table 7.3 (see appendix).

Pharmacokinetic-pharmacodynamic modelling

To guide the model building, exploratory analysis was carried out by plotting the time course of the biomarker levels as well as the drug concentration vs. biomarker levels profile. The PKPD analysis was subsequently carried out sequentially in two steps, with modelling of PKPD data after completion of the pharmacokinetic analysis. Details on the pharmacokinetic modelling can be found in the appendix to this manuscript. All modelling was performed in NONMEM®, version 7.2 (Icon, Dublin Ireland), using the FOCE (first order conditional estimation) method. PsN 3.5.3 was used to run NONMEM, whilst data manipulation and plots were performed in R 2.13[22].

Both for the PK and PKPD analysis, a parameter Θ for an individual i was described by the following expression:

$$\Theta_i = \Theta_{TV} * \exp^{\eta_i} \quad (1)$$

Where Θ_{TV} is the typical (population) value of the parameter, η is a random variable with zero mean and a variance ω^2 .

Inter-individual variability (IIV) was parameterised using an exponential distribution model. The square root of the variance is reported for IIV, as this is an approximation to the apparent coefficient of variation of a normal distribution on log-scale. The residual variability comprising measurement and model misspecification errors was described with an exponential model, thus for

$$Y_{ij} = F_{ij} * \exp^{\varepsilon_{ij}} \quad (2)$$

Where Y is the j^{th} observed concentration in the i^{th} individual.

F is the predicted concentration and ϵ is a random variable with zero mean and variance σ^2 . The concentration-biomarker response relationships were described by the following equation/expression for the sigmoid I_{max} model

$$Eff = I_0 - (I_0 - I_{max}) * \frac{C^\gamma}{C^\gamma + IC_{50}^\gamma} \quad (3)$$

Where I_{max} represents the maximum inhibitory response to GW406381 plasma concentrations (C), I_0 is the baseline production of PGE_2 and γ is the Hill factor. The covariate effects of baseline PGE_2 on the parameter I_0 was tested according to the following expression

$$I_{0i} = I_{0TV} * \frac{BAS_i - MED}{MED} \quad (4)$$

where I_{0i} represents the parameter value for the i^{th} individual, I_{0TV} is the population value of the parameter, BAS_i and MED represent the individual and median values of the baseline PGE_2 , respectively.

Model evaluation and validation procedures

Parameter inclusion and thus final model selection was based on the likelihood ratio test, parameter point estimates and their respective 95% confidence intervals (CI) as well as goodness of fit plots. For the likelihood ratio tests, the significance level was set at 0.01 which corresponds with a decrease of 6.63 points after the inclusion of one parameter in the minimum value of the objective function (MVOF) under the assumption that the difference in MVOF between two nested models is χ^2 distributed. Visual goodness of fit plots comprised individual vs. population or individual predictions, and weighted residuals vs. time or population predicted values. Minimisation was considered successful in case the minimisation occurred with a positive covariance step and no associated error messages.

Validation

The precision of estimated model parameters was assessed using a non-parametric bootstrap. Two thousand bootstrap samples were generated in PsN 3.5.3 [23]. Results were used to assess model stability and obtain estimates for the coefficient of variation for relevant model parameters. The mean and standard errors of the parameters obtained from bootstrapping were subsequently compared with those obtained by fitting the model to the original dataset. Finally visual predictive checks were used to visually inspect the concordance between simulated data and real observations. Using the final model parameters, 2000 datasets were simulated and the simulated data overlaid with the real observations.

Simulations

The last part of this work was to simulate analgesic doses in patients based on biomarker inhibition data from human subjects, under the assumption that pharmacodynamics in the target population are comparable, other than differences in baseline levels of inflammatory mediators due to differences in disease conditions. Most importantly, it was assumed that the analysis was based on the premise that PGE₂ inhibition represents a causal step in the pain cascade [24]. The drug effect was parameterised in terms of IC_n as per the following expression [15].

$$IC_n = \left(\frac{n}{100-n} \right)^{\frac{1}{\gamma}} * IC_{50} \quad (5)$$

where n = the degree or extent of COX-2 inhibition in percentage.

Using the final PKPD model, concentration-biomarker response profiles were simulated for a number of clinical scenarios are presented in Table 7.1 below.

Table 7.1: Factors altering drug exposure

| Scenario | Altered parameters | Remarks | Reference |
|---|--|--|--|
| Liver dysfunction | Cl: -25% Cl: -50% Cl: -75% | Mild, moderate and severe liver dysfunction-as per Child Pugh scores | Schmitt-Hoffmann <i>et al.</i> , 2009[25] |
| Systemic vasculitis (General inflammation) | I ₀ : +30% I ₀ : +50% | Significant interferon inhibitory activity attributed to increased levels of soluble interferon receptors, PGE ₂ levels and interferon inhibitory protein | Ambrus JL, <i>et al.</i> 1997[26] |
| CYP3A4 induction | Cl: +25% Cl: +50% | Induction of CYP3a4 | Maronpot <i>et al.</i> 2009[27] Hewitt <i>et al.</i> 2007[28] |
| Combination of liver dysfunction and general inflammation | Cl: -25% - I ₀ : +30% Cl: -50% - I ₀ : +50% | Lower clearance with general inflammation | Vet, N <i>et al.</i> 2011[29] |
| Once vs. twice daily dosing | None | The same regimen was compared as once vs. twice daily doses | NA |

For each scenario, concentrations at steady state were generated for 50 subjects per dose group, assuming treatment for two weeks using a q.d. regimen. The dose range used for these scenarios was 0, 20, 35, 70, 100, 150, 250 and 400 mg. Samples were collected on the first and last treatment day before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 h after administration.

The simulated scenarios were based on the anticipated clinical relevance (i.e., safety and efficacy), of different levels of COX inhibition. A summary of the simulation algorithm is depicted in Figure 7.1.

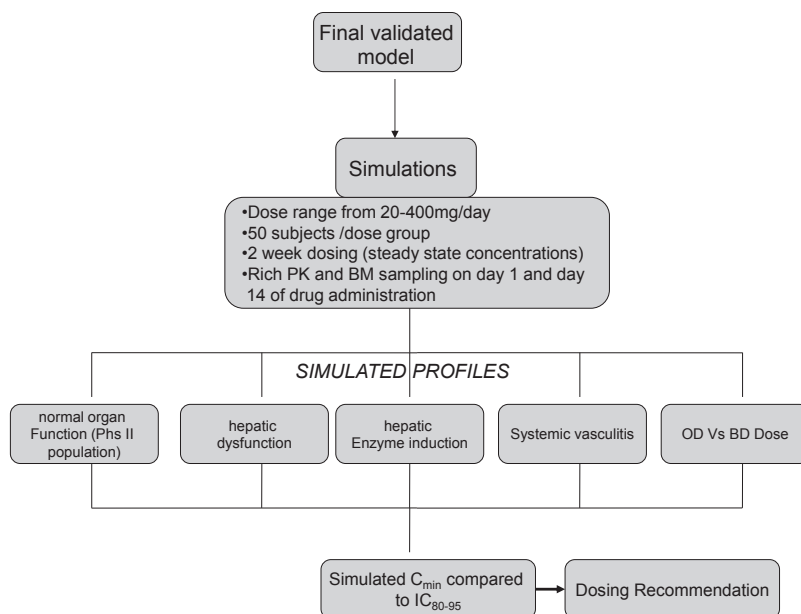


Figure 7.1: Diagram depicting the simulation protocol. Five different clinical scenarios were simulated; each having the characteristics shown in the box above the simulated profiles. The simulated trough (C_{min}) concentrations were compared to the estimated IC_{80-95} to assess deviations from the putative therapeutic window.

The simulations were also used to calculate the putative therapeutic windows at dose level. For each dose, trough concentrations at the steady state (C_{min}) were simulated and compared to the benchmark values, namely IC_{80} (efficacious levels), IC_{90} (maximum desirable response) and IC_{95} (potential safety risk). A ratio of 1 represented optimum desired concentrations while IC_{90}/C_{min} of 2.5 was assumed to be the upper margin of the therapeutic window. At concentrations of around or greater than the IC_{95} , safety events were assumed to be expected. Therefore, effective but non-toxic doses were defined as those at which the C_{min} values at steady state were around the IC_{80} , while the C_{max} was below the IC_{95} . In addition, given that for meaningful analgesic response, i.e., not only the attainment but also the maintenance of pain relief is important, the time span during which drug concentrations remained within the therapeutic window (i.e., between IC_{80} and IC_{95}) was evaluated for twice daily dosing and compared with the standard q.d. regimen.

RESULTS

Pharmacokinetic Analysis

A two-compartment model with first order absorption and elimination best described the PK of GW406381 in adults. Due to high variability in the data, higher concentrations were

found to be slightly under predicted. This discrepancy may be due to the absence of data on influential covariates, which means that not all of the observed variability could be fully characterised. Nevertheless, interindividual variability (IIV) was identified on the peripheral volume (V_3), clearance (CL), absorption rate constant (k_a) and bioavailability (F_1). Residual variability was best described using an exponential error model. The PK parameters from the final model as well as the results of a non-parametric bootstrap are presented in Table 7.2.

Table 7.2: Final pharmacokinetic and pharmacodynamic model parameter estimates and the results of a non-parametric bootstrap (n=2000).

| Model estimates | | | Bootstrap Results | | |
|--------------------|-----------------------|-------|-------------------|--------|---------|
| Parameter | Final Model estimates | CV% | Median | % CI | 95% CI |
| V_2 (L) | 252.38 | 35.06 | 242.93 | 160.39 | 416.84 |
| V_3 (L) | 959.78 | 60.54 | 954.25 | 476.81 | 2115.03 |
| CL (L/h) | 30.21 | 43.97 | 29.89 | 17.86 | 58.61 |
| K_a (h^{-1}) | 15.24 | 78.7 | 10.83 | 3.92 | 35.81 |
| Q (h^{-1}) | 37.28 | 35.82 | 35.6 | 23.83 | 62.44 |
| Lag time (h) | 0.47 | 6.05 | 0.47 | 0.41 | 0.49 |
| F_1 35 | 1.00 | FIXED | FIXED | FIXED | FIXED |
| F_1 70 | 0.49 | 44.2 | 0.48 | 0.25 | 0.93 |
| IIV V_3 | 93% | 73.05 | 98% | 30% | 145% |
| IIV CL | 56% | 76.4 | 53% | 32% | 84% |
| IIV K_a | 198% | 48.84 | 166% | 98% | 258% |
| IIV F_1 | 95% | 50.58 | 91% | 53% | 133% |
| Residual error | 0.11 | 58.99 | 0.1 | 0.05 | 0.23 |

| PD parameters | | | PD Bootstrap Results | | |
|-------------------|----------|--------|----------------------|----------|----------|
| I_0 (pg/ml) | 63196.80 | 9.74 | 63077.60 | 53861.30 | 73871.50 |
| I_{max} (pg/ml) | 479.00 | FIXED | FIXED | FIXED | FIXED |
| IC_{50} (ng/ml) | 43.25 | 12.22 | 43.69 | 36.04 | 53.21 |
| Hill factor | 1.59 | 10.37 | 1.61 | 1.42 | 1.94 |
| IIV I_0 | 44% | 28.63 | 42% | 33% | 54% |
| IIV I_{max} | 272% | 107.38 | 241% | 0% | 343% |
| Residual error | 0.002 | 10.91 | 0.002 | 0.001 | 0.002 |

Where V_2 and V_3 =central and peripheral volumes of distribution respectively, CL=clearance from the central compartment, Q=intercompartmental clearance, F_1 =relative bioavailability

Although the coefficient of variation for some of the parameter estimates was high, all findings were comparable to the bootstrap median results except for K_a , which showed 50% higher variation than the bootstrap median. The observed profiles and model fits are presented in the supplemental material (Figure 7.6), along with diagnostics of individual

and population predictions as well as an individual predicted profile. The high IIV was reflected in the visual predictive check. The medians of the predicted and observed data are very similar, however, the uncertainty around the predictions, is maximum above the 95% quartile, especially in the multiple-dose-phase for the 35 mg dose group (see Figure 7.7).

PKPD analysis

GW406381 did not have any effect on TXB_2 levels. Therefore, only PGE_2 data were analysed. The I_{\max} model was fitted using the PK parameters estimated during the PK analysis. The PKPD model was able to describe the data adequately, as assessed by the basic goodness of fit plots (see right panel Figure 7.6, in the appendix). High variability was seen in the baseline (I_0) PGE_2 , which is most conspicuous in the placebo group. Interindividual variability was modelled exponentially and IIV identified only on two parameters. Not all subjects showed high concentrations due to highly variable exposure, whereas maximum PGE_2 inhibition was observed in only 3 subjects. A summary of the PD parameters from the final model along with the estimates from the nonparametric bootstrap estimates are presented in Table 7.2. From the visual predictive checks, it can be seen that variability is inflated at the upper boundary of the confidence interval (see Figure 7.7 in the appendix).

Simulation scenarios

We have assumed that effective analgesic and anti-inflammatory effects could be achieved and maintained when PGE_2 inhibition are kept above 80%, but below 95%. Based on the predicted potency estimates, a range of doses from 20-400 mg/day was investigated. From a physiological perspective, two parameters were considered to fluctuate in the target population, depending on intrinsic or extrinsic factors, such as differences in metabolism and disease conditions, namely CL and I_0 .

Patients with Normal Organ Function

Patients with normal organ function were used as a reference for the other scenarios. The objective of this scenario was to provide the range of doses which provide clinically relevant target engagement. As can be observed from the concentration vs. time profiles, the median peak concentrations lie below the IC_{95} after a dose of 100 mg daily. Biomarker response increases in a nonlinear manner, with trough concentrations reaching IC_{80} values after 250 mg given as a once daily dose regimen. However, at this dose level peak concentrations are above IC_{95} . Consequently, to remain within the proposed therapeutic range (i.e., $\geq 80\%$ and $< 95\%$ inhibition), the median effective therapeutic dose appears to lie between 70 mg and $< 250\text{mg/day}$. (See Figure 7.2).

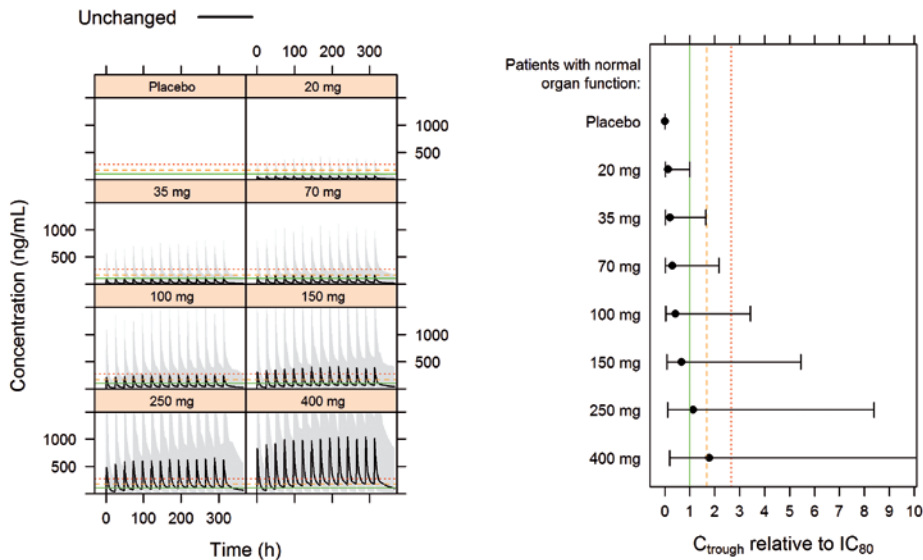


Figure 7.2: Patients with normal organ function. (Left panel) GW406381 concentration vs. time stratified by dose group. Black solid line depicts median concentrations in patients with reference parameter values whilst the shaded area represents the 90% confidence interval (right panel). Forest plots showing drug response associated with different dose levels. X-axis shows the concentration at trough, relative to the IC_{80} of GW406381 stratified by dose group. The solid black circles depict median concentration whilst solid lines represent the 90% confidence interval. Green solid line: IC_{80} . Orange dashed line: IC_{90} . Red dotted line: IC_{95} .

Patients with hepatic impairment

Given the metabolic elimination route (CYP3A4) of GW406381, patients with hepatic impairment are likely to show decreased clearance of the drug. In this scenario, we explored how changes in clearance alter drug exposure and consequently biomarker response. Patients with severe liver impairment needed a longer time to reach steady state (>5 days), especially in the higher dose groups. Furthermore, the trough concentrations were found to be higher than IC_{95} values for the dose groups receiving >250mg. The forest plot (Figure 7.3) reveals that the median trough concentration reached the IC_{80} for the mild, moderate and severe forms of liver impairment at doses of 150, 100 and 35 mg, respectively. Based on these findings, the doses of GW406381 to be used in mild hepatic impairment should be between 100-150mg, whilst for moderate and severe impairment further reductions should be considered (i.e., from 70-100 mg and 20-35 mg respectively). See Figure 7.3.

Metabolic (CYP3A4) induction

The dose required to reach IC_{80} trough concentrations was higher in this scenario, as compared to patients with normal organ function. GW406381 concentrations were overall lower, and steady state concentrations were reached soon after start of the treatment.

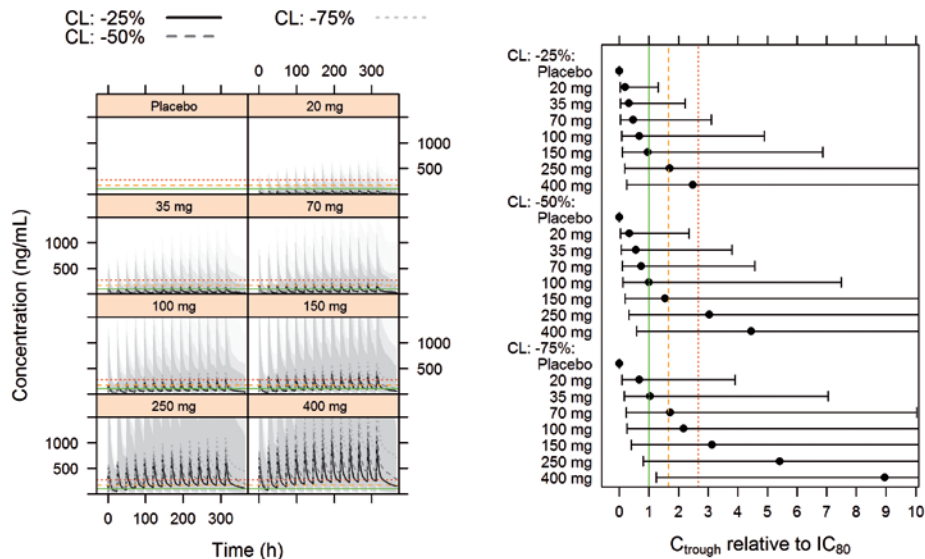


Figure 7.3: Patients with decreased liver function. Panel (a-left) GW406381 concentration vs. time stratified by dose group. Black solid line depict median concentrations in patients with hepatic impairment, whilst the shaded area represents the 90% confidence interval. Panel (b-right) Forest plots showing the different dose levels. X-axis indicates drug concentrations at trough, relative to the IC_{80} of GW406381 stratified by dose group. Percentages reflect the predicted change (%) in hepatic function. Solid black circles: median concentration. Solid lines: 90% confidence interval. Green solid line: IC_{80} . Orange dashed line: IC_{90} . Red dotted line: IC_{95} .

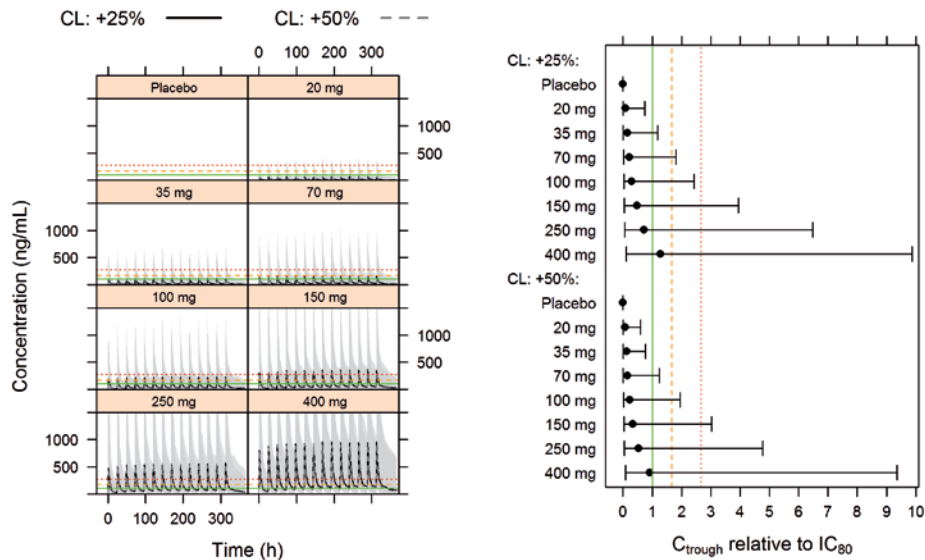


Figure 7.4: Metabolic enzyme (CYP3A4) induction, with 25% CL and 50% increase in CL. Panel (a-Right) Concentration vs. time stratified by dose group. Panel (b-Left) Forest plots showing the different dose levels. Percentages reflect the predicted change (%) in hepatic function.

The difference between 25% and 50% increase in clearance are negligible, as evident from the forest plots. The overall trough concentrations relative to the IC_{80} are lower than what was observed in the reference groups with normal organ function. IC_{80} values are reached at trough levels for doses between 250 and 400 mg when total clearances increased by 25%. Median plasma concentrations of GW406381 were below IC_{80} values throughout the simulated dose range when 50% increase in clearance was considered. See Figure 7.4.

Inflammatory conditions

A scenario was considered in which symptoms worsen as compared to standard inflammatory diseases such as in systemic vasculitis or generalised septicaemia. Interestingly, the dose range required to yield effective exposure in these patients remained the same as in patients with normal organ function. Elevations in baseline PGE_2 did not appear to alter the concentration-effect relationships. Consequently, these findings indicate that no dose adjustment is required in patients showing variable degrees of inflammatory response.

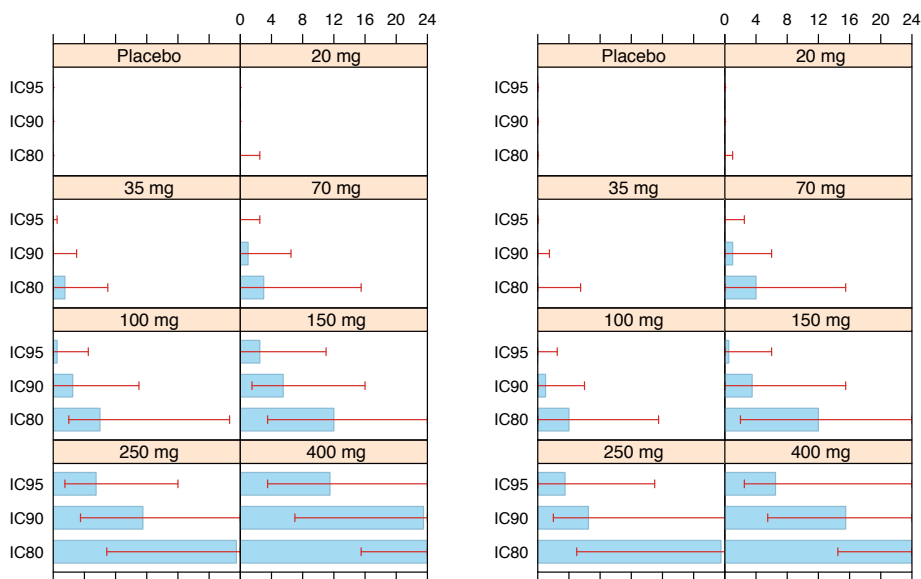


Figure 7.5: Time above the $IC_{80/90/95}$ respectively for once daily (OD, left panel) and twice daily (BID, right panel) stratified by dose group, in patients with normal organ function. Red bars depict the 5 and 95% Confidence intervals respectively.

Once daily (q.d.) vs. twice daily (b.i.d.) dosing regimens

Given the proposed therapeutic range (i.e., $\geq 80\%$ and $< 95\%$ inhibition), it was found that a b.i.d. regimen allowed peak concentrations to remain above the IC_{95} for a shorter time and at much higher dosages, without significant effect on trough concentrations, which were

comparable to those achieved with an q.d. regimen. Furthermore, important differences can be noticed in the total time plasma concentrations remain above IC_{80} , IC_{90} and IC_{95} . Whilst no differences are observed for the ratio between trough concentrations to IC_{80} , the total time above IC_{95} was significantly lower (see Figure 7.5).

DISCUSSION

The rationale for the therapeutic dose range has always been a difficult aspect of drug development. Recommended doses and dosing regimens are often defined early on in development when information on the drug's pharmacology is scarce. As can be deduced from the number of drugs for which the recommended dose has undergone revisions, dose selection remains a point of concern in even in the post marketing phase[30]. The possibility of generating data on a compound's pharmacology represents an opportunity to optimise and personalise treatment during the development programme.

Phase I studies have traditionally been designed with the aim of evaluating the maximum tolerated dose (MTD) in humans [4, 31] . Even though the scope of these studies has expanded in recent years to allow the early evaluation of pharmacodynamics, challenge models in healthy subjects, similar to animal models of pain, reproduce symptoms rather than expressing the pharmacology of the compound[32]. In fact, their translational relevance is questionable. Data from these models have been documented to be non-specific and can at times yield contradictory results [33].

Conceptually, dose selection and optimisation of pain control have been primarily determined by techniques such as titration to effect [13]. In addition, subjective scales have been endorsed as clinical endpoints of choice for the evaluation of analgesia in regulatory guidance documents for neuropathic as well as nociceptive pain[34, 35]. Not surprisingly, there is little evidence in the published literature of clinical trials in which the dose selection for appropriate analgesia has been based on pharmacological activity. Here we have illustrated the concept of biomarker driven dose selection and emphasise the importance of gaining insight into the clinical pharmacological properties of a compound to ensure accurate assessment of safety and efficacy early in the clinical development programme. Specifically, we show how such biomarkers can be used in a quantitative manner to guide the dose selection and identify the conditions requiring dose adjustment. With the help of simulation scenarios, we show how the scope of Phase I studies may be expanded to understand the pharmacology of candidate compounds, taking into account different characteristics of target sub-populations, who would be likely recipients of the drug later in the clinical development programme.

From a methodological perspective, even in circumstances where high variability exists in the data, the use of a model-based approach in conjunction with biologically relevant

model parameterisation allows one to explore the impact of individual differences in pharmacokinetics and pharmacodynamics and quantify the overall consequences (i.e., uncertainty or true interindividual variation) of variability on dose selection for different groups in the target patient population. In our example, the variability in the actual data was attributable firstly to the hepatic metabolism of GW406381, as it is a CYP3A4 substrate [17, 36]. It is well known that CYP3A4 substrates show high IIV in metabolism [37]. Secondly, considerable IIV has been reported in the degree of COX-2 inhibition and selectivity in similar assays of enzymatic activity in healthy subjects [38]. The high variability observed in healthy subjects also exposes a limitation of using *in vitro* potency as a benchmark to compare compounds in early clinical development, which does not reflect differences in selectivity or metabolic activity *in vivo*. In fact, Fries *et al.* showed that despite the higher potency of rofecoxib relative to celecoxib *in vitro*, their *in vivo* selectivity is likely to be the same [38]. Likewise, the *in vitro* potency of GW406381 was estimated to be approximately 30 times as high as rofecoxib [39]. However, the optimal recommended dose range proposed from our simulations lies between 150-250 mg, while that for rofecoxib is 25-50mg [40]. This is mostly explained by the inter-individual differences in pharmacokinetics and enzyme activity described above.

We acknowledge that such an exercise presumes the availability of biomarkers of pharmacology, which may not always be readily measurable in a different disease or therapeutic indication. Yet, there are some general principles of basic pharmacology that can be extended to clinical pharmacology studies, i.e., that target engagement determines therapeutic response and as such needs to be taken into account for the purposes of dose selection [2, 3]. Evidence of clinical efficacy and safety without further characterisation of the underlying pharmacological activity is misleading. Of particular relevance in the case of COX-inhibitors for the treatment of chronic inflammatory pain is the fact that clinical response is reached below maximum target engagement [24, 41]. Similarly, the use of target engagement or target receptor occupancy may be applied to the evaluation of various other drugs (e.g., antibodies, cannabinoids and centrally acting analgesics), subject to the availability of a suitable mechanistic biomarker of response.

In chronic pain, hypersensitivity is the result of downstream effects of COX-2 production which, in turn, is mediated by PGE₂ and TXA₂ [11, 42]. However, pain scales are considered pre-requisites for demonstrating evidence of analgesic and anti-inflammatory response [43, 44]. Various issues arise from such an empirical, fragmented approach to drug development; the most important one being the inability to define the true therapeutic window. By contrast, the use of a biomarker-driven approach provided us insight into the therapeutic window. Under the assumption that drug exposure levels leading to > 95% inhibition in biomarkers (i.e., IC_{95}) is above the therapeutic margin, we could show how the risk of adverse events (AEs) can be mitigated by ensuring drug levels at doses yielding exposure

within IC_{80} - IC_{95} values. This therapeutic window is based on the investigation of Huntjens *et al.* who have shown that analgesic therapeutic plasma concentration is directly correlated with IC_{80} [45]. At COX-2 inhibition >90%, treatment effects suppress the physiological levels of COX-2, which are also present under healthy conditions. In addition, for some drugs, COX-2 selectivity may also be lost, which would then result in adverse events associated with COX-1 inhibition. This subtle balance has been highlighted by Capone *et al.* who have shown that a correlation exists between COX-2 inhibition greater than 90% and elevated risk of cardiovascular events [46]). Clearly, our work illustrates how the pharmacodynamics of this class of compounds can be used as a proxy or predictor of clinical response.

In contrast to traditional non-steroidal anti-inflammatory drugs, selective COX-2 inhibitors do not alter TXB_2 levels, which act as a pro-coagulant [12, 47]. In fact, concerns about the safety of selective COX-2 inhibitors arise from the pharmacological activity on its primary target [48-51]. As can be seen from our simulations, the reported cardiovascular events with this class of compounds is likely to be the result of an inappropriately high dose, the selection of which was not based on pharmacological activity, but rather on the statistical significance of the differences between active and placebo treatment arms [52-55]. McGettigan *et al.* have proposed that there exists gradient of cardiovascular risk for COX-2 inhibitors which runs from protective to risk-inducing, i.e., lower doses are cardio protective, becoming risk-inducing at higher doses [56]. Furthermore, additional evidence points to a time-dependent effect, suggesting that it's the prolonged suppression of COX-2 activity that may ultimately determine adverse cardiovascular outcome [57].

Lastly, we have attempted to show how different dosing regimens affect the therapeutic window in clinical practice. Our analysis reveal that optimal exposure to GW406381 can be achieved by constraining drug concentrations to fluctuate within the range comprised between IC_{80} and the IC_{95} values. Such a requirement can be met by the administration of GW406381 according to a twice daily dosing regimen. This finding can be partly substantiated by the safety profile of celecoxib, which is also prescribed as b.i.d. regimen [58].

Methodological Limitations

Our exercise had some limitations, which for the sake of clarity are worth mentioning. We assumed that PGE_2 inhibition is required not only for the onset but also the maintenance of pain response. The role of secondary, downstream mediators known to contribute to the inflammatory process has been excluded from our analysis [59]. In addition, the data available for this exercise did not include any other intrinsic factor or covariate that might contribute to further changes in response to COX inhibition, such as differences in receptor density or other mediators that might antagonise the effects of COX-2 inhibition. We have also assumed that the disease status and processes do not alter during the time span considered for the simulation scenarios. However, it has been shown that in certain conditions, such as

systemic inflammation (vasculitis or rheumatoid arthritis), other circulating mediators such as cytokines vary over time and may therefore influence pain response over time [60].

Another obvious criticism is the lack of prospective validation of the simulation scenarios and availability of data confirming the suggested dose recommendations. As this is the crux of matter in terms of the concepts implemented here, we refer the reader to a few examples from published literature in which population-based approaches have been used for dose selection and extrapolation purposes [61-63]. The most compelling example is provided by the work of Huntjens *et al.* With the help of PKPD modelling, they have analysed human *in vitro* and *ex vivo* PGE₂ inhibition data and were able to demonstrate that IC_{80} estimates for fenopfen were similar between healthy subjects and patients with systemic lupus erythematosus (SLE)[64]. Subsequently, based on simulations the authors conclude that doses above 600-800 mg/day yield concentrations above IC_{80} for at least 80% of the dosing interval (24hrs). This compares favourably with the recommended total daily analgesic fenopfen dose of 800-1200 mg for the relief of mild to moderate pain in adults [65]. The common denominator in all these examples is that the biological substrate across the populations or experimental groups is the same.

In conclusion, the role of biomarkers expands beyond the potential diagnostic and prognostic value currently perceived by most investigators in industry and academia. In contrast to many of the translational efforts using pre-clinical species [66], biomarkers offer a mechanistic basis for the characterisation of PKPD relationships and as such provide valuable guidance for the dose selection as well as for the design of subsequent studies during drug development. Moreover, this approach contributes to further dismantling of an entrenched belief that still pervades the field of clinical pharmacology, i.e., that the maximum tolerated dose should be evaluated in subsequent efficacy trials, irrespective of any evidence of underlying target engagement.

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Conflicts Of Interest

Oscar Della Pasqua is an employee of GlaxoSmithKline UK. The authors have no other conflicts of interest.

Author Contributions

AT and SPO conducted the research, which was planned and conceptualised by ODP. MD and ODP were involved in the manuscript preparation along with AT and SPO.

APPENDIX

Table 7.3: Demographic and Study Protocol Information on Study Subjects.

| Study Phase | No of Subjects | Subject Demographics | Dose/s Administered | PK sampling Times | PD sampling times |
|---|--------------------------------|--|--|--|---|
| Single Ascending Dose (day 1) | 2 cohorts of 12 subjects each. | A healthy adult male in the age ranges 18-45. Weight range 55-95kg. BMI 19-29kg/m ² | In Cohort 1, subjects were randomized to receive 35mg(n=9) or matching placebo (n=3), while in cohort 2 the subjects received 70mg or placebo. | Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 54 hrs post-dose. | PGF ₂ Pre-dose and 2, 4, 6, 8, 12, 24,48 hrs post-dose |
| DRUG FREE PERIOD UPTO DAY 9 | | | | | |
| Multiple dose (day 9-18) | 1 cohort of 12 subjects | as above | 35mg(n=9) or matching placebo, once daily from day 9-18 | At steady state on day 18 Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 54 hrs post-dose. | At steady state on day 18 PGF ₂ Pre-dose and 2, 4, 6, 8, 12, 24,48 hrs post-dose |
| TXB2 | | | | | |
| Pre-dose, 2, 4, 6, 8, 12, 24,48 hrs post-dose | | | | | |

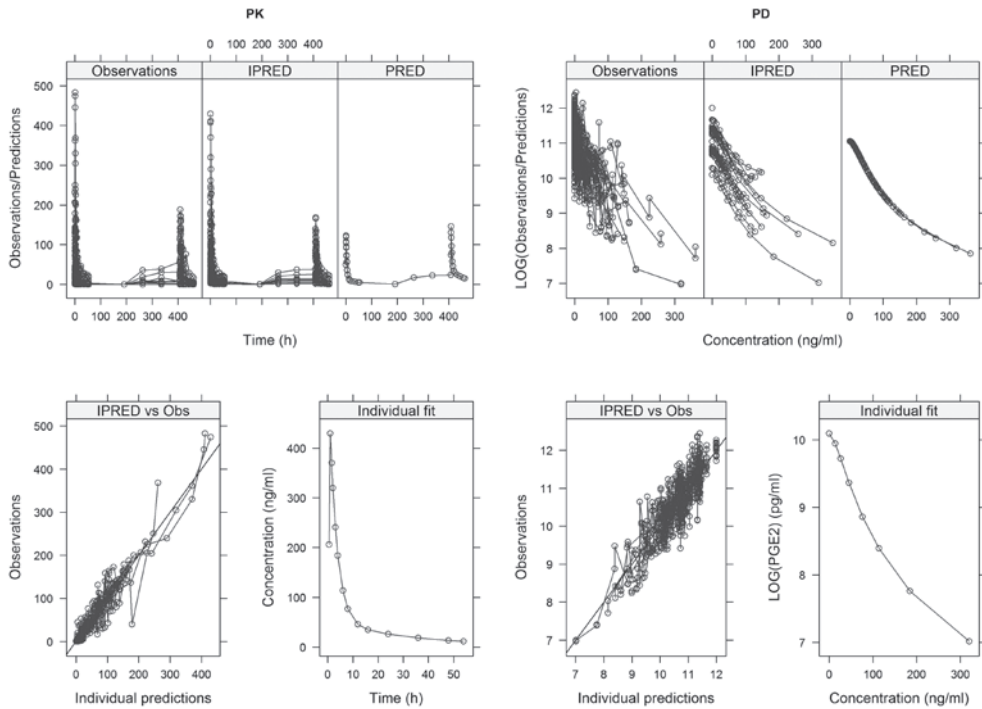


Figure 7.6: Diagnostics (goodness-of-fit plots) for the pharmacokinetic (left panels) and pharmacokinetic-pharmacodynamic (right panels) models. The upper panels show the observed data, the individual and population predictions vs. time (PK panels) or concentration (PD panels). The lower left graph shows the observed data vs. individual predictions, whereas the lower right graph depicts an individual predicted profile.

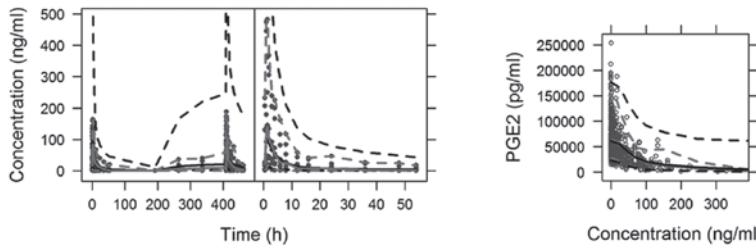


Figure 7.7: Visual Predictive checks of final pharmacokinetic model (left panels) and PKPD model (right panel). The dots represent the actual observations, whereas the lines represent the median (solid line) and the 5th and 95th percentile (dashed line) of the real data. For the VPC on the right, the black lines represent the 5th, 50th and 95th percentile of the simulated data, respectively.

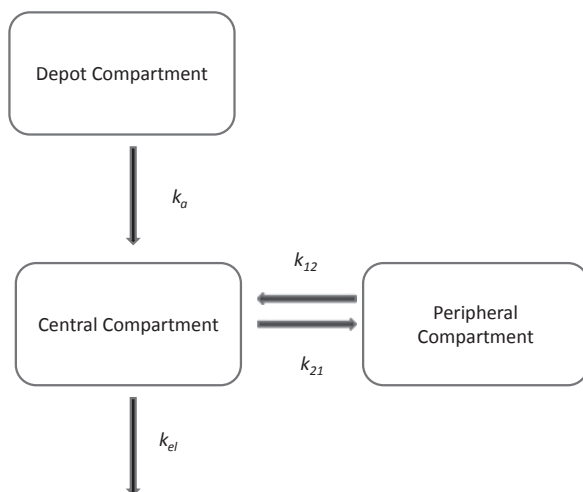


Figure 7.8: Two-compartment pharmacokinetic model with first order absorption used to describe the pharmacokinetics of GW406381 in plasma.

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CHAPTER 8

Pharmacokinetic-pharmacodynamic modelling of the core set of outcome measures (ACRn) in rheumatoid arthritis

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Submitted

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ABSTRACT

Objective: In the current investigation, we characterise the exposure response relationship of GW406381, an investigational selective COX2 inhibitor, in patients with rheumatoid arthritis. Using the change in core set of outcome measures (ACRn) as an index of improvement, we aimed to predict the effective dose range required for a future Phase 3 study.

Methods: Demographic and efficacy data from a Phase 2b study, in which four different treatment arms (0, 10, 35 and 50mg) were tested, were available for the purposes of the analysis. First, a Weibull function was used to describe the natural time course of ACRn in individual patients. Treatment effect was then characterised by an I_{max} model in which the predicted drug exposure as well as biomarker activity are used to assess the underlying exposure-response relationships. In addition, patients were divided into responders and non-responders in order to evaluate the impact of interindividual variability in pharmacodynamics and in pain response. Response was defined as a change $\geq 25\%$ in scores of at the end of the study, as compared to baseline.

Results: Both the placebo and the pharmacokinetic-pharmacodynamic model described the data accurately. The percentage of responders was 54, 70, 59 and 69, across the various dose groups, indicating that there was little discrimination amongst the doses. Despite the wide distribution observed for IC_{50} values, no covariates were identified, other than significant differences between the responder and non-responder phenotypes.

Conclusions: ACRn scores can be accurately described by a Weibull function despite fluctuations in individual response profiles during the course of treatment. Albeit empirical in nature, the proposed model illustrates how further understanding of the underlying exposure-response relationships can be used to explain heterogeneity in response and support the rationale for dose selection in subsequent trials.

INTRODUCTION

Clinical scales are the preferred endpoint for the evaluation of efficacy during the development of analgesic and anti-inflammatory drugs. Doses in Phase 2 and 3 trials are usually selected based on treatment response without taking the underlying pharmacological activity into consideration. This practice is often accompanied by a reluctance to collect information on drug exposure in patients, and consequently reliance on the nominal dose for the analysis of the efficacy [1], which prevents the quantitative characterisation of exposure-response relationships. Although clinical and regulatory guidelines endorse the use of physician and patient reported scores [2, 3], this empirical dose selection raises the following questions: 1) which doses would be selected if direct evidence were available of the underlying pharmacological activity? 2) Is it plausible to expect clinical improvement after maximum pharmacology has been reached and contrarily 3) can clinical response occur in the absence of target engagement?

A number of scales are currently available in clinical practice [4], such as the visual analogue scale (VAS) or numerical rating scale (NRS), which are easy to administer in an out-patient setting. However, they suffer from important drawbacks in that it has been shown that most often than not, no clear link has been identified between pharmacology and clinical response. This is partly due to the fact that different scales differ in sensitivity, and do not always correlate with one another or with other aspects of the disease such as functional disability [4],[5]. Most importantly, they do not provide any clue about how drug interventions alter the underlying pain signalling pathways.

In a previous investigation with a selective COX2 inhibitor GW406381 in healthy subjects, we showed that it is possible to correlate target engagement (i.e., the extent of COX-2 inhibition) with a biomarker of pharmacological activity, namely prostaglandin E_2 (PGE₂) [6]. Additionally, published literature shows that there is a relationship between COX-2 inhibition and therapeutic drug levels associated with pain relief in a range of chronic and acute inflammatory conditions [7]. Hence, we envisage the development of a biomarker-guided approach for analgesic drugs, in which target engagement is used to guide the dose rationale.

Rheumatoid arthritis is a chronic relapsing remitting systemic autoimmune disorder characterised by inflammation and joint cartilage destruction [8]. In clinical trials a number of scales have been used to assess disease activity or measure/evaluate treatment response. Amongst these are the ACR-20, the DAS-28 and the ACRn [9]. The ACRn represents an index of improvement, combining a continuous scale of percentage improvement with traditional categorical response scales such as the ACR-20, ACR-50 or the ACR-70 [10]. It is considered a robust indicator of treatment response since it is a continuous measure of different elements of the disease and can thus detect small effect sizes [10]. Although it has been considered to show construct validity when compared to other scales used in pain research, it is not

symptom or mechanism specific, as exemplified by its use in the evaluation of efficacy for different drug classes [11-13].

Even though the therapeutic dose range of many of the selective COX-inhibitors used for the treatment chronic inflammatory pain in rheumatoid arthritis has been defined according to empirical evidence of pain relief after administration of discrete dose levels in clinical trials, in the current work we attempt to illustrate the concept of biomarker-guided dose selection by evaluating how exposure to GW406381 correlates to clinical response in rheumatoid arthritis, as assessed by the time course of ACRn scores. Despite some limitations imposed by the typical experimental protocol in chronic pain, inferential methods are used to explore and predict the underlying pharmacological effects in patients. This exercise is also aimed to show how target engagement can be used to select the effective dose range in the patient population and ultimately improve the overall benefit-risk balance of a treatment by ensuring that the appropriate level of pharmacological activity is achieved and maintained during the course of therapy. From a conceptual standpoint, our approach represents a shift from the empiricism which has dominated the design of Phase 2b and 3 trials, allowing the implementation of a learn and confirm paradigm [14]. The putative PKPD relationship will ultimately be used as a basis for the selection of the dose in a future Phase 3 trial.

SUBJECTS AND METHODS

Subjects

Study data was obtained from a multicentre, randomised, double-blind placebo and active controlled phase 2b parallel group dose ranging study in patients with rheumatoid arthritis [15]. Information was available from a total of 541 patients who completed at least one trial visit. Patient demographics are presented in Table 8.1

Table 8.1: Demographic overview of the patients enrolment into the trial

| Characteristic | No. of Patients |
|--|-----------------|
| Male patients | 405 |
| Female patients | 136 |
| Mean Age (SD) years | 54.4 (11.80) |
| Race | |
| Native American | 51 |
| White/Caucasian | 408 |
| Black | 67 |
| East/South Asian | 5 |
| South Asian | 1 |
| Japanese | 1 |
| Others | 8 |
| No of centres | 77 |
| Mean (SD) Baseline scores for the core set measures. | 35.28 (11.28) |

Protocol and study procedures were approved by the respective institutional ethics committees of the participating institutions. Included patients gave their written informed consent prior to enrolment into the study.

Study design

For randomisation, subjects had to meet the following disease activity criteria at baseline when compared to their screening visit:

- A minimum of 6 tender/painful joints and an increase of at least 2 tender /painful joints (or a 20% increase whichever was greater).
- A minimum of three swollen joints at baseline and an increase of at least two swollen joints (or 20% increase, whichever was greater).
- A visual analogue scale (VAS) of at least 40 mm at baseline and an increase of 10 mm (or 20% increase, whichever was greater).

All subjects who were randomised received the study drug for a period of 42 days with follow-up visits scheduled on days 7, 14, 28, 42 and 49. The subjects were allowed to continue with their standard DMARD(disease modifying anti-rheumatoid drug) therapy, as well as paracetamol up to a maximum of 300 mg per 24 hour period as rescue analgesic medication. An overview of the enrolment and randomisation scheme is presented in Figure 8.1 (left panel).

Summary of the data available for the PKPD analysis

For the included patients, the number of recorded visits was 2737, representing 84% of the maximum scheduled visits. Stratification of the data by dose group shows that the drop-out rate was unrelated to the dose, with the total number of visits being 80% , 86% , 82% and 88 % in the placebo, 10 mg, 35 mg and in the 50 mg groups respectively.

Outliers

Data from all patients who had at least one visit after the start of the treatment were to be included in the analysis, with exception of two subjects, whose ACRn at the end of the study was >100. One was on placebo the other received 35 mg of the study drug. It is possible that in both cases, ACRn truncation was not performed as per protocol or that these results were data entry errors.

The ACRn

The ACRn is an improvement index that synthesises in a single number the percentage of improvement from baseline that a patient has experienced in analogy to ACR20, ACR50 and ACR70 responses[10, 16]. It is the lowest of the following three disease cores set measures (CSM), indicating the least improvement, 1) the percentage change from baseline in tender

joint counts; 2) the percentage change from baseline in swollen joint counts; and 3) the median percentage change from baseline in the following five assessments:

- The patients' assessment of pain, as measured by a visual analogue scale (VAS)
- The patient's global assessment of disease activity (VAS)
- The physician's global assessment of disease activity (VAS)
- The patient's assessment of physical function, as measured by the health assessment questionnaire
- C reactive protein/acute phase reactants

The percentage change for a disease core set measure (CSM) listed above is calculated as

$$\delta CSM\% = \left(\frac{CSM_{EOS} - CSM_{BAS}}{CSM_{BAS}} \right) * 100 \quad (1)$$

where CSM_{EOS} , CSM_{BAS} represents the CSM score at the end of the study (EOS) and at the baseline (BAS) respectively, in the original scale. The ACRn is simply the value representing the *least improvement* in any one of the core set measures among the individual scales listed above. Since it is a δ or change relative to baseline, the ACRn is by definition only a measure of response and conveys no information about the status of disease activity. Furthermore, the results are dependent on the state and accuracy of baseline measurements. It should also be noted that by aggregating information across a number of measures, it is not possible to discriminate between the changes of the actual measures (scales) making up the response [17] [18].

The ACRn may be presented in such a way that increasing values indicate improvement on a scale from 0 to 100, or in terms of percentage improvement [9]. It may also be presented as in our study where the scores ranged from -100 to +100, with -100 indicating maximum relief and +100 indicating a 100% increase in pain relative to baseline scores.

Withdrawal and handling of drop-outs

In the original statistical analysis plan, data analysis for subjects who dropped out during the study was based on imputation by LOCF (last observation carried forward) to the last planned study visit, i.e. day 42. For the purposes of our PKPD analysis, however, the actual time corresponding to the visit when drop-out occurred was noted together with the corresponding clinical response.

Figure 8.1 shows the number of drop-outs stratified by visit. From this graph, it appears that drop-out was non-informative. Since subjects were missing at random (MAR), we have decided that a drop-out model was not required.

Compliance

In view of the lack of additional information on treatment compliance, adherence to the prescribed dose was assumed to correspond to the number of completed visits. Subjects received 7 days of trial medication at baseline and on day 7, while on days 14 and 28 trial medication was provided for a 2-week period. Therefore, it is assumed that if a subject missed the visit on day 7, he/she could be considered 83% compliant, whilst a subject who missed the visit on day 14 or day 28 would have missed 14 doses, and therefore has a compliance of only 63%.

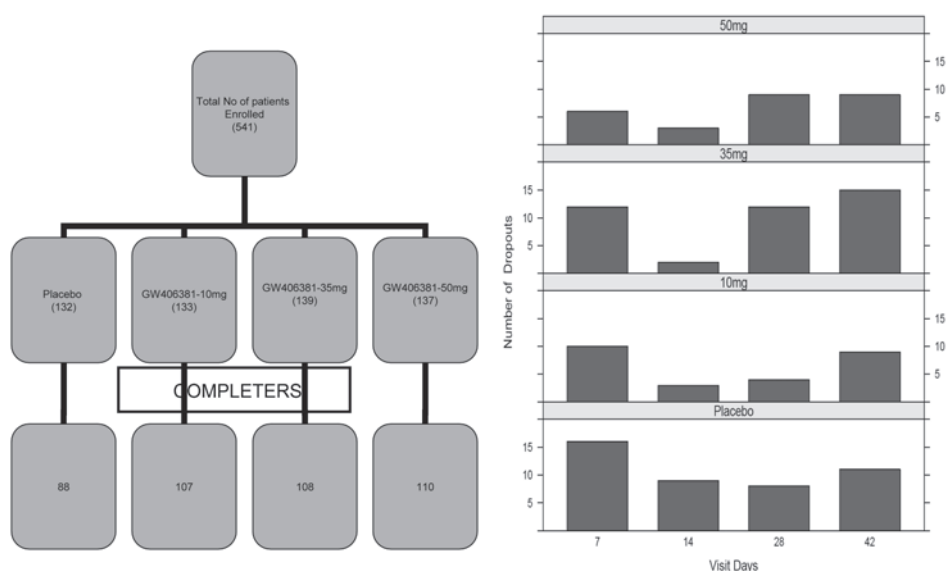


Figure 8.1: A (left panel) Overview of patient recruitment stratified by dose level and treatment completers. A completer was a subject who remained in the trial until completion of his/her last (5th) scheduled post-randomisation visit. B (right panel) Bar diagrams show the frequency of drop-out per dose level at each visit.

Late follow-up

All randomised subjects were on treatment from day 1 to day 42 post randomisation. Thereafter as per protocol, there was one post-treatment follow-up visit, planned on day 49. Of all patients, eleven subjects followed up after day 42 of which 10 on day 49 (visit 6) and one on day 56. All these subjects were analysed with the assumption that none were on rescue medication during the follow-up phase.

Simulation of systemic drug concentrations

Our investigation presupposes that estimates of treatment potency, and consequently of the drug levels associated with efficacy, are more accurate when exposure response

relationships can be derived, as compared to the traditional dose-response curves. However, in the current study, pharmacokinetic data were not collected. Instead, drug concentrations were simulated to subsequently characterise the concentration-response relationship. Mean steady state concentrations were simulated at the time points at which the clinical response was recorded. For this purpose, a population pharmacokinetic model based on healthy volunteer data was used [19]. The pharmacokinetics of GW406381 in adult male subjects was best described by a two-compartment model with first order absorption and elimination. Given the absence of covariate effects on the pharmacokinetics of GW406381 in healthy subjects, it was assumed that rheumatoid arthritis has little or no effect on drug disposition. A summary of the pharmacokinetic model parameters are presented in Table 8.2 in the appendix.

Model building strategy

The time course of the ACRn was plotted along with the corresponding core set measures (CSM) and used as the basis for subsequent model parameterisation. Placebo data was evaluated first to ensure accurate characterisation of the within and between-subject variation in response. The exploratory plots are depicted in Figure 8.2.

Pharmacokinetic-pharmacodynamic analysis

To describe the drug effect, assumptions were made with regard to the disease status of the patients entering the trial [20]. More specifically, it was assumed that the analgesic and anti-inflammatory effects of GW406381 were additive to the natural time course of response, as observed in the placebo group. Given the short duration of the trial disease progression was considered undistinguishable from placebo response and was therefore considered a single entity [21]. In addition, it was assumed that the disease remained stable over the short observation time of six weeks. Therefore the contribution of concomitant medications (particularly DMARDs and prednisolone) to the reduction in pain symptoms could be considered constant throughout the course of treatment. Furthermore, parameter estimation was performed under the assumption that 1) DMARD use was equally prevalent across all dose groups and 2) the effect of rescue medication could be neglected as it caused only transient benefit.

Structural model building was performed in a stepwise manner. First the placebo model was built using the placebo data. Drug effects were then characterised using fixed placebo-related model parameters. Eventually the combined placebo and drug effect was fitted to the total dataset.

The placebo model

Despite some erratic trajectories, the Weibull function was considered appropriate to describe the non-linear decrease in the scores from baseline, which reaches a plateau prior to completion of the treatment [22]. In fact, a steady state placebo effect appears to be reached no later than the third week of the trial. Despite its empirical nature, the use of a Weibull function was deemed one of the best choices to describe the observed profiles. This model can take into account both placebo responders as well as those with a flat (zero) placebo response. It is given by the following expression:

$$Pd_t = Basl * \left(1 - P_{csm} * \left(1 - \exp \left(- \left(\frac{t}{td} \right)^\alpha \right) \right) \right) \quad (2)$$

where Pd_t is the clinical endpoint at time t , $Basl$ =baseline value of the clinical endpoint, P_{csm} is the estimate of the CSM with placebo treatment, td = time to reach the maximum placebo effect, α =shape parameter [23]. From $Basl$ and P_{csm} we calculate a derived parameter, P_{max} or the magnitude of the placebo effect which is the difference between the two given as:

$$P_{max} = Basl - P_{csm}$$

In order to describe the time course of ACRn, modifications to the basic equation were required to constrain ACRn to be 0 at baseline, as per clinical definition. Therefore, instead of $Basl$ we use an offset term (OFF), which was parameterised as follows:

$$OFF = ACRn_{t0} + Bas_Sc \quad (3)$$

where $ACRn_{t0}$ =ACRn at time=0, Bas_Sc = core set measures at baseline (time=0).

This modification was necessary because the use of a Weibull function implies a finite non-zero parameter representing baseline conditions at time=0. The proposed modification does not however influence the model predicted time course of ACRn. Substituting OFF in equation 2, one gets:

$$Pl_t = OFF * \left(1 - P_{csm} * \left(1 - \exp \left(- \left(\frac{t}{td} \right)^\alpha \right) \right) \right) \quad (4)$$

During model building we also considered the inclusion of an additional term to equation 4 to describe worsening of the disease after initial improvement. Although the Weibull function seemed a good descriptor of a stable placebo response, it does not allow for late fluctuations in the individual trajectories [22]. Gomeni *et al.* have previously proposed an additive term (Rc) describing disease worsening, or return to baseline disease conditions [24] :

$$Pd_t = Basl * \left(1 - P_{csm} * (1 - \exp^{-(\frac{t}{td})^\alpha}) \right) \quad (5)$$

This modified version of the model was tested for subjects who had late follow-up, at visits 6 or 7.

Drug Effect Model

The effects of GW406381 on the ACRn were best described using a standard I_{max} model, as shown in the following expression:

$$I_{cox} = \frac{I_{max} * Conc}{IC_{50} + Conc} \quad (6)$$

where I_{max} represents the maximum inhibitory effect, IC_{50} is the drug potency and $Conc$ the systemic drug concentrations. The net effect on the time course of the ACRn at time t , was proportional to the placebo effect, and given by the following expression:

$$ACRn_t = OFF * \left(1 - P_{csm} * (1 - \exp^{-(\frac{t}{td})^\alpha}) \right) * (1 - I_{cox}) \quad (7)$$

Dichotomisation into Responders and Non-responders

To explore treatment and patient characteristics underlying clinical response across the different dose groups, subjects were split into responders (R) and non-responders (NR). A trial subject was categorised as a responder if he/she had decrease in ACRn > 25% relative to baseline at the last study visit (i.e., completion of treatment). A comparison was then made between model-predicted and observed median response profiles.

This approach was deemed necessary to further investigate a putative correlation between pharmacological activity and clinical response. An assumption is made with regard to the clinical response itself, in that response implies disease activity susceptible to COX-2 inhibition, whereas a non-responder presupposes not only limited drug action (e.g., low dose), but also refractoriness to COX-2 inhibition mechanisms. Given that these definitions are irrespective of the treatment allocation, the dichotomisation into responders and non-responders should yield different estimates of drug potency for each population. Therefore separate, model fitting was performed for either sub-population. Another important assumption here was that there was no misclassification for a drop-out. Thus, a subject who had dropped out earlier in the study would have continued to exhibit the same response he/she did at the time of dropping out.

Stochastic model

Inter-individual variability (IIV) in model parameters was assumed to be log normally distributed. The square root of the variance is reported for IIV, as this is an approximation to the apparent coefficient of variation of a normal distribution on log-scale. A parameter value of an individual i (post hoc value) is given by the following expression:

$$\Theta_i = \Theta_{TV} * \exp^{\eta_i} \quad (8)$$

where Θ_{TV} is the population value of the parameter, η is a random variable with zero mean and a variance ω^2 .

The residual variability was described by an additive error model. It was assumed to comprise measurement error and any model misspecification:

$$Y_{ij} = F_{ij} + \epsilon_{ij} \quad (9)$$

Where Y is the j th observed response in the i th individual. F is the predicted response and ϵ is a random variable with zero mean and variance σ^2 .

Covariates

To explore the potential influence of demographic covariates on response, post-hoc estimates were plotted against the respective covariate vectors. In case a linear covariate parameter relationship was identifiable, non-linear relationships were further tested. Covariate factors included race, sex and age.

Model evaluation and validation procedures

The pharmacokinetic-pharmacodynamic analysis was performed using nonlinear mixed-effect modelling in which the typical values of the parameters as well as their inter-individual variances were estimated. Parameter inclusion and model selection were based on the likelihood ratio test, parameter point estimates and their respective 95% confidence intervals (CI) as well as on the visual inspection of goodness of fit plots. For the likelihood ratio tests, the significance level was set at $p < 0.01$, which corresponds to a decrease of 6.63 points in the minimum value of the objective function (MVOF) after the inclusion of one parameter under the assumption that the difference in MVOF between two nested models is χ^2 distributed. Minimisation was considered successful when runs were completed with a positive covariance step and no associated error messages.

The precision of model parameters was assessed using a non-parametric bootstrap. Two thousand bootstrap samples were generated in PsN 3.5.3 [25]. The model was fitted to the replicates and a distribution of the parameter estimates hereby obtained. The relative standard error (RSE) of the bootstrap estimates was then calculated. The bootstrapped parameter estimates were then compared to the final model parameter estimates. Secondly, visual predictive checks were used to visually inspect the concordance between

simulated data and real observations. Using the final model parameters, 2000 datasets were simulated and the simulated data overlaid on the real observations. In addition, mirror plots were generated to assess the predictive performance of a model when used for simulation purposes. Mirror plots depict fitting profiles using simulated data, which provide information about the variance-covariance structure of the model [26]. These plots are based on the principle that the selected model should accurately describe the variance pattern observed in the original data.

Separate validation procedures were performed for the analysis of responders and non-responders, which included visual predictive checks (n=1000) and NPDE (normalised prediction distribution errors), which are based on the assumption that the normalised (decorrelated) prediction distribution errors (discrepancies) are normally distributed [27]. One hundred datasets were simulated using the final model, which was then tested for the assumption of normality of the prediction distribution errors.

Software

All analyses were performed using non-linear mixed effects modelling, as implemented in the NONMEM version 7.2 (Icon, Eliott City, Maryland USA). The first order conditional estimation method (FOCE) with INTERACTION was used to estimate all parameters. PsN 3.5.3 was used to run NONMEM. Data manipulation, graphical and statistical summaries were performed in R 2.13 [28].

RESULTS

Exploratory analysis

The population used for modelling of the exposure-response relationship comprised 541 patients who received placebo (n=132) or active treatment, namely 10 mg (n=133), 35 mg (n=139) or 50 mg (n=137) mg doses of GW406381. Visual inspection of the data showed an exponential decrease in the ACRn score, indicating pain relief over the first few weeks of therapy, which tended to plateau, typically after the third week of the trial. Exploratory plots of the time course of the observed ACRn are shown in Figure 8.2, stratified by dose level for responders and non-responders. For the sake of clarity, the time course of the corresponding core set measures is also presented for the overall population in the same figure. The trajectory of the core set measures provides insight into the individual response pattern and its variability. As shown in Figure 8.6 (appendix), the distribution of baseline scores was comparable across dose levels, suggesting no trends due to different disease status at the start of treatment.

Simulation of systemic drug concentrations

As pharmacokinetic samples were not collected during the trial, mean concentrations of GW406381 in plasma were simulated for each patient, under the assumption of random interindividual variation around the population mean.

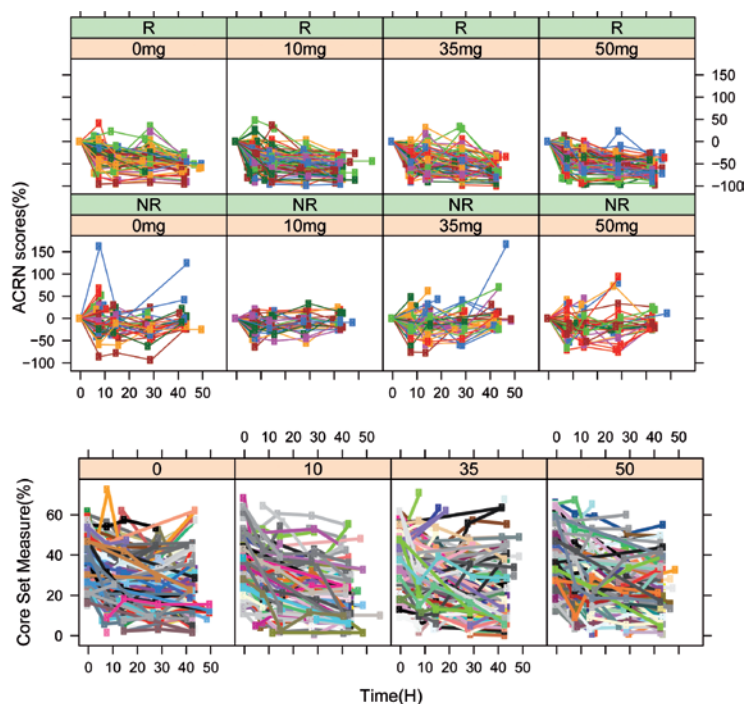


Figure 8.2: (Upper panel) Time course of the observed ACRn stratified by dose level for responders and non-responders. (Lower panel) Time course of the core set measures (CSM) for the overall population.

Table 8.2: Final parameter estimates for the Weibull model used to describe the exposure response relationship for GW406381, including the results of a non-parametric bootstrap (n=2000).

| Exposure-response model | | Bootstrap Results | | | |
|-------------------------|-------|-------------------|--------|-------|--------|
| | | CV% | Median | %5 CI | 95% CI |
| Bas_Sc (%) | 36.5 | 1.5 | 36.50 | 34.31 | 38.44 |
| P_{csm} (%) | 2.05 | 9.04 | 2.024 | 1.760 | 3.75 |
| K_{on}^* | 0.386 | 25.40 | 0.306 | 0.413 | 0.423 |
| α | 0.351 | 40 | 0.32 | 0.380 | 0.79 |
| I_{max} (%) | -100 | FIXED | FIXED | FIXED | FIXED |
| IC_{50} (ng/ml) | 7780 | 38.20 | 113 | 7848 | 16763 |
| IIV P_{csm} (CV%) | 38.3 | 5.80 | 30.65 | 38.21 | 46.71 |
| Residual Error(%) | 17.46 | 32 | 16.80 | 17.35 | 18 |

where P_{csm} is the estimate of the CSM after placebo treatment, * This is the reciprocal of td , td = time to reach the maximum placebo effect, α =shape parameter

Exposure-response (PKPD) modelling

As described in the methods section, the baseline CSM was estimated along with the ACRn scores. The exposure-response population for model development comprised 2737 observations from 541 patients. A Weibull function was found to best describe the time course of ACRn scores in placebo treated patients and was subsequently used as the basis for characterising drug effects. Despite some individuals showing symptomatic worsening at the end of treatment, the inclusion of an additive term to the Weibull function did not result in further improvement of the fitting. The estimated placebo response for the core set measures in a typical patient was 2.05 (9.0) % on the normalised CSM scale. This corresponds to a maximum improvement due to placebo response (P_{max}) of 34.4 %.

Drug effects could be described adequately by the I_{max} model, as assessed by the basic goodness-of-fit plots (see Figure 8.7 appendix). No unexpected trends could be determined in the individual predictions (Figure 8.8, appendix). Given that maximum pain relief was not achieved in the study, a linear model was found to equally describe the data. However, the I_{max} model was selected to ensure a clear pharmacological interpretation of the drug effects. In addition, from a pharmacological perspective, the maximum expected relief observed for this class of drugs can be anticipated. I_{max} was therefore fixed to -100, which is the maximum decrease relative to baseline scores. Final model parameter estimates are shown in Table 8.2. Despite the large variation in ACRn, inter-individual variability (IIV) was found to be identifiable only on P_{csm} , indicating clear differences in the time course of the core set measures ($p < 0.001$) between subjects. The remaining residual variability could be modelled accurately using an additive error term. Additional efforts to identify covariates did not reveal any trends or correlations with demographic or disease covariates that could explain variability in the data.

Dichotomisation by response level

In view of the role of placebo response as well as the known interindividual differences in susceptibility to pain relief, patients were further split into responders and non-responders according to the extent of changes in ACRn scores at completion of treatment (Figure 8.3). The number of observed responders per dose level was 54, 70, 59 and 69.3% in the placebo, 10, 35 and 50 mg treatment groups, respectively.

Overall the model was able to predict median trend in the observed data for both responders and non-responders, except for the late study upswing, as shown in the lower panels of Figure 8.3. These fluctuations in ACRn can be explained by the late follow-up, i.e., these patients were off-trial medication at the time of their last visit. In addition, it should be noted that response level observed here differs from the estimated maximum placebo response because of the clinical definition of response used for dichotomisation of the population into responders and non-responder.

Dichotomisation of the population into responders and non-responders has not only shown evidence of different susceptibility to the treatment, but also provided estimates of apparent IC_{50} values for each group, with parameter values suggesting that the fitting of the entire dataset is more likely to represent drug potency in the so-called non-responder population. In fact, the distribution of IC_{50} values obtained by bootstrapping seems to reflect the sensitivity of the two populations to the drug effects (Figure 8.9 appendix). Thus, the IC_{50} (CV%) value estimated for responders was 2,853 (19.14%) ng/ml. This was obtained after fixing the IC_{50} for non-responders to the value obtained for the entire population i.e. 7774 (38.09%). Conversely, the data did not support estimation of a non-responder IC_{50} with bizarre estimates being produced. We also tried to estimate separate IC_{50s} for the two phenotypes, without success.

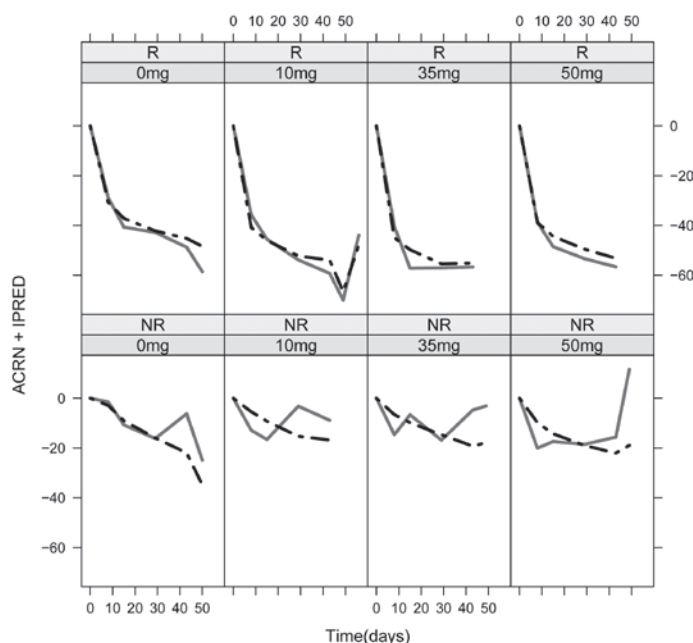


Figure 8.3: Median time course of observed (solid) and predicted (dashed) ACRn score in responders (R) and non-responders (NR) stratified by dose level.

Model validation

The visual predictive checks suggest that the Weibull function can be used to adequately describe the time course of the pain response (see Figure 8.4), except for the fluctuation observed in the last 2 visits, a limitation which is reflected in the broader prediction intervals at those time points. Despite these discrepancies, it should be noted that less than 10% of the observations lie beyond the prediction intervals, indicating good agreement between the

observed data and model predicted response. Further stratification of the population into responders and non-responders (Figure 8.5) reveals that the model predicts the time course of ACRn in responders better than in non-responders. In addition, the bootstrap procedures for the overall population show parameter values comparable to the parameter estimates obtained with the final model. Estimates also showed a low coefficient of variation, with the exception of the exponent (α) and IC_{50} . As indicated previously, this apparent imprecision is more likely to be a reflection of the differing susceptibility of the two populations to the treatment response. The distribution of IC_{50} values and the corresponding standard errors from 1000 bootstrap runs is depicted in Figure 8.9 (appendix).

In addition to the numeric and visual predictive checks, mirror plots were obtained to demonstrate model performance in subsequent simulations. These plots reveal no trends in the individual profiles, indicating accurate characterisation of the variance-covariance structure. (Figure 8.10, appendix). Yet, some of the predicted ACRn scores reach values below -100, which is probably explained by the use of an additive random error. Lastly, model performance in subsequent simulations was evaluated by NPDEs, which clearly show that apparent potency estimates are required to best describe the population of responders and non-responders (Figure 8.11, appendix).

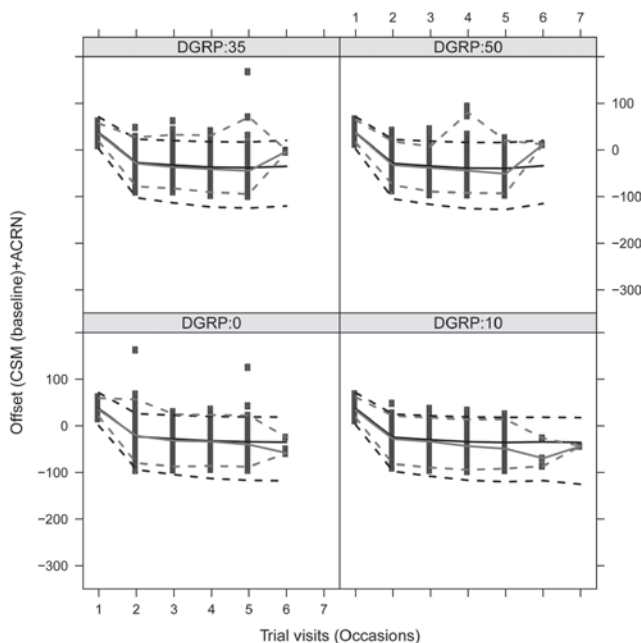


Figure 8.4: Visual Predictive Check by Dose for the Final response model. Red lines represent the median, 5 and 95% confidence intervals for the real data and the black lines for the predicted data. The open circles are the observations.

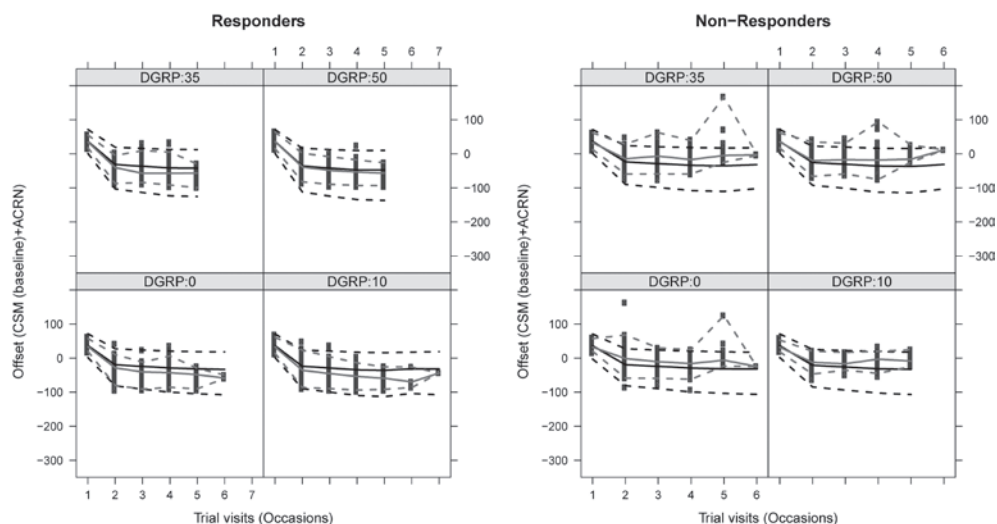


Figure 8.5: Visual predictive check for responders (left panels) and non-responders (right panels).

DISCUSSION

To our knowledge, this is the first time that nonlinear hierarchical modelling has been used to analyse clinical response to a selective COX-2 inhibitor in rheumatoid arthritis. Similar concepts have been used previously for the evaluation of the effects of gabapentin in patients with neuropathic pain. In that instance, a Weibull function was also deemed suitable to describe the time course of pain [29]. Despite the empirical nature of the Weibull function, our analysis shows how modelling can be used to describe drug effects on core set measures, as assessed by the changes in ACRn. We also emphasise that whilst other modelling efforts in pain research have relied on individual clinical scales, not much work has been done on composite measures such as ACRn [12]. The ACRn captures both worsening as well as improvement of the symptoms, making it a sensitive measure of treatment response in rheumatoid arthritis [30], [31]. Such characteristics support our modelling strategy, in that lack of response may be reflective of differences in individual susceptibility to the mechanism of action, i.e., COX-inhibition, as well as of insufficient exposure to the drug. Stratification of the population into responders and non-responders allowed us therefore to identify differences in drug-specific parameters (i.e., apparent potency), whilst taking into account the putative differences in drug exposure.

From a modelling perspective, another important point to consider was the nature of the pharmacological activity and choice for the descriptor of drug exposure. First, it should be highlighted that a direct response model was considered the most appropriate choice to describe improvement in core set measures. This choice was based on the fact that selective

COX-2 inhibition is immediate and the overall treatment duration was relatively short in comparison to the rate of disease progression in rheumatoid arthritis. This contrasts with the use of indirect response models, which have been considered the preferred approach for modelling the effects of DMARDS in rheumatoid arthritis [13],[16]. We have also decided on instantaneous drug concentrations, i.e., $C(t)$ instead of other summary measures such as, C_{min} or AUC for similar reasons. Although parsimony rules ought to be taken into account when defining model parameterisation, drug effects were described according to an I_{max} model, instead of relying on a linear function, which was also able to describe the data accordingly. This choice was essential to ensure appropriate interpretation of the drug-specific effects and subsequent differentiation between individual susceptibility and the potential impact of pharmacokinetic variability.

Despite high *in vitro* potency and selectivity for COX-2, model fitting of the total data set yielded apparently conflicting results, as suggested by the IC_{50} estimates obtained here. [32]. These findings were further corroborated by the wide distributions for potency estimates observed in the bootstrap analysis. Clearly, this was not a statistical issue, i.e., imprecision in parameter estimation or identifiability. Rather it provides evidence for two underlying groups or phenotypes amongst the trial patients with differing sensitivities to COX-2 effects. Such heterogeneity in drug potency is not implausible given the potential differences in disease state, which are not controlled for by the inclusion/exclusion criteria used in typical experimental protocol [12, 13]. We acknowledge however, that such issues have not been previously evaluated using exposure-response modelling and further investigation will be required to confirm these findings. Nevertheless, wide inter-individual variation in pain response has been attributed to differences in the underlying disease status and molecular mechanisms, including as well variable gene expression [33]. Interestingly, we were not able to identify the two populations using purely statistical criteria, as implemented in a mixture model. In addition, we could not demonstrate whether these two populations of responders and non-responders correlated with placebo response or any influential covariates [34]. The effect of demographic factors such as age, sex, ethnicity was not statistically significant. However, besides inflammation-induced gene expression there are multiple factors (covariates) such as gender, and which are determinants of response [35].

Lastly, we point out that our approach has also circumvented the unidentifiability of inter-individual variability in model parameters, which in turn prevented a more robust covariate analysis. Inter-individual variability was identifiable only on the placebo-related parameter. However, as shown in Figure 8.2, it may be appreciated that there is considerable variability in the data, which might be explained by parameters such as P_{csm} .

Whereas the evidence of two distinct distributions in the NPDEs strongly suggests that two populations or phenotypes exist, our findings suggest that other biological or clinical factors need to be identified and captured during a clinical trial for the development of mechanistic

models. However, from a drug development perspective, we should also highlight that the doses used in the trial were sub-optimal, as indicated by our previous findings in which the doses required for PGE₂ inhibition > 80% across the overall population should be at least 150 mg [19]. These doses were derived taking into account pharmacokinetic variability in CYP3A4 as well as biological variability in COX-2 activity in an ex-vivo assay. In the current study, the difference in the proportion of responders relative to placebo treatment was approximately 15%.

Limitations

We acknowledge that a mechanism-based approach would have been preferable to accurately characterise the correlation between drug exposure and clinical response. Unfortunately, pharmacokinetic data and biomarker response have not been collected in this trial. Undoubtedly, availability of individual plasma concentrations and biomarker response (e.g., PGE₂ and TXB₂ levels) would have allowed for a more comprehensive evaluation of the pharmacokinetic-pharmacodynamic relationships. Despite these limitations our approach shows that modelling of exposure-response is by far more informative than hypothesis testing based on a pair-wise comparison between active and placebo treatment arms.

From a methodological point of view, we reiterate that the use of a Weibull function imposes limitations on the trajectory of individual pain responses. In fact, the inclusion of all data in the analysis, including late follow-up visits reveal clear fluctuations in ACRn after completion of the treatment, which may be explained by the fact that patients are not taking the study medication. The use of all the data available also imposes assumptions about adherence to treatment, which may not have been 100%, in particular during the follow up period.

Finally, we emphasise that even though the use of clinical phenotypes (i.e., responder vs. non-responder) as classifiers for differentiating subjects in a population may seem arbitrary at first sight, a similar approach has been previously used by Dervieux and Kooloos *et al.* as basis for the characterisation of patterns of interaction between genetic and non-genetic attributes and methotrexate efficacy in rheumatoid arthritis [36, 37]. These factors are incorporated into a model in which response and toxicity to methotrexate can be predicted. Most important, it was the clinical evidence of toxicity or non-response in some patients that triggered their research strategy. We hope that similar efforts may be undertaken to further explain response to selective COX-2 inhibitors and other non-steroidal anti-inflammatory drugs.

In summary, we have shown that modelling concepts can be used to characterise the exposure-response relationship of a selective COX-2 inhibitor. Our efforts also unravel an intrinsic refractoriness to treatment which reflects disease heterogeneity, irrespective of the exposure levels.

APPENDIX

Table 8-3: Final parameter estimates for the pharmacokinetic model used to simulate GW406381 plasma concentrations in patients with rheumatoid arthritis, including the results of a non-parametric bootstrap (n=2000).

| Pharmacokinetic model | |
|-----------------------|---------------------------|
| Parameter | Final parameter estimates |
| V_2 (L) | 252.38 |
| V_3 (L) | 959.78 |
| CL (L/h) | 30.21 |
| K_a (h^{-1}) | 15.24 |
| Q (h^{-1}) | 37.28 |
| Lag time (h) | 0.47 |
| F_1 35 | 1.00 |
| F_1 70 | 0.49 |
| IIV V_3 | 93% |
| IIV CL | 56% |
| IIV K_a | 198% |
| IIV F_1 | 95% |
| Residual error | 0.11 |

Where V_2 and V_3 =central and peripheral volumes of distribution respectively, CL=clearance from the central compartment, Q=intercompartmental clearance, F_1 =relative bioavailability

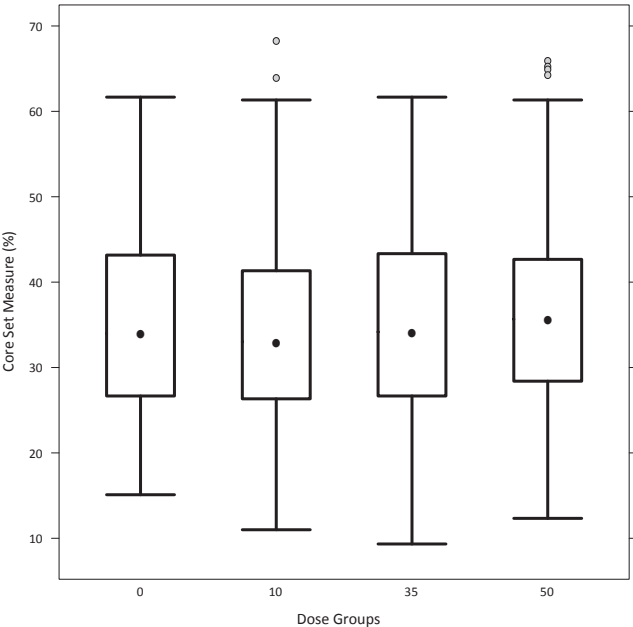


Figure 8.6: Distribution of core set measures stratified by dose at baseline.

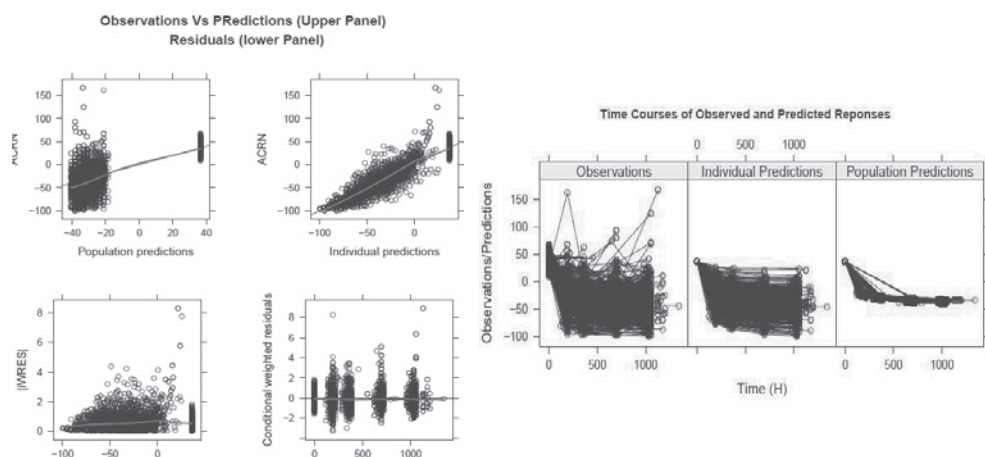


Figure 8.7: (left 4 panels) Goodness-of-fit plots for the final exposure-response model. Left upper 2 panels show the observed vs. predicted offset (CSM (bas) +ACRN) for the population and for individual patients. Weighted residuals vs. individual predictions and time indicate random distribution of the discrepancies between observed and predicted scores (left lower panels). (Right 3 panels) Time course of the observed, individual and population predicted responses. IWRES=individual weighted residuals, CWRES=conditioned weighted residuals.

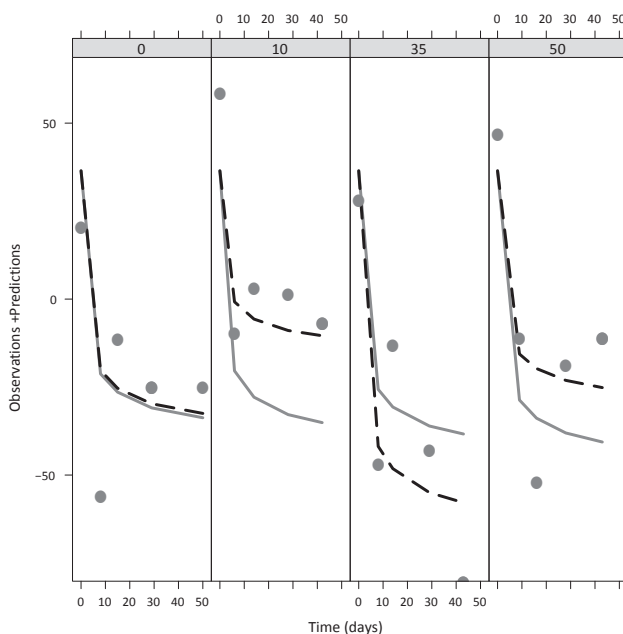


Figure 8.8: Example of the individual and predicted offset (CSM (bas) +ACRN) vs. time stratified by dose level. The dashed line represents individual model predictions, whereas the solid line depicts the mean population prediction. Dots are the individual observations from the study.

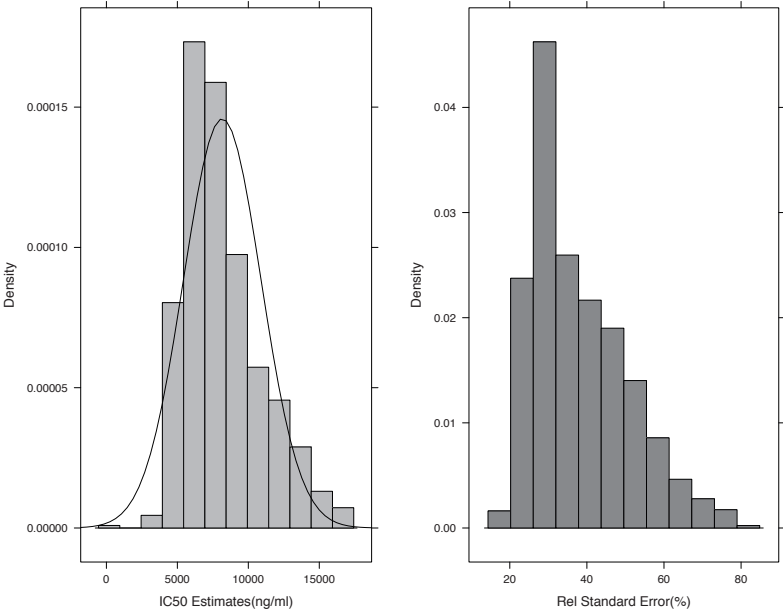


Figure 8.9: Distribution of estimated IC_{50} values obtained by bootstrapping (left panel) and the corresponding relative standard error (right panel) for the whole population. $N=2000$.

Time Course of ACRn Observed & Simulated Data

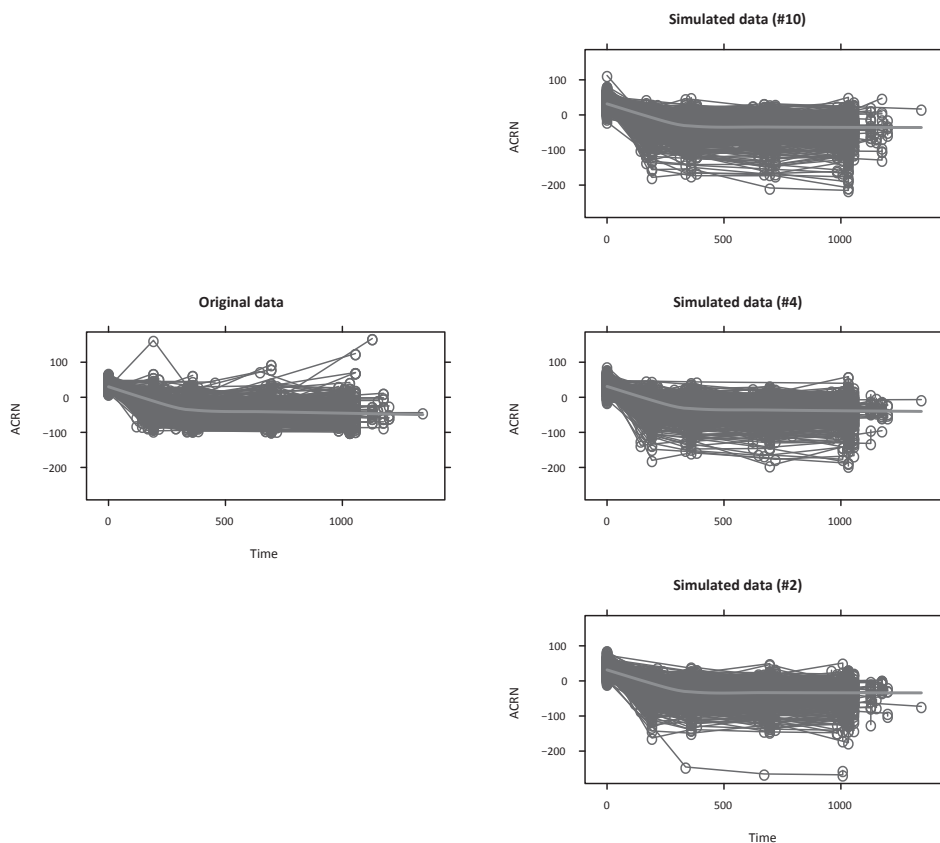


Figure 8.10: Mirror plots showing the time course of the offset, with the original data in the left panel and simulated on the 3 vertical panels on the right. The offset was used to constrain initial positive values at baseline, given that, by definition, ACRn is 0 at $t=0$. See equation 2 for details.

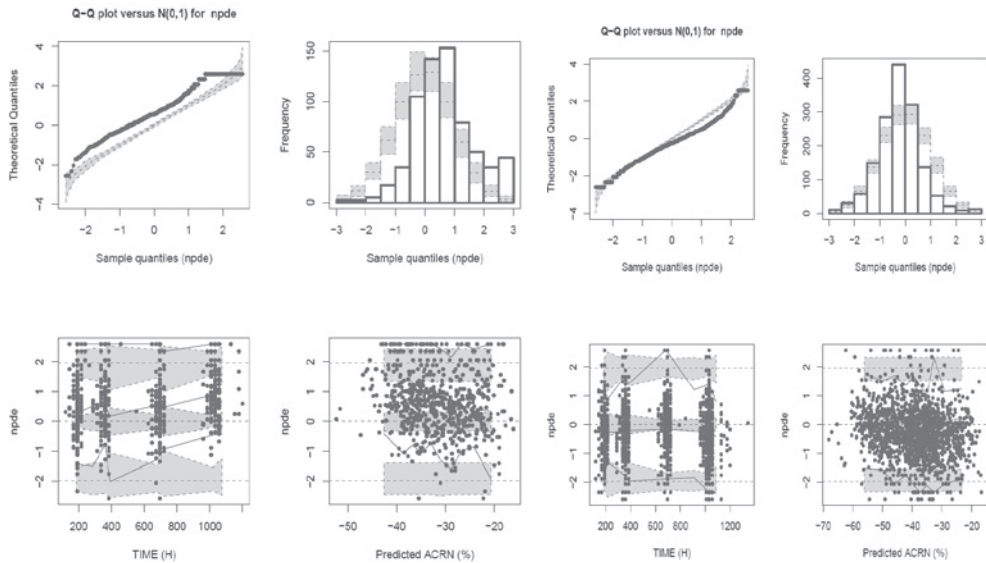


Figure 8.11: NPDEs for non-responders (left panels) and responders (right panels). The blue shaded areas are the prediction intervals of the upper and lower confidence (5 and 95%) intervals of the simulated errors; the pink shaded area is the prediction interval of the median. The solid lines represent the medians and the 5 and 95% CIs of the prediction errors respectively (lower panels). The blue shaded areas in the histograms and the QQ plots show the upper and lower confidence (5 and 95%) intervals of the simulated errors.

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CHAPTER 9

Exploratory analysis of the correlation between pain relief and target engagement in rheumatoid arthritis

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ABSTRACT

Objective: Pain relief in clinical trials in rheumatoid arthritis and other chronic inflammatory pain conditions is assessed primarily by clinical measures without taking into account the underlying pharmacodynamic response. In this investigation we illustrate how a mechanism-based approach can be used to characterise the relationship between drug exposure, prostaglandin inhibition and symptoms relief following treatment with a COX-2 inhibitor. Such an approach may provide better insight into the causes of variability in treatment response in phase 3 trials.

Methods: Using nonlinear hierarchical models previously developed in which drug exposure, prostaglandin (PGE_2) inhibition and pain response, as assessed by the ACRn scores were characterised, we simulate pain response profiles across a wide dose range for GW406381. A typical protocol was considered in which treatment is allocated according to parallel, placebo-controlled design. A spline was plotted through the simulated data to assess the correlations between ACRn and percentage PGE_2 in both responders and non-responders, as defined by ACRn changes from baseline. This was done for both the median as well as the total data.

Results: The proportion of responders increased nonlinearly across the range of doses tested. Our simulations show that target inhibition ($>80\%$) may be expected at doses higher than 250 mg, whilst a median ACRn drop of $>50\%$ occurs at a dose >100 mg GW406381. A putative relationship between PGE_2 inhibition and ACRn could be identified, which can be used to partly explain variability in response.

Conclusions Our analysis shows that the availability of exposure and biomarker data can provide a stronger basis for the dose rationale, enabling identification of patient subgroups in a trial population, for whom dose adjustments may be required. Whilst biomarkers of pharmacological activity may not always be available, mapping of clinical response to pharmacodynamics yields evidence of target engagement during the course of treatment. Unravelling such correlations can have important implications for further understanding of long term safety and efficacy.

INTRODUCTION

In an earlier study in patients with stable rheumatoid arthritis, we have described the exposure-response relationships of GW406381, an investigational COX-2 inhibitor [38]. By contrast, the dose rationale for the treatment of chronic inflammatory conditions has been traditionally driven by empirical evidence of symptom relief. In fact, doses in Phase 2 and 3 trials are selected without taking the underlying pharmacological activity of the compound into consideration. Such a practice is endorsed by clinical and regulatory guidelines and often accompanied by a reluctance to collect information on drug exposure in patients [1], [2, 3].

Undoubtedly, this paradigm needs to be revisited if the long term safety and efficacy are contemplated. The very nonlinear nature of exposure-response relationships is at the heart of the problem. Doses that are efficacious and apparently safe following a relatively short course of therapy may turn out to be unsafe when considered over a longer period of time. The rationale for dose selection is best demonstrated when evidence is generated of the appropriate level of target engagement together with the corresponding levels of pharmacological activity at the proposed therapeutic doses. A recent example of this concept has been shown by Rohatagi *et al.*, who have evaluated the predictive value of pharmacokinetic-pharmacodynamic modelling using data from Caucasian subjects to predict pharmacodynamics (COX-1 and COX-2 activity) for a new investigational drug in subpopulations of interest (including Japanese subjects), and correlate pharmacodynamic parameters to safety outcomes. There was good correlation between COX-1 inhibition and the incidence of 7-day gastroduodenal mucosal injury [39]. Based on their findings, the authors have been able to identify a dose level that yields adequate inhibition of COX-2 activity with a low risk of gastrointestinal mucosal injury.

Hence, it should be clear in areas such as chronic pain research that the use of the maximum tolerated dose concept does not necessarily mean adequate target engagement or pharmacology[40]. Moreover, it leads to inaccurate assessment of the benefit-risk balance. Here we attempt to establish a putative relationship between pharmacology, as assessed by prostaglandin inhibition (PGE_2) and response, as defined by the ACRn scores and thereby get better insight into the causes of variability in treatment response in phase 3 trials in rheumatoid arthritis. The ACRn is considered a robust continuous index of treatment response especially when compared to the traditional end point the ACR20 [9]. The ACRn is able to capture both improvement as well as worsening of disease and has been shown to provide a more accurate description of the placebo response as compared to the ACR20 [10]. Whilst its utility as an index of clinical response is beyond doubt, the correlation between ACRn and the underlying pharmacological activity following administration of a non-steroidal anti-inflammatory drug has never been established.

Previously we have also modelled the inhibition of PGE_2 by GW406381 as biomarker of pharmacological activity in healthy subjects. The concentration-effect relationship obtained in healthy subjects was then used to explore the impact of different sources of variability and to assess their implication for the dose rationale in later efficacy trials. [19]. Our working assumption has been that the optimal benefit-risk balance is likely to be achieved when COX-2 inhibition is maintained above 80% but below 95% [41, 42]. Using clinical trial (CT) simulations, we have combined the different models describing pharmacokinetics, pharmacodynamics and response to assess trial outcome as well as the putative correlation between response (ACRn) and pharmacological activity (PGE_2 inhibition). We envisage that such an approach will make it possible to predict long-term outcome and most importantly highlight the relevance of pharmacokinetic-pharmacodynamic data as prognostic markers in drug development.

SUBJECTS AND METHODS

Simulation Protocol

Our exercise comprised a pharmacokinetic model describing drug concentrations in plasma, a pharmacodynamic-model describing prostaglandin (PGE_2) inhibition and a disease model describing the placebo and drug effects on the clinical response in patients. An overview of the simulation procedures is depicted in Figure 9.1.

Simulation of systemic drug concentrations

Drug concentrations were simulated to subsequently characterise the biomarker-response relationship. Mean steady state concentrations were simulated at the time points at which the clinical response was recorded. To this purpose, a population pharmacokinetic model based on healthy subject data was used [19]. The pharmacokinetics of GW406381 in adult male subjects was best described by a two-compartment model with first order absorption and elimination. Given the absence of covariate effects on the pharmacokinetics of GW406381, in healthy subjects, it was assumed that rheumatoid arthritis has little or no effect on drug disposition. A summary of the pharmacokinetic model parameters are presented in Table 9.1. Only mean population concentrations were simulated for each dose level.

Simulation of drug effect on biomarker

The relationship between drug concentrations and biomarker were described by the following equation/expression for the sigmoid I_{max} model

$$Eff = I_0 - (I_0 - I_{max}) * \frac{C^V}{C^V + IC_{50}^V} \quad (1)$$

Where I_{max} represents the maximum inhibitory response to GW406381 plasma concentrations (c), I_0 is the baseline production of PGE_2 and γ is the Hill factor.

Simulation of the pain response

The placebo model

A Weibull function was deemed one of the best choices to describe the non-linear decrease in the scores from baseline, which reaches a plateau prior to completion of the treatment. This model can take into account both placebo responders as well as those with a flat (zero) placebo response. It is given by the following expression:

$$Pd_t = Basl * \left(1 - P_{csm} * \left(1 - \exp \left(- \left(\frac{t}{td} \right)^\alpha \right) \right) \right) \quad (2)$$

where Pd_t is the clinical endpoint at time t , $Basl$ =baseline value of the clinical endpoint, P_{csm} is the estimate of the CSM with placebo treatment, td = time to reach the maximum placebo effect, α =shape parameter [23]. P_{max} or the magnitude of the placebo effect is calculated as the difference between $Basl$ and P_{csm} .

In order to accurately describe the time course of ACRn, modifications to the basic equation is required to constrain ACRn to be 0 at baseline, as per clinical definition. Therefore, the model includes an offset term (OFF), which was parameterised as follows:

$$OFF = ACRn_{t0} + Bas_Sc \quad (3)$$

Where $ACRn_{t0}$ =ACRn at time=0, Bas_Sc = core set measures at baseline (time=0). This modification accounts for the need for a finite non-zero parameter representing baseline conditions at time=0:

$$Pl_t = OFF * \left(1 - P_{csm} * \left(1 - \exp \left(- \left(\frac{t}{td} \right)^\alpha \right) \right) \right) \quad (4)$$

Drug Effect Model

The effects of GW406381 on the ACRn were best described using a standard I_{max} model, as shown in the following expression:

$$I_{COX} = \frac{I_{max} * Conc}{IC_{50} + Conc} \quad (5)$$

where I_{max} represents the maximum inhibitory effect, IC_{50} is the drug potency and $Conc$ the systemic drug concentrations. The net effect on the time course of the ACRN at time t , was proportional to the placebo effect, and given by the following expression:

$$ACRn_t = OFF * \left(1 - P_{csm} * \left(1 - \exp^{-\left(\frac{t}{td}\right)^\alpha} \right) \right) * (1 - I_{cox}) \quad (6)$$

Dichotomisation into Responders and Non-responders

To explore treatment and patient characteristics underlying clinical response across the different dose groups, subjects were split into responders (R) and non-responders (NR). A trial subject was categorised as a responder if he /she had decrease in ACRn > 25% relative to baseline at the last study visit (i.e., completion of treatment).

A summary of the placebo and drug model parameters used for simulations are presented in Table 9.1.

Trial Execution Components

A virtual study protocol identical to the original trial was used for the purposes of this analysis. Treatment duration was 42 days with visits at baseline, on days 7, 14, 28 and 42 days after the start of treatment and follow-up approximately 7 days after completion of treatment. For the sake of clarity, data missingness, dropout rate and treatment compliance were also assumed to be comparable to the original trial. Pharmacokinetic and pharmacodynamic sampling times were collected at the same time of the assessment of the clinical response.

Study Population: Patients were assumed to have the same demographic characteristics of those enrolled in the original clinical trial. However, this information was considered for completeness only, given that demographic covariates showed no influential effects on the pharmacokinetics, pharmacodynamics or clinical response to GW406381.

Study design: A parallel placebo controlled study design was used in which treatment was assigned to eight different groups, who received placebo and 10, 35, 50, 100, 150, 250 and 400 mg q.d. doses of GW406381. Five hundred patients were enrolled into each treatment arm.

Sampling scheme for ACRn and Core Set Measures: Individual predicted (IPRED) values of the ACRN were simulated at each scheduled time point. Due to the use of an additive random error structure in the model, response values that were less than -100 were excluded from further analysis. The disease core set measure (CSM) values were calculated from the simulated individual ACRn values according to the following expression.

$$IPRED_{ACRN} = \left(\frac{CSM_{EOS} - CSM_{BAS}}{CSM_{BAS}} \right) * 100 \quad (7)$$

Where CSM_{EOS} , CSM_{BAS} represented, respectively, the CSM score at the end of the study (EOS) and at the baseline (BAS) in the original scale.

Given that interindividual variability has not been identified on baseline, individual patient data from the original trial was used to ensure comparable distribution during the simulations.

Assessment of pharmacokinetic-pharmacodynamic correlations

To explore the link between clinical response and biomarkers of pharmacological effect, correlations between the predicted median prostaglandin inhibition and ACRn were evaluated. This was done by fitting a spline which described the relationship between these two variables. The spline belongs to the family GAM (generalised additive models) which lack any explicit functional form and are useful when no a priori assumption is made about the parametric form of the function to be fitted to the data. Instead, smoothing functions, which are polynomial functions, are fitted to the predictor variable(s) in the model. This approach is appropriate for prediction/interpolation as well as for exploratory analysis of the response[43, 44].

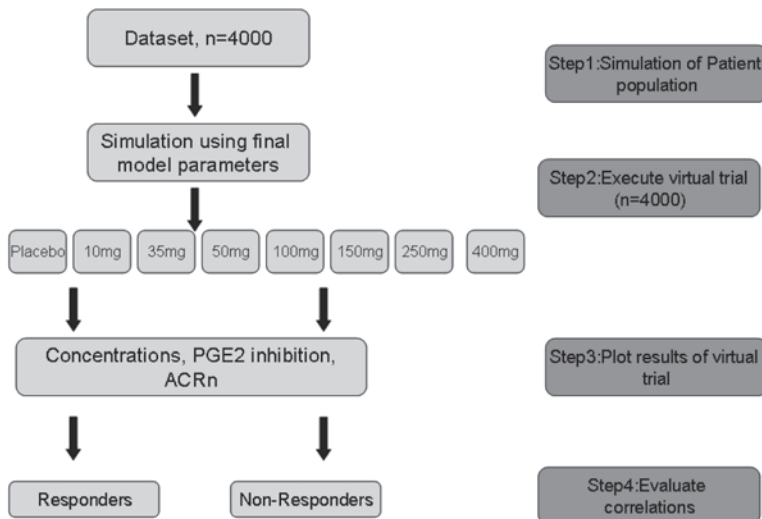


Figure 9.1: Overview of the simulation procedures.

Table 9.1: Final parameter estimates used for the simulations of pharmacokinetics, pharmacodynamics and clinical response.

| Pharmacokinetic Model | | |
|-----------------------------------|-----------------------|--------|
| Parameter | Final Model estimates | CV% |
| V_2 (L) | 252.38 | 35.06 |
| V_3 (L) | 959.78 | 60.54 |
| CL (L/h) | 30.21 | 43.97 |
| K_a (h^{-1}) | 15.24 | 78.7 |
| Q (h^{-1}) | 37.28 | 35.82 |
| Lag time (h) | 0.47 | 6.05 |
| F_1 35 | 1.00 | FIXED |
| F_1 70 | 0.49 | 44.2 |
| IIV V_3 | 93% | 73.05 |
| IIV CL | 56% | 76.4 |
| IIV K_a | 198% | 48.84 |
| IIV F_1 | 95% | 50.58 |
| Residual error | 0.11 | 58.99 |
| Pharmacodynamic (Biomarker) Model | | |
| I_0 (pg/ml) | 63196.80 | 9.74 |
| I_{max} (pg/ml) | 479.00 | FIXED |
| IC_{50} (ng/ml) | 43.25 | 12.22 |
| Hill factor | 1.59 | 10.37 |
| IIV I_0 | 44% | 28.63 |
| Parameter | Final Model estimates | CV% |
| IIV I_{max} | 272% | 107.38 |
| Residual error | 0.002 | 10.91 |
| Disease Model | | |
| Parameter | Final Model estimates | CV% |
| Bas_Sc (%) | 36.5 | 1.5 |
| P_{csm} (%) | 2.05 | 9.04 |
| K_{on}^* | 0.386 | 25.40 |
| α | 0.351 | 40 |
| I_{max} (%) | -100 | FIXED |
| IC_{50} (ng/ml) | 7780 | 38.20 |
| IIV Bas_Sc (CV%) | 38.3 | 5.80 |
| Residual Error(%) (additive) | 17.46 | 32 |

where V_2 and V_3 =central and peripheral volumes of distribution respectively, CL=clearance from the central compartment, Q=intercompartmental clearance, F_1 =relative bioavailability,

I_{max} =represents the maximum inhibitory response to GW406381 plasma concentrations (C), I_0 =i baseline production of PGE₂ and γ = Hill factor, IC_{50} =concentration at which half-maximal PD (prostaglandin inhibition/decrease in ACRn) occurs

Bas =baseline value of the clinical endpoint, P_{csm} is the estimate of the CSM with placebo treatment, K_{on}^* , this is the reciprocal of $td=$ time to reach the maximum placebo effect, α =shape parameter

Software

Simulations were performed using non linear mixed effects population modelling methodology as implemented in the NONMEM v7.2 (Icon Development Solutions, Eliott City, Maryland, USA).. Data manipulation, statistical and graphical summaries were made in R 2.13[28, 45].

RESULTS

Our simulations show a wide variation in the time course of the core set measures, irrespective of the initial baseline conditions. An overview of the individual profiles for all 4000 patients is summarised by dose level in Figure 9.2. The ACRn scores, which represent the improvement from baseline, are depicted in Figure 9.3 together with predicted prostaglandin levels (PGE_2) for placebo and three dose levels of the active treatment, namely, 50, 150 and 400 mg GW406381. It should be highlighted that the model reproduces the variability observed previously in clinical trial data.

Given that missingness and drop-out in the data set affected up to 16 % of the patients, with treatment terminating at different points during the course of 42 days, one can see an increase over time in ACRn and PGE_2 concentrations for some individuals. This fluctuation indicates worsening of symptom severity with respect to the preceding visits.

To establish a correlation between biomarker and clinical response, an initial evaluation was performed by plotting the predicted median time course of ACRn and the corresponding PGE_2 concentrations for each dose group. As shown in Figure 9.4 ,at doses up to 50mg, there is little PGE_2 inhibition (<10%). This is accompanied by ACRn scores which do not appear to differ significantly from placebo. However, at doses $\geq 150\text{mg}$, progressively greater decreases are observed in ACRn, which seems to plateau at 400mg, reaching levels of approximately 90%. In parallel, PGE_2 inhibition was predicted to reach over 20%, at a dose of 50 mg, increasing to > 60% at 150 mg and > 80% at the highest dose level (i.e., 400 mg). Based on these predictions, putative nonlinear correlation was identified between the median ACRn and the level of PGE_2 inhibition, expressed as % change from baseline. Subsequently, patients were dichotomised into responders and non-responders according to the predefined criterion (i.e., decrease in ACRn > 25% relative to baseline at the last study visit). As shown in Figure 9.5, PGE_2 inhibition levels observed in responders is much higher than in non-responders. The proportion of responders increased nonlinearly with the dose, showing a plateau at the highest dose of 400 mg.

The median prostaglandin inhibition for non-responders was 4% and that for responders was 26% across all doses. The prostaglandin inhibition range was similar for both phenotypes being 0-92%.

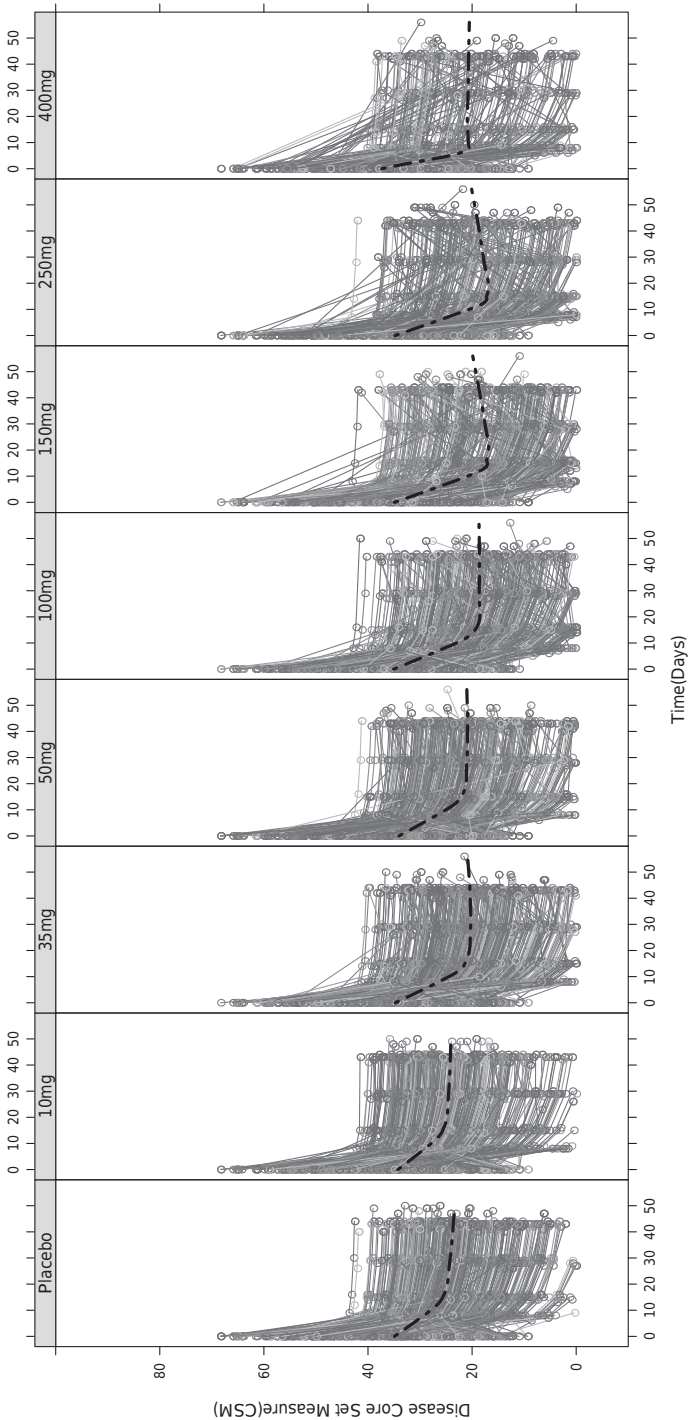


Figure 9.2: Time course of simulated disease core set measures (CSM) on a normalised scale from 0-100 stratified by dose group for all 500 patients. The black dotted line depicts the trend line.

In Figure 9.6 in the appendix, percentage prostaglandin inhibition is plotted against the corresponding ACRN scores depicted for 4 doses, namely, placebo, 100, 250 and 400 mg along with a spline indicating the line of best fit. This figure confirms the findings of Figure 9.5, in that there are a non-linear sigmoid relationship between prostaglandin inhibition and ACRN scores, at all doses. Considerable variability is appreciable in both variables, and particularly in case of ACRN, for many individuals, maximum pain relief is attained at low doses. However with prostaglandin inhibition, a clearer dose dependency is visible with the top two panels showing lower inhibition rates compared to the lower panels. In comparison to Figure 9.5 which shows the median trends, the individual variability is visible here.

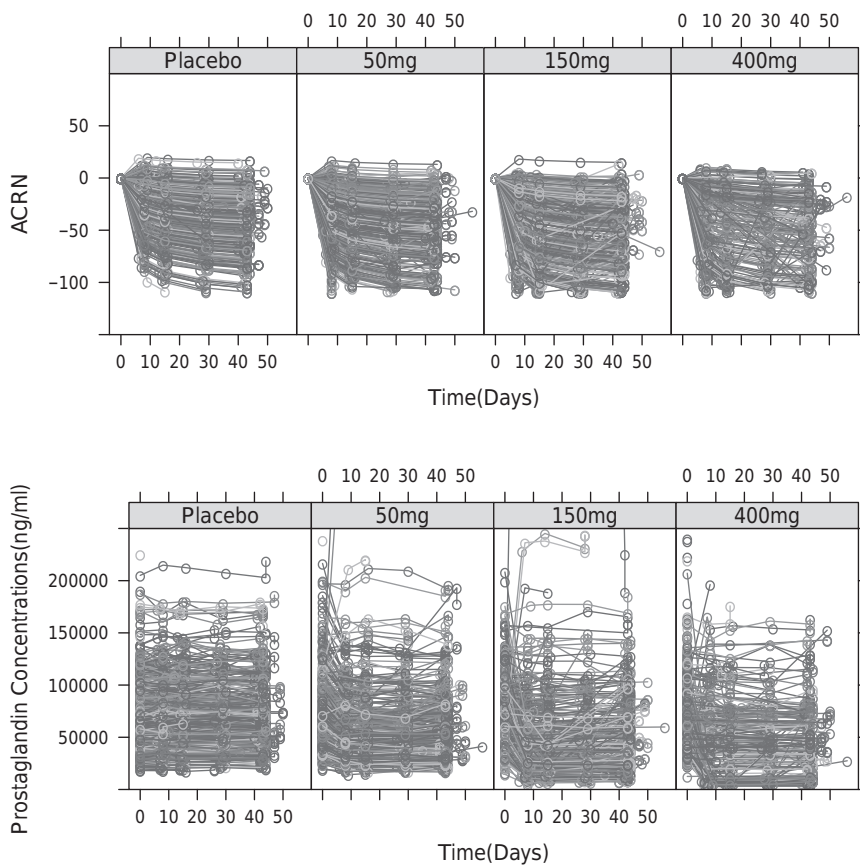


Figure 9.3: Predicted time course of simulated ACRN and the corresponding prostaglandin concentrations after administration of placebo, 50, 150 and 400 mg GW406381.

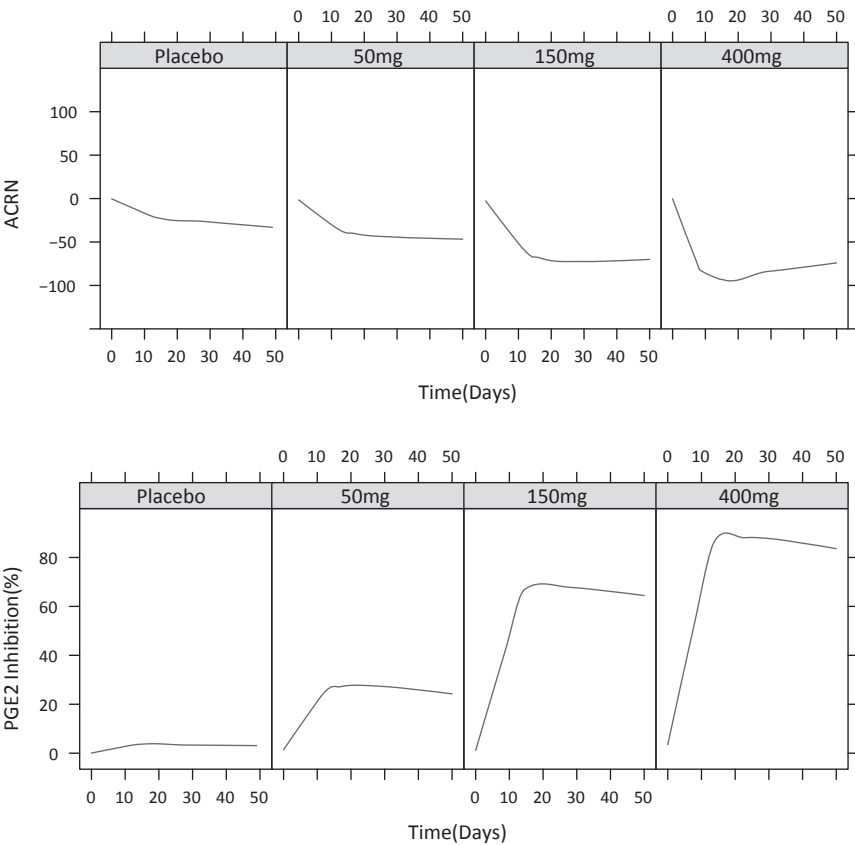


Figure 9.4: Predicted median time course of the simulated ACRn and corresponding PGE₂ concentrations after administration of placebo, 50, 150 and 400 mg GW406381.

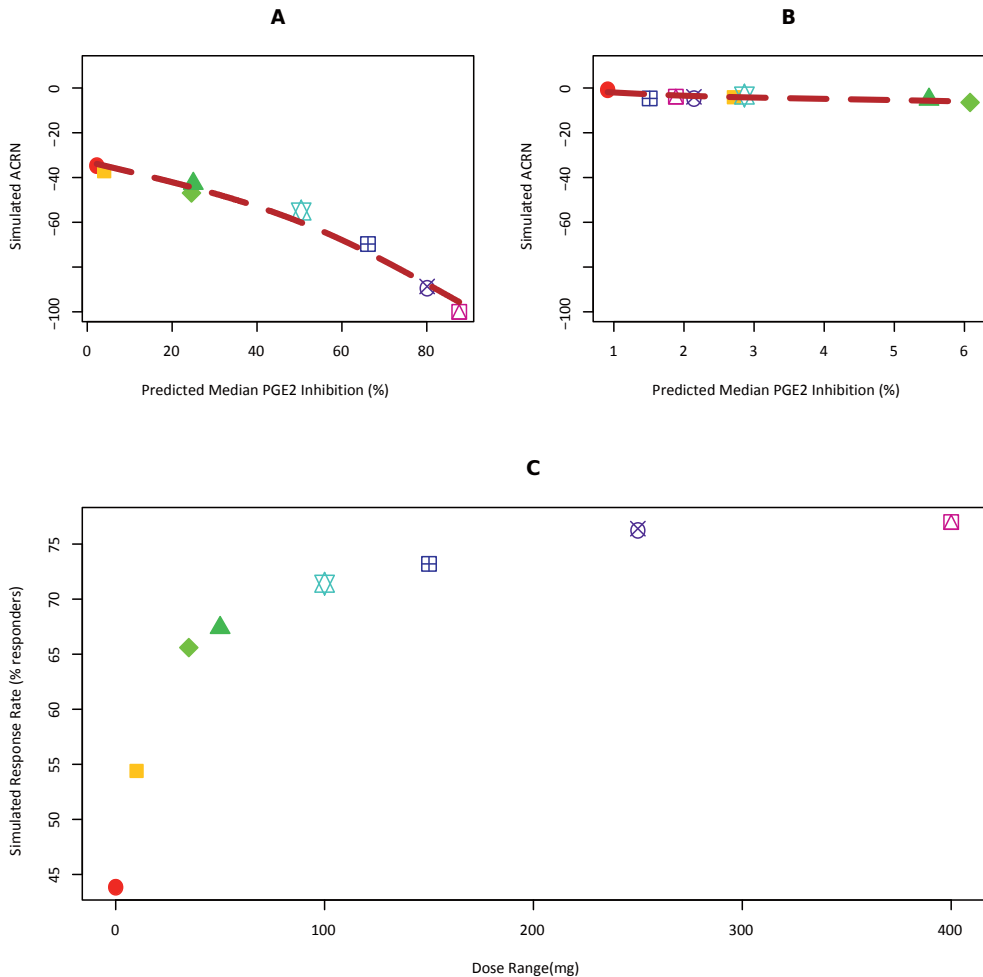


Figure 9.5: Fitting of the median PG inhibition (predictor variable) and ACRn (response variable) for responders (upper panel, A) and non-responders (upper panel, B) respectively using a nonlinear spline function. A clear correlation is observed between the relative proportion of responders and the dose of GW406381 (lower panel C). Symbols refer to individual doses namely, red dot-placebo, orange filled square-10mg, green filled diamond-35mg, green filled triangle-50mg, blue open star-100mg, blue crossed square-150mg, purple crossed circle-250mg, pink open square-400 mg. Note with the scales being harmonised for responders and non-responders, the non-linear correlation between the ACRn and the PGE_2 is not appreciable.

DISCUSSION

There are numerous factors contributing to high failure rate in the development of drugs in chronic pain conditions. A detailed analysis of the problem reveals that these factors may be categorised into three main classes: disease, drug and trial design-related factors. The main goal of the analysis described in here was to explore how *in silico* methodologies

can contribute to further understanding of drug and design-related factors. In fact, the importance of trial design-related factors has been highlighted by Katz *et al.* who have investigated the role of placebo effect and other confounders of treatment response[46]. Clearly, irrespective of the advancements in the understanding of disease and the possibility to identify biomarkers for chronic pain, our ability to demonstrate efficacy and differentiate novel treatments requires methodological research aimed at minimising this unacceptable false negative rate, whilst being able to select doses that yield the anticipated level of target engagement and consequently the expected pharmacological activity.

Our results show that the clinical response to a COX-2 inhibitor, as assessed by the ACRn, can be correlated to the underlying pharmacological activity. The availability of such a correlation represents a stronger basis for the dose rationale in efficacy trials, which have been primarily been determined by evidence of treatment response based on overt symptoms, rather than on the underlying concentration-effect relationships. The correlation is also evident after dichotomisation of the patients into responders and non-responders. However, it is clear that the results from the responders group may have strong prognostic value, since that improvement greater than 25% relative to baseline may be truly reflective of drug effect, as opposed to placebo response, which is significant in this indication [47, 48]. In addition, our simulations reveal that the number of responders in the dose range used in the original clinical trial would result in a response proportion of less than 70% at doses of 50mg, which corresponds to the observed data[38].

In an earlier work, we have predicted the therapeutic dose range was likely to lie above 150 mg administered either a once daily or twice daily dosing regimen. [19]. Our simulations suggest that pharmacological activity corresponding to clinically relevant changes in ACRn are observed between 150 and 400 mg, administered as a q.d. regimen. Although compliance and dropout level in this exercise has been considered a fixed factor based on historical data, we believe that the observed levels of data missingness do not affect the conclusions about the required dose to ensure optimal clinical response in this patient population.

Another important point to consider in this investigation is how prior knowledge about the compound's pharmacological properties can be formally incorporated into statistical inferences taking into account uncertainty and use the results to improve decisions deriving from the observed treatment effect in a clinical trial. In a field where most clinical trials have a conservative design, this methodology offers an unique opportunity not only to assess the scientific rationale of the study, but also to explore innovative designs, including a formal evaluation of design features such as (a) sample size (number of patients), (b) randomisation ratio across treatment arms, (c) frequency of assessments (number of visits), (d) dropout mechanisms, (e) clinical endpoint and (f) statistical methods for the analysis[49].

Despite the potential advantages highlighted above, one should acknowledge that while the ACRn has been shown to be a robust index of response to treatment, it is not pathway

specific [17, 50]. Therefore, this scale can be used to evaluate response to COX inhibitors as well as for DMARDs in rheumatoid arthritis [11]. This forms an important limitation to the implementation of a comprehensive mechanism-based approach, in that changes in the ACRn provides no evidence about the targets and pathways involved in the symptomatic response [35]. We raise this point to call attention to the challenges one faces in the implementation of physiologically meaningful parameters. In contrast, there are clinical scales, such as those used in neurodegenerative diseases (e.g. multiple sclerosis, Alzheimer's or Parkinson's disease), for which a correlation can be established (either directly or inversely) with markers of pharmacology. In Parkinson's disease for example, the UPDRS (unified Parkinson's disease rating scale) as well as the Hoehn and Yahr scale have been shown to have a high degree of correlation with striatal and overall dopamine transporter (DAT) binding. [51-53]. This has been confirmed by PET scanning of dopamine transporters in affected patients[53].

In chronic pain conditions however, we are essentially interested in knowing how perturbations of the different pathways influence or determine clinical surrogate end points, which are defined as characteristics or variables that measures how a patient feels functions or survives[54].

A caveat here is that our findings relate only to drugs acting via the COX pathway, thus the NSAIDs and specifically the COX2 inhibitors. For DMARDs other pathways may be recruited and hence this correlation may not be applicable, though the concept is still valid.

In conclusion, the use of a model-based approach such as presented here offers the opportunity for protocol design optimisation and improved dose selection in efficacy trials. Most importantly, it provides a quantitative framework for establishing the connection between clinical responses to the underlying pharmacology.

APPENDIX

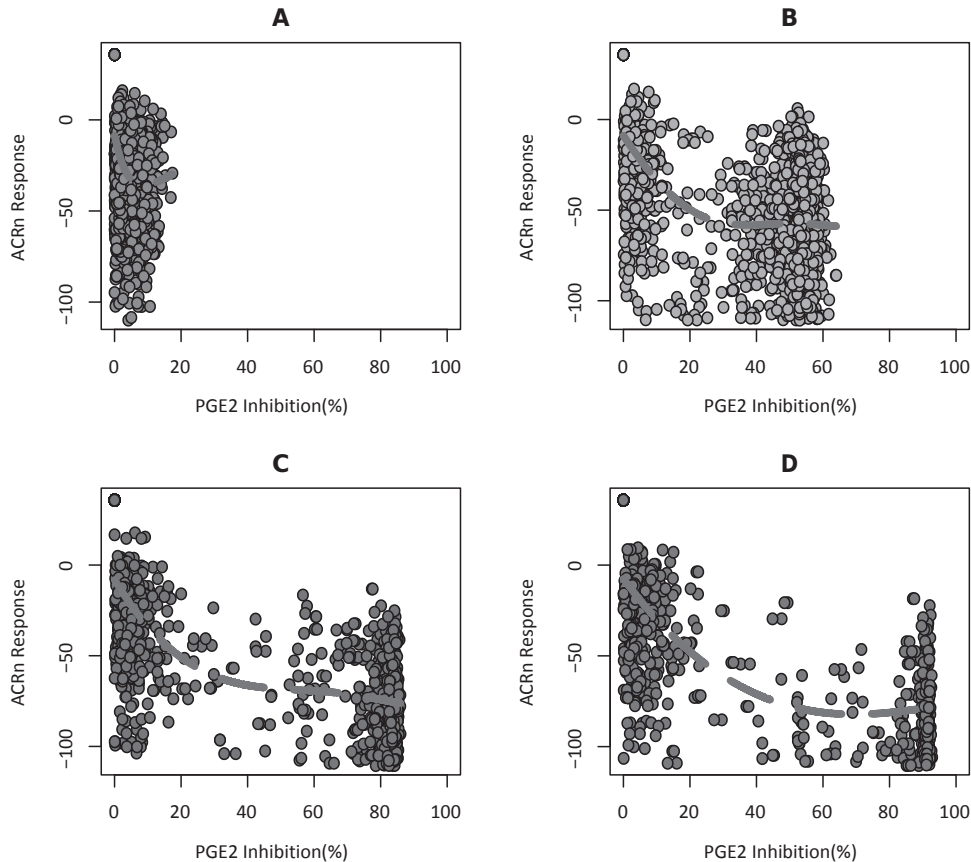


Figure 9.6: The non-linear correlation between PGE₂ inhibition and the ACRn scores, presented for the entire dataset. The spline indicates the best fit.

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SECTION IV

CONCLUSIONS AND PERSPECTIVES

CHAPTER 10

Analgesic drug development at the crossroads -
Where do we go from here?
Thesis summary, conclusions and perspectives

ANALGESIC DRUG DEVELOPMENT AS IT STANDS TODAY

Chronic pain is a significant health problem that greatly impacts the quality of life of individual patients and imparts high costs to society. Despite intense research effort and progress in our understanding of the mechanistic and molecular basis of pain, chronic pain remains a significant clinical problem that has few effective therapies [1]. In fact, existing analgesics are relatively ineffective, have a high side-effect burden and do not reduce pain in all treated individuals. While a statistically significant reduction in pain in pivotal trials can result in an investigational analgesic obtaining regulatory approval, these treatments are palliative in nature making pain symptoms more manageable, with global pain scores reducing by 30% at best in responders [2-4]. However, even in successful phase 3 analgesic trials, at the end the majority of subjects are still eligible to enter the same trial [5].

Experts across different disciplines acknowledge the unmet needs in analgesia and recommend strategies for enhancing analgesic drug development. Nevertheless, both treatment and research into chronic pain are greatly compromised by the fact that there is no objective diagnostic test that can complement the subjective assessment of chronic pain conditions. Currently, there are no concrete diagnostic measures that enable early diagnosis, prevention or prophylaxis of a syndrome that endures for long periods of time.

While there has been exploration of different treatment options, including novel putative mechanisms of action for analgesia, there has been wide-spread reluctance on the part of the pharmaceutical industry to take novel products further into development without demonstrating the efficacy of analgesics based on behavioural measures, which reflects the current status at which this pathological condition or syndrome is clinically detectable. As a consequence, efforts in drug development have been geared to the design of the clinical trials (e.g., FDA's Analgesic Clinical Trials Innovation, Opportunities, and Networks - ACTION Initiative)[6], under the assumption that better trial designs will yield more successful results. This hypothesis is questionable in view of the frequent failures of clinical efficacy trials of opioid drug products, considering the well-established effectiveness of these products from literally thousands of years of clinical experience. In this thesis, we have brought a different perspective to the evaluation of chronic pain by emphasising the role of pharmacology and target engagement as the basis for translational research and clinical evaluation of novel molecules in humans. Throughout the various chapters we have highlighted some important conceptual and experimental flaws in the way that pain signalling and pharmacological activity are characterised and translated across species and disease conditions. The common denominator of the work presented here is the requirement for accurate characterisation of exposure-response relationships, without which the dose rationale for the progression of a molecule cannot be justified, whether drugs are aimed at symptomatic relief, disease modification or prophylaxis. In addition to a comprehensive review of the mechanisms underlying pain signalling and symptoms, the work developed here focuses on three

different aspects of research underpinning the use of pharmacokinetic-pharmacodynamic relationships. First, we have explored the requirements for the characterisation of behavioural measures of pain during the early screening of candidate molecules, shedding light onto the shortcomings of experimental protocols commonly used in preclinical research. Then we introduced the prerequisites for the parameterisation of pain behaviour to ensure accurate translation of the pharmacological properties across species as well as for bridging across different phases of development. Lastly, an attempt was made to model clinical response in chronic inflammatory pain and to establish correlations between symptom improvement and the underlying pharmacological effects using biomarkers. In addition our work showed how clinical trial simulations can be used as a design tool, enabling the evaluation of a variety of scenarios that disentangle the contribution of pharmacology from the confounding effects of placebo and disease dynamics.

CHRONIC PAIN AS A NOCICEPTIVE SIGNALLING DISORDER

An overview of the ongoing research efforts in neuropathic and chronic inflammatory pain was presented in **Chapter 1**. We have shown that in addition to practical challenges, there are still several methodological issues that hinder the development of novel medications for the treatment of chronic pain. The most common problem is clearly the lack of construct validity of the experimental protocols used to assess drug effects. Pain experiments focus on transient behavioural models of pain, which do not necessarily reflect what is occurring in chronic pain patients. A new paradigm is proposed for the identification of relevant targets and candidate molecules in which pain is coupled to the cause of sensorial signalling dysfunction rather than to the symptoms. We have also shown that early diagnosis, timing of the intervention and reversibility of the underlying processes cannot be disentangled from each other and ultimately determine the success or failure of a treatment. It is therefore essential to understand the changes in the nervous system that result in the pain experience and consider the need for interventions before symptoms evolve. Consequently, one needs appropriate measures of patient response that are crucial in establishing patterns of response. On the other hand, such experimental protocols will remain of limited value unless relevant pathophysiological changes can be detected at the prodromic phase, i.e., before the metamorphosis from acute injury to chronic pain. This discussion is further extended to the limitations and flaws in the current paradigm for the screening and selection of compounds in drug development and used as a foundation for the subsequent chapters in this thesis. Here we have also highlighted the fact that the measures of pain perception currently used in experimental models do not bring to light all involved pathways, which creates an important translational gap across species.

MODEL-BASED ANALYSIS OF PAIN BEHAVIOUR IN PRE-CLINICAL INVESTIGATIONS

As discussed in **Chapter 2**, the translational value of pre-clinical models of pain has been the subject of heated debate [7]. The generally held view is that these models lack construct or predictive validity and are only able to replicate symptoms [8, 9]. Moreover coarse behavioural endpoints such as the threshold to paw withdrawal (allodynia) or paw withdrawal latency (hyperalgesia) are employed as surrogates for evaluating analgesic activity. Despite these limitations, we have highlighted how further understanding of the pharmacology and target engagement can be relevant for the progression of a molecule into humans.

Using recent examples from published literature, we have emphasised the requirements for the assessment of concentration-effect relationships in pre-clinical species, including important changes in experimental procedures in standard screening protocols. Among other factors, we have indicated the importance of pharmacokinetic sampling and expressing drug properties such as potency in terms of exposure (EC_{50}), rather than dose (ED_{50}). Poor experimental design can lead to inaccuracies in parameter estimation and consequently to biased selection and ranking of candidate molecules during screening. In this context, we have introduced how mathematical and statistical models can be used as a tool to describe biological system and drug properties in a quantitative manner. Hierarchical or population models were proposed as the basis for identifying the different sources of variability as well as to assess treatment effects and disease progression. In addition, here we bring in the concept of biomarkers as an intermediate step between drug action and behaviour, which can be integrated in a systematic manner by pharmacokinetic-pharmacodynamic modelling, enabling the characterisation of exposure-response relationships and consequently providing mechanistic underpinning, be it for the purpose of interspecies translation or determination of the therapeutic dose levels in patients.

EXPERIMENTAL PAIN MODELS: UNTRANSLATABLE YET INFORMATIVE

An important assumption underlying the work presented in this thesis was that some of the limitations of the current practice of pre-clinical research may be overcome by model-based analyses, in that **it facilitates the evaluation and discrimination between drug and system-specific properties**. Furthermore, it can be envisaged that estimates of potency that are based on concentrations rather than doses will be more informative, allowing for more accurate comparisons between compounds of the same class and possibly enhancing the predictive value of such estimates. Another premise of our work was that standardised experimental protocols are not designed to ensure the informative value of the data collected or optimally discriminate between molecules or dose levels. Given the empirical

nature of pain experiments, standardisation procedures simply attempt to increase the reproducibility of measurements. Hence, efforts were made to evaluate the impact that such improvements could represent to the development of novel molecules for the treatment of chronic pain.

As indicated previously in the scope of this thesis, the lack of suitable markers of pharmacology is compounded by poor data integration at the time candidate molecules are selected. We have shown that whilst the assessment of concentration-response relationships across the different phases of development is a *sine qua non* condition to understand treatment effects and variability, accurate estimation of PKPD parameters is not guaranteed. PKPD relationships must have predictive validity or value for the target population, both in qualitative and quantitative terms. Such considerations are not formally embedded in the requirements for evidence generation, and consequently lead to experimental protocols, which are often not informative or eventually even biased[8].

Two experimental models of pain behaviour were selected to illustrate the aforementioned concepts. First, the threshold to paw withdrawal to a normally non-noxious stimulus was measured as a marker of the anti-allodynic effect in the complete Freund's adjuvant (CFA) model, a well-known experimental animal model of inflammatory pain. In the second case, the change in flinching frequency observed after injection of formalin was selected as a marker of drug effect. The pain response in this model is based on spontaneous behaviour, rather than on the threshold for a painful stimulus. In addition, the pain reaction produced by formalin results from a conditioned motor response, reflecting higher cognitive function than simple withdrawal responses [10]. It has also been demonstrated that drugs with different mechanisms of action can be differentiated in terms of the type and magnitude of the effects on the flinching behaviour. We have hypothesised that for this reason the formalin-induced model is likely to show some construct validity as compared to other experimental models based on short duration stimuli [11]. From a methodological perspective, we have demonstrated that the choice of parameterisation is as critical as the precision of model parameters. Undoubtedly, the limited availability of data during the screening of compounds leads to two important statistical issues, namely parameter identifiability and poor precision.

In **Chapters 4 and 5** we showed therefore how efficacy can be reliably assessed for gabapentin and pregabalin. To prevent model and parameter identifiability issues, an approach was proposed based on the use of a binary response as parametric filter for drug screening. Our analysis showed that it is possible to dissect system from drug-specific features, allowing the assessment of potency as parameter of interest for two paradigm compounds. The aim was to decrease the uncertainty on EC_{50} estimates as well as on the corresponding inter individual variability. ED-optimality was therefore applied in combination with a logistic regression model describing the relationship between drug exposure and response

to evoked pain in rats, enabling us to take parameter uncertainty into account [12]. The design variables selected for optimisation included the dose levels and sampling times required for the characterisation of the analgesic effects. Moreover, information regarding system-specific model parameters and relative *in vitro* potency data were also incorporated as priors during optimisation. Our analysis also shed light onto the implications of the empirical choice of dose levels, which are often too high to allow accurate estimation of drug potency *in vivo*. Moreover, despite the wide range of doses used in the experiments, simulated concentration profiles of gabapentin were not significantly different from each other. Relative bioavailability was found to decrease nonlinearly from 100 % at 10 mg/kg to 9 % at 300 mg/kg. In contrast to results from typical experiments in which ED_{50} is calculated, irrespective of the underlying exposure levels, protocol optimisation procedures clearly showed the importance of optimised sampling and dose schemes. EC_{50} values for gabapentin and pregabalin were 1400 and 897 ng/ml, confirming that the relative difference potencies observed *in vitro* are also seen *in vivo*. Overall, these findings demonstrate that protocol optimisation does represent an improvement in terms of parameter precision and bias. We anticipate that these procedures will provide the basis for further validation of experimental models of pain in terms of sensitivity and specificity, i.e., enabling the characterisation of the false positive and false negative rates during the screening process.

The advantages of model parameterisations based on drug- and system-specific properties was further explored in **Chapter 6**, where we investigated the feasibility of developing a semi-mechanistic model to describe the effects of a wide dose range of gabapentin (i.e., 0 – 100 mg/kg) on formalin-induced pain, which is characterised by two peaks of flinching behaviour. The first peak of hypersensitisation was described by a mono-exponential declining function, whereas the second, prolonged phase of nociception was best parameterised by an indirect response model with a time variant synthesis rate. Drug effect was parameterised as an I_{max} function. Here, however, the drug effect represented a covariate on pain response. The approach contrasts to traditional parameterisation of drug effects in indirect response models where it is applied to the synthesis or the elimination rate of the disease [13]. Yet, our choice of parameterisation has a mechanistic basis, given that gabapentin is known to have no disease-modifying effects. Interestingly, the mean IC_{50} values of 7510 ng/ml were found to be in the same log order of magnitude as those reported by Lockwood *et al.* and Whiteside for gabapentin in clinical studies [14, 15]. On the other hand, despite high exposure levels, gabapentin did not completely suppress the behavioural effects induced by formalin. This apparent discrepancy appears to reflect the somewhat limited efficacy in neuropathic pain patients.

Whilst concrete improvements in the estimation of drug- and system-specific parameters were demonstrated by the use of a model-based approach, our endeavour thus far to characterise behavioural endpoints as a measure of drug effects has highlighted the

implications of the lack of biomarkers as an intermediate step for the translation of the pharmacological properties across different phases of development. Undeniably, there is a pressing need to obtain early signals of efficacy by means of biomarkers, which could be used not only as “*scaling factors*” to translate drug effects from pre-clinical species to humans, but also as a tool for extrapolating drug effects across populations during drug development, i.e., from health to disease conditions. The points-to-consider for the use of biomarkers as the basis for the dose rationale were presented in the subsequent section of the thesis.

Biomarker-guided dose selection and prediction of clinical response

As indicated in the introduction, to address the escalating attrition rate in drug development, focus must be given to two key approaches in parallel [16]. The first is better target selection, taking into account the pathways as well as the timing of diagnosis and intervention. The second is the routine pursuit of early proof-of-concept studies, preferably already in Phase I or in human tissue, in which biomarkers or surrogate endpoints could be employed as markers of efficacy and safety. Despite the evidence from other therapeutic areas supporting such an approach [17, 18], chronic pain protocols have remained primarily based on clinical scales without any reference to the underlying pharmacology or target engagement [19, 20]. Clearly, the integration of biomarkers of pharmacology into drug development also offers the possibility to eliminate part of the bias that arises from empirical evidence using nonspecific behavioural measures.

In **Chapter 7**, we made therefore an attempt to show that opportunities exist for truly characterising the clinical pharmacological profile of novel molecules in humans when biomarkers are used as *predictors* of efficacy, enabling mechanistic insight into the exposure-response relationships and consequently better rationale for the therapeutic dose range. Moreover, the assessment of pharmacokinetic-pharmacodynamic relationships using biomarkers can provide a stronger basis for personalised medicine.

Here data from GW406381, a cyclo-oxygenase (COX) inhibitor was used to illustrate the concept of biomarker-guided dose selection and emphasise the importance of gaining insight into the clinical pharmacology of the compound as the basis for the dose rationale. The choice of the COX-2 system as a paradigm was dictated by the various reports arising from the withdrawal of different drugs from the market, for which the clinical pharmacology profile was clearly known to determine efficacy and safety. Data from a phase I, randomised double-blind single dose followed by a 10-day repeated dose study in healthy male subjects were available for the analysis. Doses of 35 or 70 mg GW406381 were administered orally under fasting conditions. Plasma concentrations of GW406381 and PGE₂ (prostaglandin E₂) as well as thromboxane B₂ (TXB₂) were measured at regular intervals throughout the study.

The analysis was performed in two stages. First, a compartmental pharmacokinetic model with first order absorption was used to describe the time course of drug concentrations in the plasma. Then an I_{max} model was fitted to the prostaglandin data to describe the exposure-response relationships.

From a methodological standpoint, by integrating pharmacokinetic data from a Phase I trial with PGE₂, as determined by an *ex vivo* assay, we have illustrated how biomarkers can be used to guide dose selection in subsequent phases of drug development. It is noteworthy to mention that our analysis was successful despite the high variability in PGE₂ data. Mean potency estimates (IC₅₀) were 43.25 ng/ml, with IC₈₀ and IC₉₅ values reaching 103.43 and 275.58 ng/ml respectively. In addition, the high variability in pharmacokinetics and pharmacodynamics observed in healthy subjects exposed an important limitation of using *in vitro* potency as a benchmark to compare compounds in early clinical development, which does not reflect differences in selectivity or metabolic activity *in vivo*. In fact, Fries *et al.* showed that despite the higher potency of rofecoxib relative to celecoxib *in vitro*, their *in vivo* selectivity is likely to be the same [21]. Likewise, the *in vitro* potency of GW406381 was estimated to be approximately 30 times as high as rofecoxib. However, the optimal recommended dose range proposed from our simulations lies between 150-250 mg, while that for rofecoxib is 25-50mg. This is mostly explained by the inter-individual differences in pharmacokinetics. Given the proposed therapeutic range (i.e., <80% and < 95% inhibition), it was found that a b.i.d. regimen allowed peak concentrations to remain above the IC₉₅ for a shorter time and at much higher dosages, without significant effect on trough concentrations, which were comparable to those achieved with a q.d. regimen.

With the help of simulation scenarios we have also demonstrated how biomarkers can be used to explore dose adjustment in special populations. The simulated clinical scenarios included factors known to have potential effect on the pharmacokinetics and pharmacodynamics of GW406381, namely hepatic impairment, metabolic induction and systemic inflammation. Our results showed that moderate to severe hepatic impairment or metabolic induction do lead to significant changes in exposure and consequently in dose adjustments to ensure target exposures are achieved and maintained during the course of therapy. Conversely, increases in baseline levels of PGE₂ appeared to require no changes in dosing regimen.

In brief, this analysis allowed for the evaluation of the dose rationale taking into account the benefit-risk balance, which depends not only on the total dose level, but also on the dosing regimen [22]. Such a balance is likely to be achieved when PGE₂ is maintained above 80% but below 95%. Under these conditions, chronic treatment would effectively block the inducible fraction of the available COX-2 pool, whilst allowing for the residual or basal activity of PGE₂ and other COX-2 related prostacyclins, which have an essential role in normal tissue homeostasis and repair.

In the last part of this thesis, we have expanded the concept of biomarker-guided dose selection to Phase 2 clinical trials to gain further insight into the relationship between biomarkers and overt pain symptoms. By applying the mechanistic classification proposed by Danhof *et al.* [23], it was possible to unravel a putative exposure-biomarker response relationship for GW406381. To this purpose, ACRn scores from a large clinical trial in rheumatoid arthritis patients were used. Even though the utility of the ACRn as an index of clinical improvement is beyond doubt, its correlation with the underlying pharmacological activity following administration of a COX-2 inhibitor has not been established. In **Chapters 8** we have progressed with the use of biomarkers from health to disease and attempted to ascertain whether biomarkers could be correlated with ACRn. In spite of the limited number dose levels that were tested in the trial (0, 10, 35 and 50 mg, administered orally as a once daily dosing regimen), our objective was to show how longitudinal modelling can provide the basis for inferences about the pharmacological effects underlying clinical response. An integrative approach was therefore proposed in which information was derived about the whole time course of treatment response. This contrasts with current practice, in which efficacy is determined by comparing the differences in clinical response at completion of treatment only. The time course of the pain response was best characterised by a Weibull function with an I_{max} model describing the drug effects. We could identify a responder phenotype in the population, which included all individuals who displayed at least a 25% decrease in ACRn from baseline at the end of the study. Interestingly, the percentage of responders was not dose proportional, which reflects the impact of high pharmacokinetic and pharmacodynamic variability. Whilst direct evidence of efficacy could not be obtained from higher dose levels, these findings are in agreement with the predicted levels of target engagement, as determined by the PGE₂ inhibition. Based on the premise that pharmacological activity translates into clinical analgesia when PGE₂ inhibition reaches a threshold of at least 80%, it appears that the doses selected for the clinical study were sub-therapeutic. According to the analysis of the biomarker response, the therapeutic doses for GW406381 were likely to be between 100 and 250mg/day.

Our endeavour to evaluate the predictive value of a model-based approach for the dose selection of analgesic drugs would not be complete without further characterisation of the correlation between biomarker and clinical response [24]. In **Chapter 9** we explore therefore the putative relationship between prostaglandin inhibition (PGE₂) and the ACRn scores in rheumatoid arthritis patients. In addition to demonstrating the suitability of PGE₂ as a proxy for target engagement (i.e., COX-2 inhibition), inferences about the correlation between biomarker and clinical response provide better insight into the causes of variability in a large population. Using the pharmacokinetic and pharmacokinetic-pharmacodynamic models previously developed in chapters 7 and 8 simulations were performed of the time course of the clinical response (ACRn) and PGE₂ concentrations. The hypothetical experimental

protocol was based on a typical clinical trial in rheumatoid arthritis, with treatment duration of 6 weeks and five hundred patients per dose level. A range of doses from 10 to 400 mg GW406381 was considered in our exercise to ensure accurate characterisation of the potential sources of variability. Patients were classified as responders or non-responders based on the simulated response at completion of treatment. Our results showed a wide variation in the time course of the core set measures, irrespective of the initial baseline conditions, with predicted PGE_2 inhibition over 80% at doses greater than 250mg. Furthermore, the proportion of responders increased in a non-linear fashion across the simulated dose levels, with a decrease of 50% in the median ACRn at doses >100mg/day. The availability of such a correlation represents a stronger basis for the dose rationale in efficacy trials, which have been primarily been determined by evidence of treatment response based on overt symptoms, rather than on the underlying concentration-effect relationships.

In conclusion, we have shown that modelling and simulation can facilitate the translation of experimental findings across different phases of drug discovery and development, improving the dose selection and the design of experimental protocols. To this end, we have demonstrated that the availability of longitudinal data is paramount. However, experimental protocols must be based on sampling schemes that allow for the accurate characterisation of pharmacokinetics, biomarkers and efficacy. Moreover, the choice of appropriate model parameterisations, which enable discrimination between drug and system-specific parameters, offers the possibility to systematically explore drug effects taking into account the historical evidence for system-specific properties. In fact, we have shown that statistical priors can be used to reduce uncertainty during parameter estimation and protocol optimisation and as such enable further understanding of the underlying variability in experimental data. Furthermore, using paradigm compounds with known analgesic activity, we have underlined how essential concentration-effect relationships are for the dose rationale in humans. It provides the basis for evaluating the effect of influential covariates on drug exposure and response.

Albeit a preliminary exercise, in which modelling and simulations are used as inferential tools, the approach presented throughout this thesis represents a shift from the empiricism which has dominated selection of candidate molecules, the rationale for the therapeutic doses and the design of Phase 2b and 3 trials for chronic pain conditions. The critical point here is that drug response cannot be expected without sufficient target engagement. On the other hand, further increases in exposure do not yield additional efficacy when maximum pharmacology has been reached. Undeniably, the use of pharmacokinetic-pharmacodynamic models makes target engagement a central component in the development of analgesic drugs.

Future perspectives

Drug development has traditionally been considered a linear process beginning with target selection and ending with regulatory approval [1, 25]. This creates a sequential approach to decision making, in which there should be learning at each step, i.e., knowledge from a previous step informs the subsequent one (see Figure 10.1). Currently, however, as shown throughout this thesis each step appears to occur in isolation and there is little or no integration and accrual of information from preceding steps.

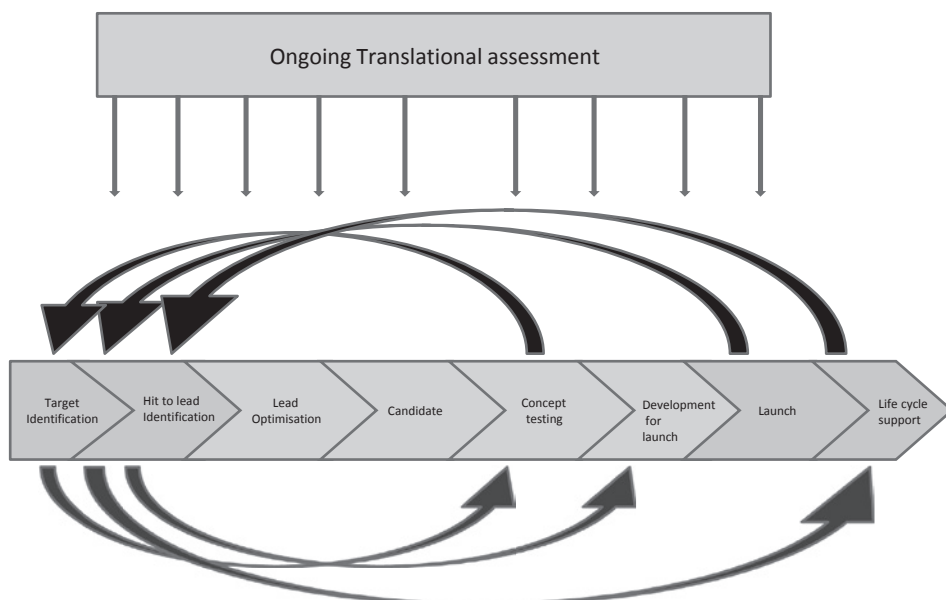


Figure 10.1: Translational steps in drug discovery and development. The standard linear model of drug development is depicted, for illustrative purposes only. Translational activity is bidirectional. Translational assessment needs to start early and requires constant updating according to progress in both directions ‘including reverse translation’. Adapted with permission from [25].

From a clinical and biological perspective, an approach is required that allows us to depart from the current fragmented strategy, which focuses on individual signal transduction pathways, to studying pain as a system based-approach [26]. Moreover, efforts must be made to detect the underlying signalling disorder that precedes the overt pain experience. Prophylactic or pre-emptive treatments are needed to ensure normal tissue homeostasis is maintained after onset of injury and subsequent acute inflammatory response. In this context, drug development programmes will have to consider co-development of diagnostic markers.

Figure 10.2: A proposed new analgesic development pathway. The preclinical and clinical distinction must be abolished. The choice of a target must be driven by data from patients using unbiased screening techniques. Screening and validation must focus on the native human target expressed in human cells relevant to the target's action in pain; preclinical toxicity studies must be done in human cells; Phase 1 must include pharmacokinetic data showing engagement or target occupancy. Phase 2b must be designed to differentiate true efficacy by detailed phenotyping, use of appropriate biomarkers and outcome measures. Phase 3 should be a confirmatory step to identify responders and assess the clinical impact of patient heterogeneity. iPS= induced pluripotent cell, PET=positron emission tomography, POP=proof of principle, fMRI= functional magnetic resonance imaging. *Adapted with permission from [1].*

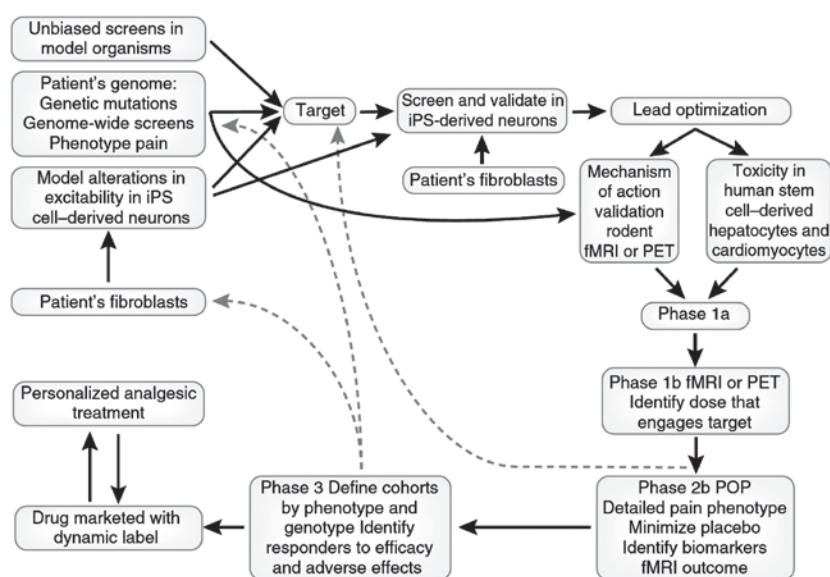


Figure 10.2: A proposed new analgesic development pathway. The preclinical and clinical distinction must be abolished. The choice of a target must be driven by data from patients using unbiased screening techniques. Screening and validation must focus on the native human target expressed in human cells relevant to the target's action in pain; preclinical toxicity studies must be done in human cells; Phase 1 must include pharmacokinetic data showing engagement or target occupancy. Phase 2b must be designed to differentiate true efficacy by detailed phenotyping, use of appropriate biomarkers and outcome measures. Phase 3 should be a confirmatory step to identify responders and assess the clinical impact of patient heterogeneity. iPS= induced pluripotent cell, PET=positron emission tomography, POP=proof of principle, fMRI= functional magnetic resonance imaging. *Adapted with permission from [1].*

Currently there is no validated biomarker for chronic pain. In fact, the identification and use of biomarkers for CNS disease are more challenging than other diseases [27] due to the inaccessibility of the brain. A number of reviews have summarized the current state of the art of imaging pain in the brain [28]; [29, 30] but none have evaluated the potential of using imaging to discover and define biomarkers of pain and their potential application in drug development.

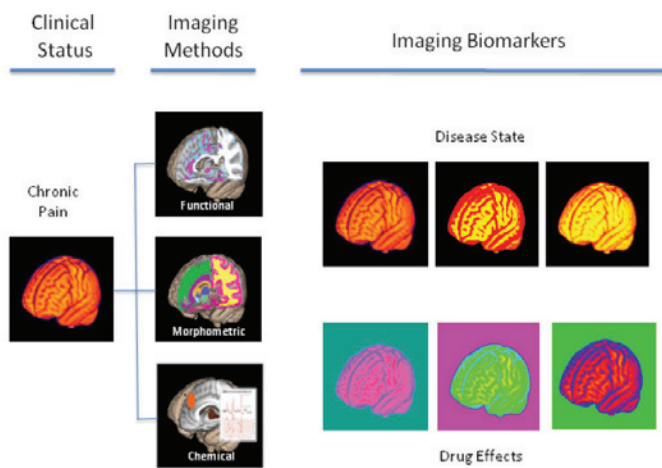


Figure 10.3: Imaging biomarkers of disease state and drug effects. Functional biomarkers may include specific fMRI signals for pain based on evoked or resting state connectivity patterns. Morphometric biomarkers include changes in volume or thickness of brain gray matter or alterations in white matter integrity. Chemical biomarkers include specific chemical changes in brain regions related to disease state or drug effect (*adapted with permission from [37]*).

Developments in functional imaging will unquestionably facilitate the identification of biomarkers of pain and help us to better understand the role of various brain areas in the expression of pain, especially in processes that are associated with neuroplasticity, which is a critical step for the development of chronic pain [31]. In conjunction with physiological (sensory) challenges it may be possible to anticipate signalling disorders before symptoms emerge. Collectively, a refined understanding of abnormal activity or connectivity of synaptic elements may allow us to more effectively target interventions in patients who are likely to later experience chronic pain [32]. In the context of drug development, these advances may also improve translational go/no go decision making between the laboratory and early clinical trials. However, a reductionist approach needs to be avoided in that further understanding of the pain syndrome cannot be achieved by functional imaging alone. When integrated with clinical subjective assessments [33], genetic [34], metabolomics [35], or proteomics [36], such evidence could provide orthogonal views that together may improve the predictive value of pain biomarker strategies. The challenge to be overcome as for any

chronic disease that requires prolonged dosing is that one will need to differentiate the early direct effects of the drug from chronic effects on brain systems that are associated with longer-term effective treatment.

Irrespective of the advancements in the field of imaging and proteomics as an important step to generate evidence of target engagement and possibly of markers of disease progression, pharmacokinetic-pharmacodynamic models can play an essential role as translational tool, providing guidance for the introduction of novel research protocols aimed at reducing uncertainty about the potential clinical relevance of candidate molecules and enabling selection of the putative therapeutic dose range and transition from the pre-clinical phase to humans. Through the use of systems pharmacology, pathways associated with signalling processes can be better mapped and system-specific parameters disentangled from drug-specific properties. Under the assumption of common substrates in experimental and disease conditions, modelling can be used to predict target engagement and consequently the downstream pharmacological effects. It is also conceivable that *scaling factors* could be identified to describe potential differences, such as receptor density, between experimental protocols and disease conditions in humans. Similar concepts have been recently applied for antipsychotics, for which *in vitro* receptor occupancy in rats has been scaled all the way to clinical efficacy [38, 39]. As already proposed in this last section of this thesis, quantitative techniques could then be used further explore the role of phenotypic differences and other influential factors (covariates) on pharmacokinetics and response, thereby supporting dose selection and other label claims (Figure 10.4).

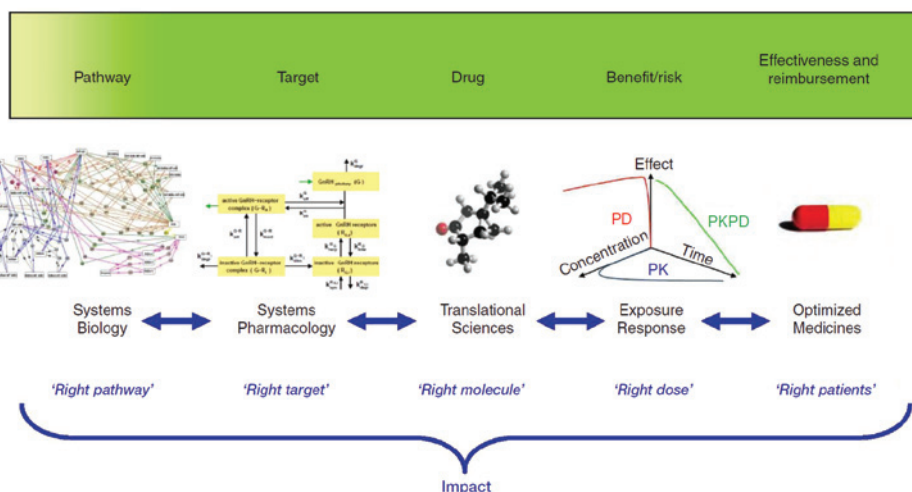


Figure 10.4: An integrated model-based approach across drug discovery and development, from systems biology all the way to exposure-response modelling. Adapted with permission from [40].

A slightly different scenario can be envisaged with regard to the challenges one faces in the evaluation treatment effects in chronic inflammatory pain, for which a few biomarkers exist and imaging technology is already being used. The challenge will be to reach consensus about the importance of revisiting current guidelines, which dismiss the role of pharmacological activity as the basis for dose selection and exclude the concept of learning and confirming as the paradigm for drug development. Undoubtedly, the use of simulation scenarios will play an increasingly important role in the evaluation of the impact of heterogeneity in target population as well as of variability in pharmacokinetics, pharmacodynamics and response to intervention. In this context the integration of statistical models that describe trial design factors, such as drop-out and censoring, with mechanism-based models describing the underlying progression of the disease will be of great value [41]. Finally, we envisage that further advancements in the prediction of pain response can be obtained by expanding the concepts to multiple endpoints as well as by incorporating fully mechanistic models to the pharmacometric framework proposed in this thesis.

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CHAPTER 11

Nederlandse samenvatting
(Summary in dutch)

PKPD RELATIES EN KEUZE VAN DE DOSIS BIJ DE ONTWIKKELING VAN ANALGETICA – STREVENDE NAAR HET VOORSPELLEN VAN RECEPTORBEZETTING

Chronische pijn wordt beschouwd als een aanzienlijk probleem in de geneeskunde. Een belangrijk klinisch en maatschappelijk gevolg van deze aandoening is de vermindering van de kwaliteit van het leven voor de betrokkenen en de daarbij behorende hoge kosten voor de gezondheidszorg. Ondanks doorlopend onderzoek en vordering in de kennis van de moleculair-mechanistische basis van pijn, blijft het een complex probleem waarvoor tot op heden geen effectieve behandeling bestaat. De bestaande geneesmiddelen worden voornamelijk gebruikt voor de behandeling van pijnsymptomen. Ze zijn en als zodanig relatief ineffectief, aangezien analgesie vaak gepaard gaat met veel bijwerkingen. Bovendien blijken deze medicijnen beperkt werkzaam te zijn in patiënten die reeds met andere medicatie behandeld werden, en dit vormt een groot probleem voor de ontwikkeling van nieuwe analgetica.

Om tot de markt toegelaten te kunnen worden, moet namelijk in klinische studies aangetoond worden dat een nieuw pijnstillend geneesmiddel statistisch significant beter is dan bestaande middelen. Doorgaans leidt de werking van de gangbare pijnstillers slechts tot een vermindering van de verschijnselen van pijn, waardoor de aandoening beter verdragen wordt. Hierbij kan er maximaal een reductie van 30% in de pijnscore van patiënten bereikt worden. Bij het merendeel van patiënten die deelneemt in de zogeheten fase 3 klinische studies, is de verlichting van pijn symptomen gering. Aan het einde van de studie voldoen zij vaak nog aan dezelfde criteria en eisen als voorafgaand aan de deelname in de studie. Deskundigen van verschillende disciplines zijn het met elkaar eens over de grote behoefte aan effectievere farmaca en trachten daarom strategieën te ontwikkelen voor het verbeteren van het ontwikkelingsproces voor een nieuwe geneesmiddel. Onder anderen worden zowel pijnbestrijding als het onderzoek naar chronische pijn momenteel gehinderd door het ontbreken van objectieve diagnostische testen op basis waarvan de subjectieve waarneming van pijn kan worden vastgesteld en eventueel aangevuld met pathofysiologische criteria. Daarnaast zijn er tot op heden geen diagnostische methodes beschikbaar die tot een tijdige diagnose en daarmee tot preventie van chronische pijn kunnen leiden. Als gevolg daarvan worden alle inspanningen gericht op het verbeteren van het ontwerpen en uitvoeren van klinische studies, waarbij wordt aangenomen dat verbeteringen van de studieopzet en protocol voldoende kunnen zijn voor het identificeren van de benodigde dosis voor adequate pijnbestrijding. Deze laatste veronderstelling wordt echter in vraag gesteld door het frequente mislukken van klinische studies voor opiaten, waarvan de effectiviteit door de jaren heen is bewezen door ervaring in de praktijk.

In dit proefschrift hebben we geprobeerd een nieuwe benadering te ontwikkelen voor de evaluatie van de werking van analgetica waarbij de nadruk ligt op de mate van

receptorbezetting als basis voor translationeel onderzoek en de klinische evaluatie van nieuwe geneesmiddelen. In de verschillende hoofdstukken in dit proefschrift hebben we opmerkelijke conceptuele en experimentele vergissingen en fouten in de manier waarop pijnsignalen en farmacologische activiteit worden vastgesteld belicht. Tenslotte hebben we geprobeerd de vertaalslag te vinden tussen species en ziektes .

De rode draad van dit proefschrift is dat nauwkeurige karakterisering van de concentratie-effect-relatie een absolute eis is voor het bepalen van de therapeutische dosis van ieder geneesmiddel, ongeacht zijn werkingsmechanisme. Naast de inhoudelijke uiteenzetting van de onderliggende mechanismen van pijnsignalen en symptomen, worden er drie aandachtsgebieden bestudeerd. Deze benadrukken het belang van farmacokinetiek-farmacodynamiek (PKPD) relaties. Allereerst hebben we de eisen voor het karakteriseren van gedragsmetingen van pijn onderzocht bij het vroeg screenen van geneesmiddelkandidaten. Daarna hebben we de tekortkomingen van de huidige experimentele protocollen belicht. Vervolgens worden de vereisten voor de parameterisering van pijn gedrag toegelicht. Dat maakt de nauwkeurige vertaling van farmacologische eigenschappen tussen species mogelijk, evenals de overbrugging tussen de verschillende fasen van het ontwikkelingsproces. Ten slotte hebben we de klinische respons in chronische inflammatoire pijn gemodelleerd en de correlatie onderzocht tussen de verlichting in de verschijnselen en de onderliggende farmacologische effecten. We hebben aangetoond hoe clinical trial simulaties gebruikt kunnen worden voor de evaluatie van uiteenlopende scenario's om onder andere de bijdrage van het farmacologisch effect te kunnen onderscheiden van zowel het placebo effect als de onderliggende ziekteprocessen.

Samengevat zijn de vraagstellingen van dit proefschrift als volgt:

1. Is het mogelijk om door middel van modellering en simulatie een verband te leggen tussen bevindingen in verschillende fasen van de ontwikkeling van een nieuw geneesmiddel waardoor de voorspellende waarde van specifieke experimenten wordt verbeterd?
2. Is het mogelijk om bestaande data te gebruiken voor het berekenen van parameterverdeling (priors) en deze vervolgens toepassen bij het vaststellen van de werkzaamheid van een nieuw geneesmiddel?
3. Welke experimentele en methodologische eisen zijn van belang voor het optimaliseren van de evaluatie van nieuwe stoffen?
4. Wat kan het karakteriseren van de concentratie-effect relatie betekenen voor de keuze van de therapeutische dosis ?
5. Kunnen clinical trial simulaties toegepast worden voor het optimaliseren van de therapeutische dosis, en op basis daarvan de farmacologische effecten te kunnen onderscheiden van andere versturende effecten in de bestudeerde populatie of doelgroep?

Om de bovengenoemde doelstellingen helder te behandelen is dit proefschrift in drie secties ingedeeld.

In sectie I, de algemene inleiding, wordt de onderliggende pathofysiologische basis van chronische inflammatoire pijn en zenuwpijn toegelicht. Daarnaast zijn de meest voorkomende methodes voor het ontwikkelen van een analgetisch geneesmiddel beschreven.

In de hierop volgende sectie bestuderen we de technische eisen voor de parameterisering van een PKPD model en aan de hand hiervan definiëren we eisen voor het optimaliseren van de opzet van een preklinisch screeningsexperiment, met de nadruk op het gebruik van binaire metingen tijdens het screenen en het ordenen van kandidaat-moleculen voor zenuwpijn.

Ten slotte hebben we zowel biomarkers als de klinische respons in chronische inflammatoire pijn gemodelleerd. Met behulp van een klinische pijn-respons model zijn er correlaties tussen symptomatische pijnverlichting en de onderliggende farmacologische effecten vastgesteld aan de hand van mechanistische biomarkers. Hierbij hebben we de toegevoegde waarde van clinical trial simulaties aangetoond. In de hierop volgende alinea's worden de verschillende hoofdstukken die dit proefschrift omvat één voor één belicht.

Chronische pijn als een stoornis in het signaleren van de pijnwaarneming

Hoofdstuk 1 bestaat voornamelijk uit een overzicht van de vorderingen in het onderzoek naar zenuwpijn en chronische pijn. We hebben aangetoond dat er naast praktische hindernissen meerdere methodologische aspecten zijn, die de ontwikkeling van nieuwe farmaca voor pijnbestrijding verhinderen. Het meest voorkomende probleem is het ontbreken van de zogenaamde constructvaliditeit of begripsvaliditeit van preklinische proeven die tegenwoordig worden gebruikt voor het bepalen van de pijnstillende activiteit van een nieuw geneesmiddel. Pijn-experimenten zijn ontworpen om kortdurende uitingen van pijngedrag te meten, die niet noodzakelijkerwijs de ziekte bij chronische pijn patiënten weerspiegelen. In dit hoofdstuk hebben we een nieuwe benadering voorgesteld waarbij de identificatie van relevante doelwitten is gekoppeld aan potentiële stoffen, en de verschijnselen van pijn zijn gekoppeld aan de onderliggende oorzaak van de stoornis. We hebben aangetoond dat een tijdige diagnose, de timing van de ingreep en de omkeerbaarheid van relevante pathofysiologische processen niet kunnen worden verwaarloosd. Uiteindelijk bepalen deze zaken het succes of het falen van de therapie. Het is daarom essentieel om de onderliggende veranderingen in het centraal zenuwstelsel die betrokken zijn bij het waarnemen van pijn te begrijpen. Idealiter zou men moeten ingrijpen voordat de pijn symptomen tot uiting komen. Zonder deze mogelijkheid blijven experimentele protocollen van beperkte waarde aangezien de onderliggende veranderingen niet tijdig gedetecteerd kunnen worden in de prodromische fase, oftewel vòòr de overgang van acuut letsel naar chronische pijn.

Tevens benadrukken we ook dat de metingen van de waarneming van pijn, zoals deze bij proefdieren wordt uitgevoerd, niet alle betrokken pathways in kaart brengen. Hierdoor ontstaat een belangrijke translationele (interspecies) kloof. Dit heeft verdere gevolgen voor de ontwikkeling van nieuwe farmaci, met name voor het screenen en selecteren van nieuwe moleculen. Bovenstaande punten vormen de grondslag van dit proefschrift.

Modelmatige analyse van pijn gedrag in preklinische onderzoek

Zoals bediscussieerd in hoofdstuk 2, is er een verhit debat geweest over de translationele waarde van preklinische modellen. De algemene opinie is dat deze modellen geen construct of begripvaliditeit hebben, en dat deze enkel de verschijnselen van pijn kunnen nabootsen. Sterker nog, grove eindpunten zoals het terugtrekken van de poot / de voetdrukdrempelwaarde en de tijd tot het terugtrekken van de poot, zijn toegepast als surrogaten voor de evaluatie van de verwachte analgetische activiteit in de kliniek. Ondanks de bovengenoemde beperkingen hebben we benadrukt hoe een beter begrip van het concept van receptorbezetting relevant kan zijn om preklinische gegevens te kunnen vertalen naar de mens en daarmee de therapeutische dosering van een nieuw molecuul beter te voorspellen. Met behulp van recente voorbeelden uit de literatuur hebben we de eisen voor de beoordeling van concentratie-effect relaties in preklinische diersoorten belicht. We hebben het belang van het nemen van bloedmonsters voor farmacokinetische analyse aangeduid, en het samenvatten van de farmacokinetisch-farmacodynamische eigenschappen van het molecuul door middel van parameters die de blootstelling weergeven (EC_{50} in plaats van ED_{50}).

Daarnaast laten we zien dat de zogenoemde standaard experimentele opzet die gebruikt wordt tijdens screening, tot onnauwkeurige parameterberekeningen kan leiden, waardoor onjuiste keuzes worden gemaakt met betrekking tot de potentie en ordening van kandidaat-moleculen. In dit verband hebben we aangetoond hoe wiskundige en statistische modellen toegepast kunnen worden om zowel het biologische systeem als de geneesmiddeleigenschappen op een kwantitatieve manier te beschrijven. Hiërarchische of populatie modellen zijn voorgesteld als basis voor het identificeren van de verschillende bronnen van variabiliteit, evenals voor het beoordelen van de effecten van de behandeling en de progressie van de ziekte. Hiernaast stellen we het concept van biomarkers voor als een intermediaire stap tussen geneesmiddeleffect en de uiteindelijke verschijnselen, die op een systematische manier geïntegreerd kan worden.

Het vaststellen van concentratie-effect relaties op basis van PKPD modellering biedt de mogelijkheid om deze relaties mechanistisch te onderbouwen en de verschillen tussen interspecies mee te nemen bij het bepalen van de therapeutische dosis.

Experimentele pijn modellen. Onvertaalbaar maar toch informatief

Een belangrijke veronderstelling voor het werk in dit proefschrift is dat sommige beperkingen van de manier waarop hedendaags preklinisch onderzoek wordt verricht, ondervangen kunnen worden met behulp van een modelmatige aanpak. Daarmee wordt het mogelijk om onderscheid te maken tussen geneesmiddel-specifieke en systeem-specifieke eigenschappen. Bovendien kan verwacht worden dat parameterwaarden die gebaseerd zijn op concentraties meer informatief kunnen zijn dan die waarbij de dosis wordt gebruikt. Hierdoor is de vergelijking tussen diverse stoffen nauwkeuriger en zijn daardoor betere voorspellingen mogelijk.

Een andere aanname van ons werk was dat gestandaardiseerde experimentele protocollen niet noodzakelijkerwijs ontworpen zijn om optimaal data te genereren of onderscheid te maken tussen moleculen en tussen dosisniveaus. Aangezien pijnexperimenten per definitie empirisch zijn, zullen standaardprocedures simpelweg proberen de reproduceerbaarheid van de metingen te verhogen. Daarom zijn er pogingen gedaan om het resultaat van verbeteringen in de ontwikkeling van nieuwe farmaca voor de behandeling van chronische pijn te evalueren. Zoals eerder in dit proefschrift is vermeld, wordt het gevolg van ontbrekende markers van farmacologie verergerd door gebrekkige data-integratie en geschikte analyse methodes gedurende de selectie van kandidaat-moleculen. We hebben aangetoond dat, terwijl het bestuderen van concentratie-effect relaties gedurende de ontwikkelingsfase van farmaca een *condicio sine qua non* is voor het vaststellen van het effect van behandeling en de variabiliteit daarvan, zulke nauwkeurige berekeningen niet altijd vanzelfsprekend zijn. PKPD relaties moeten voorspellende waarde hebben voor de doelgroep, in kwalitatieve en kwantitatieve termen. Echter tot op heden zijn bovengenoemde overwegingen geen formele voorwaarden voor het genereren van experimentele data, waardoor experimentele protocollen vaak niet informatief genoeg zijn of sterker nog, zelfs onjuist.

Om de probleemstelling en bovengenoemde concepten nader te illustreren, hebben we twee experimentele modellen van pijngedrag geselecteerd. Eerst werd de drempelwaarde voor het terugtrekken van de poot gemeten na een normaal niet-pijnlijke prikkel in het zogeheten complete Freund adjuvant (CFA) model in de rat. In het tweede geval werd de verandering in de frequentie van terugdeinzen (flinching) na injectie van een pijn opwekkende stof, namelijk formaline, gekozen als een marker voor het pijnstillende effect. In tegenstelling tot het CFA model, is de pijnrespons bij deze laatste methode een spontaan gedrag. Daarvoor wordt geen pijnprikkel gebruikt. De pijnlijke reactie die veroorzaakt werd door formaline is het resultaat van een geconditioneerde respons, die de betrokkenheid van hogere cognitieve functies weerspiegelt. Geneesmiddelen met uiteenlopende werkingsmechanismen kunnen onderscheiden worden aan de hand van het type en de omvang van de effecten op het gedrag. Daarom veronderstellen we dat het formaline-geïnduceerde pijnmodel meer constructvaliditeit toont ten opzichte van andere modellen

die gebaseerd zijn op kortdurende stimulaties. Vanuit een methodologisch oogpunt hebben we tevens aangetoond dat de juiste parametrisering evenals de nauwkeurigheid van de geschatte waarden (modelparameters) van cruciaal belang zijn. Ongetwijfeld zijn de beperkte beschikbare data gedurende de screeningsfasen van farmaca de oorzaak van twee belangrijke statistische kwesties voor de toepassing van een modelmatige benadering, oftewel de identificeerbaarheid en precisie van de parameters.

In hoofdstuk 4 en 5 hebben we aangetoond hoe de werkzaamheid van twee analgetische stoffen (gabapentine en pregabaline) kan worden vastgesteld en eventuele verschillen tussen hen geïdentificeerd. Om de toepassing van onze benadering in toekomstig onderzoek te vereenvoudigen werd werkzaamheid gedefinieerd in termen van een binaire (ja of nee) respons. Op basis van een logistisch regressiemodel hebben we systeem-specifieke eigenschappen weten te onderscheiden van stof-specifieke eigenschappen. Hierdoor kon de werkzaamheid geschat worden en de twee geneesmiddelen met elkaar vergeleken worden. Het was ons doel om de schatting van de EC_{50} en de interindividuele variabiliteit (IIV) nauwkeuriger te maken. Daarvoor gebruikten we de zogeheten ED optimaliteit techniek om de relatie tussen bloedconcentraties en de pijnrespons te beschrijven, rekening houdend met de onzekerheid rondom de geschatte parameterwaarden. Om praktische en theoretische redenen wilden we zowel de dosis als de bloedmonster-afname-tijdpunten optimaliseren. Informatie betreffende de systeem-specifieke eigenschappen werd geïntroduceerde als priors (bestaande informatie) voor de optimalisatie procedures. Onze analyse heeft ook de consequenties van de empirische dosisselectie in kaart gebracht, die vaak te hoog zijn waardoor de berekening van de EC_{50} aanzienlijk minder precies wordt. Ondanks het grote bereik van de doseringen die gebruikt werd in de oorspronkelijke protocollen, verschilden de gesimuleerde concentratieprofielen niet significant van elkaar. De relatieve biologische beschikbaarheid daalde van 100% bij de 10 mg/kg dosis tot 9% bij 300mg/kg. In tegenstelling tot experimenten waarbij ED_{50} wordt berekend ongeacht de plasmaspiegels, toonden protocol-optimalisatie procedures de waarde van de juiste bloedafname tijd en dosisschema. EC_{50} waarden voor gabapentine en pregabaline waren respectievelijk 1400 en 897 mg/ml. Wij stellen hierdoor vast dat de verschillen in werkingssterkte in *in vitro* experimenten ook aantoonbaar zijn in de *in vivo* studies. Al met al toonden deze bevindingen aan dat protocol-optimalisatie een verbetering betekent voor de precisie en een vermindering in de systematische fout. Wij verwachten dat deze procedures een basis zullen verschaffen voor de validatie van experimentele diersystemen, waardoor niet alleen de gevoeligheid en specificiteit van de protocollen verhoogd kunnen worden, maar ook vals-positieve en vals-negatieve resultaten beter gekarakteriseerd kunnen worden.

In hoofdstuk 6 hebben we getracht een semi-mechanistisch model te ontwikkelen om de effecten van gabapentine op formaline-geïnduceerde pijn in een rat diersysteem te kunnen

beschrijven. De pijn-inducerende effecten van formaline in dit diemodel zijn aangeduid als een verhoogde frequentie van terugdeinzen waarin er twee pieken van maximale pijnintensiteit voorkomen. In ons semi-mechanistische model werd de eerste piek beschreven met behulp van een mono-exponentiële afnemende functie terwijl de tweede, meer aanhoudende piek werd beschreven met een indirect respons model met een in de tijd variërende parameter die de synthese van pijn gerelateerde mediators weerspiegelt. De effecten van het geneesmiddel werden geparametriseerd door een I_{\max} functie, die als een covariabele de pijn-respons beïnvloedt.

Onze parametrisering van geneesmiddeleffecten wijkt af van effecten die traditioneel beschreven worden in het desbetreffende model, waarin effecten worden toegepast op de synthesesnelheid of eliminatiesnelheid van de ziekte. Ondanks dit verschil heeft onze weergave van de pijnrespons en geneesmiddeleffecten een mechanistische basis omdat gabapentine geen ziekteremmende effecten heeft, en het slechts de pijn enigszins kan verminderen. Interessant is dat de gemiddelde IC_{50} waarden die we hebben gerapporteerd (7510 ng/ml) van dezelfde orde van grootte zijn als die gepubliceerd door Lockwood en Whiteside *et al.* voor gabapentine in klinische studies. Anderzijds, ondanks hoge bloedspiegels werden de pijnsymptomen (frequentie van terugdeinzen) niet volledig onderdrukt door gabapentine. Deze discrepantie geeft duidelijk aan, dat de werkzaamheid van gabapentine beperkt is in deze aandoening.

Terwijl we concrete verbeteringen hebben kunnen aantonen door het invoeren van een modelmatige benadering, hebben we ook in kaart gebracht dat biomarkers van kritische belang zijn om een vertaalslag te kunnen maken tijdens de ontwikkeling van een geneesmiddel. Het is onbetwist dat er een behoefte is aan het verkrijgen van aanwijzingen van de werkzaamheid in de vroege stadia van het ontwikkelingstraject. Dit kan door het inschakelen van biomarkers die als schaaufactoren kunnen dienen bijvoorbeeld bij de interspecies-translatie, en tevens ook als hulpmiddelen voor de extrapolatie van effecten in verschillende populaties zoals gezonde vrijwilligers en patiënten.

In de volgende secties worden de overwegingen voor het gebruik van biomarkers als basis voor het bepalen van dosisschema's gepresenteerd.

BIOMARKER-GESTUURDE DOSISSELECTIE EN HET VOORSPELLEN VAN KLINISCHE RESPONSE

Om het percentage geneesmiddelen dat faalt tijdens de ontwikkeling terug te dringen moet men focus geven op twee strategieën. Ten eerste een verbeterde selectie van targets, rekening houdend met de betrokken pathways evenals de timing van de diagnose en

ingreep. Ten tweede dienen routinematig zogeheten proof-of-concept onderzoeken plaats te vinden, bij voorkeur gedurende de fase 1 trials of in experimenten (bijvoorbeeld in humaan weefsel) waarbij het farmacologische effect (farmacodynamiek) op de doelgroep kan worden nagebootst. Op deze manier kunnen biomarkers gebruikt worden als maatstaf voor werkzaamheid en/of veiligheid. Ondanks de aanwijzingen uit andere therapeutische gebieden die deze benadering ondersteunen, wordt dit concept verwaarloosd in onderzoeksprotocollen voor chronische pijn. In het algemeen wordt onderzoek naar pijnstillende farmaca voornamelijk gebaseerd op klinische schalen, zonder de onderliggende farmacologie of receptorbezetting in ogenschouw te nemen.

De integratie van biomarkers die het farmacologische effect voorspellen al in de vroege stadia van het ontwikkelingstraject geeft de mogelijkheid de therapeutische dosis nauwkeuriger te selecteren dan wanneer gebruik wordt gemaakt van empirisch bewijs afkomstig van specifieke metingen van gedrag.

In hoofdstuk 7 hebben wij laten zien dat, wanneer biomarkers gebruikt worden voor het voorspellen van de werkzaamheid, het klinisch-farmacologisch profiel van nieuwe moleculen gekarakteriseerd kan worden, waardoor een mechanistisch inzicht wordt gegeven in de relatie tussen blootstelling en effect, wat op zijn beurt leidt tot een betere onderbouwing voor het therapeutische doseringsbereik. Bovendien kan het kwantificeren van de farmacokinetisch-farmacodynamische relatie waarbij gebruik wordt gemaakt van biomarkers een meer solide basis geven voor behandeling van individuele patiënten.

Het concept van biomarker-gestuurde dosisselectie werd geïllustreerd door middel van data van GW406381, een cyclo-oxygenase (COX) remmer, waarbij de nadruk werd gelegd op het verwerven van inzicht in de klinische farmacologie van het middel om de optimale dosering al voor phase 2 vast te kunnen stellen. De keuze voor deze klasse geneesmiddelen als paradigma werd ingegeven door het van de markt halen van diverse middelen waarbij voldoende informatie betreffende het farmacologisch profiel voorhanden was om de veiligheid en werkzaamheid te kunnen bepalen.

Ons onderzoek is gebaseerd op data van een gerandomiseerde, dubbel blinde fase 1 studie in gezonde mannelijke vrijwilligers waarbij een enkele dosis werd gevolgd door meermalige doses gedurende 10 dagen. De nuchtere proefpersonen kregen een orale dosis van 35 of 70 mg GW406381 toegediend, waarbij plasma concentraties van het geneesmiddel, alsmede de waarden van de biomarkers prostaglandin E_2 (PGE_2) en thromboxane B_2 (TXB_2) op vaste tijdstippen werden bepaald. De analyse werd verricht in twee stappen: eerst werd de farmacokinetiek van GW406381 beschreven met behulp van een één-compartiment-model met eerste-orde absorptie. Vervolgens werd aan de hand van de prostaglandine concentraties een I_{\max} model ontwikkeld om de blootstelling-respons relatie te beschrijven. Door integratie van de farmacokinetische data uit de fase 1 studie met de PGE_2 -data, zoals bepaald in een *ex vivo* analyse, werd getoond hoe biomarkers kunnen leiden tot

verbeterde dosis selectie in de opeenvolgende fasen van geneesmiddelontwikkeling. Het is noemenswaardig dat onze analyse succesvol was ondanks het hoge variabiliteit in de PGE_2 data. De gemiddeld werkingssterkte (IC_{50}) bedroeg 43 ng/ml met een IC_{80} en IC_{95} van respectievelijk 103 en 276 ng/ml.

Hiernaast toonde de hoge variabiliteit in zowel farmacokinetiek als farmacodynamiek in de gezonde vrijwilligers een belangrijke beperking van het gebruik van de *in vitro* potentie als criterium om geneesmiddelen te vergelijken in vroege fasen van ontwikkeling, omdat die de verschillen in selectiviteit of metabole activiteit *in vivo* niet reflecteren. Fries *et al.* lieten zien dat, ondanks de aanzienlijke hogere *in vitro* potentie van rofecoxib ten opzichte van celecoxib, de *in vivo* potentie weinig verschilt. Ook de *in vitro* potentie van GW406381 werd 30 maal hoger geschat dan die van rofecoxib. Echter, de aanbevolen optimale dosis gebaseerd op onze simulaties ligt tussen 150-250 mg terwijl die van rofecoxib 25-50 mg bedraagt. Dit wordt grotendeels verklaard door interindividuele (metabolische) verschillen in de farmacokinetiek. Verder hebben wij aangetoond dat, met de voorgestelde dosis leidend tot 80-95% inhibitie, tweemaal daags doseren gunstiger was dan eenmaal daags; piekconcentraties bleven korter boven de IC_{95} bij hogere doses, zonder dat een significant effect had op de dalconcentraties.

Aan de hand van uiteenlopende simulatiescenario's lieten we zien hoe, door gebruik te maken van biomarkers, dosis aanpassingen bij speciale populaties konden worden gedaan. Dit betrof patiënten met leverfunctiestoornissen, metabole inductie en gegeneraliseerde inflammatie. Hieruit kwam naar voren dat metabole inductie evenals leverfunctiestoornissen leiden tot significante verschillen in de totale blootstelling waardoor dosis aanpassen cruciaal zijn om een vergelijkbare blootstelling te verkrijgen en te behouden gedurende de behandeling. Omgekeerd behoeft een verhoogde uitgangskonzentratie PGE_2 geen dosisaanpassingen. Samengevat maakte dit werk de evaluatie van doseringen mogelijk, rekening houdend met de balans tussen de baten en de risico's van de behandeling, die naast de totale dosis ook afhangen van het doseerschema. Zoals aangetoond wordt deze balans bereikt bij een inhibitie van PGE_2 tussen 80 en 90%. Onder deze omstandigheden wordt bij chronische therapie de induceerbare fractie van de beschikbare COX_2 geblokkeerd, terwijl voldoende residuele activiteit van PGE_2 en andere COX_2 -gerelateerde enzymen behouden blijft voor homeostase en weefselherstel.

In de laatste gedeelte van dit proefschrift hebben we het concept van biomarker-gestuurde dosisselectie verruimd naar fase 2 klinisch onderzoek om de relatie tussen biomarkers en de symptomen van pijn te onderzoeken. Door de mechanistische classificatie toe te passen, die beschreven werd door Danhof *et al.*, was het mogelijk om de veronderstelde relatie tussen blootstelling en biomarker van GW406381 te beschrijven. Hiertoe werden ACRn scores gebruikt, die verzameld waren in een grote klinische studie naar de effecten van

COX2 remmers bij reumatoïde artritis. Hoewel de rol van ACRn als een maat van klinische verbetering vaststaat, is de relatie met de onderliggende farmacologische activiteit na toediening van COX2 remmers niet eerder bepaald. In hoofdstuk 8 werd de rol van biomarkers verder onderzocht door deze bij gezonde personen en patiënten te correleren aan ACRn.

Ondanks het beperkte aantal doses welke tijdens de studie onderzocht waren (0, 10, 35 en 50 mg eenmaal daags oraal), lieten wij zien hoe simulaties en longitudinale farmacokinetisch-farmacodynamische modellen inzicht verschaffen in de farmacologische effecten welke ten grondslag liggen aan de klinische respons.

Een integrale benadering werd ontwikkeld waarbij informatie over het gehele tijdsverloop van het behandelingseffect werd gebruikt, terwijl het in de huidige praktijk gebruikelijk is de werkzaamheid tussen groepen te bepalen op basis van de respons na afloop van de behandeling. Het tijdsverloop van de pijnrespons werd het best beschreven door een Weibull functie met een I_{max} model voor het geneesmiddel effect. Twee fenotypes konden worden geïdentificeerd in de populatie, waarbij patiënten met een minimale daling van 25% in ACRn scores ten opzichte van basislijn werden beschouwd als responders. Opvallend genoeg was het percentage responders niet evenredig met de dosis, wat de hoge variabiliteit in farmacokinetiek en farmacodynamiek reflecteert. Hoewel informatie over hogere doses ontbrak, zijn deze bevindingen in lijn met het voorspelde percentage receptorbezetting, zoals bepaald door PGE_2 inhibitie.

Gebaseerd op de aanname dat klinische analgesie optreedt bij een prostaglandine-inhibitie van minimaal 80%, kan voorgesteld worden dat de geselecteerde doses gebruikt in deze klinische studie niet optimaal en meestal sub-therapeutisch waren. Volgens onze analyse van de biomarker respons ligt de therapeutische dosis van GW406381 waarschijnlijk tussen 100 en 250 mg per dag.

Om de voorspellende waarde van een modelmatige benadering voor de dosisselectie van analgetische geneesmiddelen te evalueren werd verder ingegaan op de relatie tussen biomarker en klinische respons. In **hoofdstuk 9** werd de veronderstelde relatie tussen inhibitie van PGE_2 en ACRn onderzocht in patiënten met reumatoïde artritis. Er werd niet alleen aangetoond dat PGE_2 kon worden gebruikt als surrogaat voor receptorbezetting (d.w.z. COX₂ inhibitie), ook gaf de relatie tussen biomarker en klinische respons inzicht in de verschillende oorzaken van de variabiliteit in een heterogene populatie. Met behulp van de in hoofdstuk 7 en 8 ontwikkelde farmacokinetische en farmacokinetisch-farmacodynamische modellen kon het tijdsverloop van de klinische respons (ACRn) en PGE_2 worden gesimuleerd. Dit hypothetische experimentele protocol was gebaseerd op een typerende klinische studie bij reumatoïde artritis waarbij 500 patiënten per dosisgroep gedurende 6 weken behandeld werden. Om de verschillende bronnen van variabiliteit te kunnen karakteriseren varieerden de doses GW406381 van 10 tot 400 mg per dag. Patiënten werden geclassificeerd als

responders of non-responders op basis van de gesimuleerde uitkomst na afloop van de behandeling. Onze resultaten lieten een grote variatie in het tijdsverloop van de pijnscores zien, ongeacht de uitgangswaarde, waarbij de voorgespelde PGE_2 inhibitie boven 80% lag bij doses hoger dan 250 mg. Verder nam het aantal responders niet-lineair toe bij toemende gesimuleerde doses, waarbij een 50% reductie van ACR_n werd bewerkstelligd bij een dosis van meer dan 100 mg/dag. De aangetoonde concentratie-effect relatie geeft een robuustere basis voor het bepalen van het doseerschema, welk traditioneel worden bepaald door het effect van de behandeling op basis van zichtbare symptomen.

Concluderend hebben we aangetoond dat modelleren en simuleren de vertaling van experimentele bevindingen gedurende de diverse fasen van ontdekking en ontwikkeling van geneesmiddelen kan vergemakkelijken, waardoor de dosisselectie alsmede de ontwikkeling van experimentele protocollen verbeterd kunnen worden. Hiervoor is de beschikbaarheid van longitudinale data van cruciaal belang. De experimentele protocollen moeten worden gebaseerd op schema's welke het karakteriseren van farmacokinetiek, biomarkers en werkzaamheid mogelijk maken. Hiernaast kunnen, wanneer in het model onderscheid wordt gemaakt tussen geneesmiddel- en systeem-specifieke eigenschappen, de geneesmiddel-specifieke eigenschappen systematisch onderzocht worden, rekening houdend met bestaande kennis over het systeem.

We hebben aangetoond dat statistische veronderstellingen gebruikt kunnen worden om de onzekerheid in de schatting van de parameters te verminderen, om zodoende de variabiliteit in de experimentele data te onderzoeken. Door een geneesmiddel te gebruiken met aangetoonde analgetische activiteit konden wij het belang van de concentratie-effect relatie voor doseeradviezen onderstrepen. Dit vormt de basis voor de evaluatie van het effect van covariabelen op de blootstelling en de invloed daarvan op de respons op het geneesmiddel.

De in dit proefschrift gepresenteerde benadering, waarin modellering en simulatie worden gebruikt als gereedschap om conclusies te trekken, vertegenwoordigt een verschuiving van het empirisme dat de selectie van kandidaat moleculen, de onderbouwing van dosisselectie en het ontwerp van fase 2b en 3 klinische studies voor chronische pijn heeft gedomineerd. Het lijkt geen twijfel dat de klinische respons niet mogelijk is zonder voldoende receptor bezetting. Anderzijds heeft een toename van blootstelling geen toename van de respons tot gevolg wanneer de maximale of benodigde receptor bezetting bereikt is. Tenslotte laten onze resultaten ook zien dat door gebruik te maken van farmacokinetisch-farmacodynamische modellen kan niet alleen receptorbezetting maar ook biomarkers systematisch worden bestudeerd tijdens de ontwikkeling van analgetische geneesmiddelen.

TOEKOMSTIGE PERSPECTIEVEN

Doorgaans wordt de ontwikkeling van een geneesmiddel beschouwd als een lineair proces dat begint met het identificeren van een target en eindigt met markt toelating. Dit creëert een sequentiële aanpak bij de besluitvorming waardoor de gelegenheid ontstaat om te kunnen leren bij iedere stap in de ontwikkelings keten. In de werkelijkheid echter vindt iedere stap geïsoleerd plaats en is er bij elke stap nauwelijks integratie of toename van informatie van de voorafgaande stappen vanuit een klinisch of biologisch perspectief. Er is daarom een aanpak vereist die afwijkt van de huidige gefragmenteerde strategie gericht op geïsoleerde targets of werkingsmechanismes. Er moet rekening worden gehouden met de onderliggende systemen en ziekteprocessen die neurale overgevoeligheid voor pijn (hypersensitisatie) opwekken. Verder moet geprobeerd worden om de onderliggende stoornissen in signalen te detecteren die voorafgaan aan de zichtbare belevenis van pijn.

Profylactische behandelingen zijn noodzakelijk om te zorgen dat de homeostase in het weefsel is hersteld na het optreden van letsel en de daarop volgende acute inflammatoire respons. In deze context moet overwogen worden om parallel aan geneesmiddelontwikkeling ook diagnostische biomarkers te ontwikkelen. Een belangrijk vraagstuk dat telkens opduikt is of bestaande diermodellen ons ooit de benodigde informatie kunnen leveren om een vertaalslag tussen dieren en mensen te kunnen maken. Een aanpak gebaseerd op modellen is essentieel om de opzet van experimenten te optimaliseren en de kwaliteit van informatie te verbeteren. Wiskundige modellen kunnen gegevens afkomstig van een breed scala aan experimentele protocollen, waaronder in-vitro experimenten, op systematische wijze integreren.

Woolf en collega's hebben een nieuwe aanpak voor geneesmiddelontwikkeling voorgesteld, waarbij genetica wordt toegepast om potentieel analgetische targets te valideren. Voorbeelden zijn de genetische polymorfismen van het voltage-gated kanaal Nav1.7 en het enzyme GTP cyclohydrolase-1 die betrokken zijn bij verminderde gewaarwording van pijn. Op dezelfde manier kunnen andere aanpakken worden overwogen, waarbij een vroege extrapolatie van de farmacologie naar mensen wordt toegepast, ongeacht verschillen in de keuze van onderzoeksmethoden of protocollen.

Alle voorstellen voor aanpak zijn in overeenstemming met de rol van mechanistische biomarkers in geneesmiddelontwikkeling. Anderzijds zijn er nog steeds geen gevalideerde biomarkers voor chronische pijn. De identificatie en het gebruik van biomarkers voor CNS aandoeningen is moeilijker dan voor andere ziekten. Een aantal overzichtsartikelen hebben de huidige kennis over kwantitatieve beeldvorming van de hersenen belicht maar geen daarvan heeft de mogelijkheden van deze technieken onderzocht voor het ontdekken en definiëren van biomarkers van pijn en hun toepassing bij het ontwikkelingstraject van geneesmiddelen. Nieuwe ontwikkelingen in functionele imaging zullen ongetwijfeld het identificeren van biomarkers van pijn mogelijk maken en zullen daarbij helpen de rol van

verschillende hersengebieden beter te begrijpen. Dit is vooral nuttig voor processen waarbij neuroplasticiteit optreedt, wat een voorloper is van de ontwikkeling van chronische pijn. Tezamen met fysiologische verschijnselen zou het mogelijk kunnen zijn te anticiperen op signaalstoornissen voordat deze zichtbaar worden.

Alles bij elkaar zal een verhoogd en verfijnd begrip van abnormale activiteit, met name van de synaptische connectiviteit resulteren in de ontwikkeling van effectieve en meer doelgerichte behandelingen. Deze vorderingen zullen het beslissingsproces tussen de experimenten in het laboratorium en de eerste klinische studies zowel versnellen als verbeteren. Echter, een diepgaand begrip van het pijnsyndroom is niet haalbaar op basis van functionele imaging alleen. Dit zal geïntegreerd moeten worden met uiteenlopende disciplines zoals metabolomics en proteonomics om beter inzicht te verschaffen in hoe de voorspellende waarde van biomarkers verhoogd kan worden. Het zal cruciaal zijn om de vroege directe effecten van het geneesmiddel te onderscheiden van latere chronische effecten geassocieerd met langdurige behandeling. Ongeacht de vooruitgang in de disciplines van imaging en proteonomics, spelen PKPD modellen een belangrijke rol als hulpmiddelen om de klinische relevantie van een kandidaat geneesmiddel in te schatten en bij te dragen aan het bepalen van de therapeutische dosis. Daarnaast zullen PKPD modellen ons in staat stellen om een vertaalslag te maken tussen dieren en mensen. Door middel van systems pharmacology kunnen pathways geassocieerd met signaal processen beter in kaart gebracht worden. Door aan te nemen dat betrokken pathways in experimentele omstandigheden en ziekte hetzelfde zijn, kan modellering gebruikt worden om de receptor bezetting en tevens de daardoor veroorzaakte farmacologische effecten te voorspellen. Het is aannemelijk dat schalingsfactoren geïdentificeerd kunnen worden om de invloed van potentiële verschillen zoals receptordichtheid tussen proefdieren en patiënten te verklaren. Een soortgelijk concept is recentelijk succesvol toegepast voor antipsychotica waarvoor *in vitro* receptorbezetting bij ratten werd vertaald naar klinische werkzaamheid.

Hiernaast kunnen kwantitatieve technieken ingevoerd worden om de rol van fenotypische verschillen en andere invloedrijke factoren (covariabelen) op de farmacokinetiek en respons in kaart te brengen. Door deze factoren mee te nemen in de rationale voor dosisselectie en aanbevolen indicaties zal de variabiliteit in klinische respons verminderd worden. Wat betreft de uitdagingen bij het geneesmiddelonderzoek voor chronische inflammatoire pijn, is er een verschil met neuropathische pijn. In inflammatoire pijn zijn reeds enkele biomarkers bekend en worden imaging technieken al gebruikt. Er is behoefte aan overeenstemming tussen alle betrokkenen over het aanpassen van de regulatorische richtlijnen die momenteel geen onderzoek van de onderliggende farmacologie vereisen en het paradigma van 'leren en bevestigen' niet toepassen. Ongetwijfeld zal simulatie een belangrijke rol gaan spelen bij de evaluatie van heterogeniteit in de doelgroep evenals bij het evalueren van de variabiliteit in de respons op de behandeling. In deze context zal de integratie van statistische modellen

die factoren zoals drop-out, censurering en andere studieopzet aspecten beschrijven samen met mechanisme-gebaseerde modellen van ziekteprogressie, uitermate belangrijk zijn. Tenslotte voorzien we dat er vooruitgang behaald kan worden in het voorspellen van de respons op pijn door het bestuderen van meerdere eindpunten alsmede door het incorporeren van volledig mechanistische modellen in het farmacometrische kader neergezet in dit proefschrift.

APPENDICES

**ACKNOWLEDGEMENTS/DANKWOORD
LIST OF PUBLICATIONS**

ACKNOWLEDGEMENTS/DANKWOORD

After achieving her dream and creating a world record by crossing the 154 kilometer wide Florida Straits, endurance swimmer Diana Niyad remarked, 'it looks like a solitary sport but it is a team effort. The completion of this thesis would not have been a reality without the seminal contributions of many.

Oscar, we have had our share of disagreements and even heated debates, but looking back we can both be proud of having done good science. This meant challenging established beliefs and being confronted and disregarded by certain reviewers repeatedly, but that is how a scientist's insight evolves. Finally but for your trust in an 'unknown variable', or a voice at the end of a telephone line, it would not have begun! *Ti ringrazio di tutto.*

Dealing with pain research represented an opportunity to optimise some rudimentary aspects of current protocols. Optimal design theory is considered daunting even by hardened mathematicians and I had the good fortune of being mentored by one of the gurus in the field, Joakim Nyberg, from the University of Uppsala. It was also a pleasure to work with Inaki Troconiz on the formalin project, which in the beginning seemed undoable. I express my gratitude for his guidance through the concepts of indirect response modeling and for making himself always available for telecons even at short notice.

It is said that if you want to teach someone to build a boat, teach him a love for the sea. From our insightful discussions, Vincenzo di Iorio, I have learnt immensely about how to parameterise a problem. You were able to infect me with your passion for R and programming in general. I may not have become a complete nerd as yet, but I will get there someday. You will agree with me that it is indeed possible for an old dog to learn new tricks. *Grazie mille.* Maurice Wang, with your unassuming nature and astute mind, you were an asset in the PKPD group and like many others, I benefitted from our interactions. Tarj Sahota, you have a special ability to explain difficult concepts in a lucid manner, I could always get leads to the problems that I was confronted with. I am also grateful to my internal review committee, comprising of Massimo, Francesco, and Li for taking the time out to review the various sections of my thesis.

Venkatesh and Martin, it was quite a coincidence that three Indians, all from the Indian pharmaceutical industry (of which two from the same company), started at almost the same time in TI Pharma. I enjoyed our camaraderie over the years and thanks for all the helpful hints when I moved to Groningen.

All in all, the nearly five years I spent in the Division of Pharmacology were as instructive as they were fun. It was a vibrant atmosphere with academic interactions and journal clubs interspersed with social events, department picnics, bowling evenings and more. In short 'gezelligheid' exemplified. Thanks to all those who made it possible.

For being my sheet anchor and for just being there, dear Aanchal, no words would suffice. At times when the going got really tough, it was your support that got me through those times. It was not easy to set up a life and a home in a foreign country, with Avnay still small, and me being preoccupied with my research, but yet you made it work. Lastly, thanks to mom and dad for giving me your blessings to seek a challenging path when I was at the cross-roads of my career. The values you have instilled in me define who I am today. Manik, there is a part of you that will always be a happy-go-lucky school boy, but there is nobody else I would rather have as my kid brother. Nanu nani, for your unconditional love and support we were able to take this step in our lives. I count myself lucky to have a family such as you all. To close this section my favourite *sher Hazaron kwaishen aisi ki har khwaish pe dam nikale Nikale armaan bahut se par phir bhi kam nikale*.

De reis van een duizend mijl begint met een enkel stap (Chinese gezegde). In tegenstelling tot de Chinese filosoof, hoefde ik mijn duizend mijl niet in mijn eentje af te leggen, maar er waren veel mensen die een steentje hebben bijgedragen om de reis leuk en aangenaam te maken.

Meindert, mede dankzij uw leiderschap konden problemen met data beschikbaarheid, verblijfvergunningen en afnemende interesse van de partners afgehandeld worden, waardoor ik dit onderzoek kon volbrengen. Ondanks dat u gelijktijdig meerdere functies moest uitvoeren, wist u altijd op de hoogte te blijven van de stand van zaken van iedere AIO in de groep. Ik zal de regenachtige avond in mei dit jaar niet gauw vergeten toen ik kleddernat op uw stoep belandde om de puntjes op de "i" van mijn proefschrift te zetten.

Als project manager van de eerste TI Pharma PKPD platform, wist jij, Margot, op een efficiënte wijze project-gerelateerd problemen op te lossen. Daarnaast zorgde je ervoor dat er geen gebrek was aan hard- of software. Dankzij allerlei cursussen en workshops die je regelde, konden we snel aan de slag met ons onderzoeksprojecten. Anderzijds heb ik de gelegenheid gehad om te leren hoe frusterende modelleren kan zijn. Vincent, Ibrahim en Rick, de tijd dat we hebben doorgebracht leidde tot het vormen van hechte banden; dat heeft me geholpen om mijn teleurstellingen tegen te gaan. Vincent, je behulpzame instelling stel ik zeer op prijs.

Van een stagiair wordt verwacht dat hij of zij een steentje bijdraagt aan het onderzoek van een promovendus. Sean je overtrof mijn verwachtingen met gemak. Al gauw had je de fijne kneepjes van het vak begrepen en ik hoefde alleen maar een ondersteunende rol te spelen. Met jouw natuurlijke aanleg voor de beta-wetenschap en jouw bekwaamheid in programmeren had je überhaupt weinig begeleiding nodig. En als je ooit met vakantie in India bent, dan bezorg ik je een rit op een echte olifant!

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Paranimfen waren oorspronkelijk bedoeld om het proefschrift te verdedigen wanneer de promovendus daar zelf niet toe in staat was. Daarom viel de keuze op twee van de stoerste jongens in de afdeling. Rick en Sven, dank hiervoor, en voor jullie bijdrage aan mijn Nederlandse samenvatting. Annelien, het was leuk om jouw te leren kennen en enkele perspectieven over het leven en onderzoek uit te wisselen. Ook bedankt voor het nakijken van mijn Nederlands samenvatting ondanks dat je naar een congres moest. Hans Proost, ik wil je bedanken voor je begrip toen ik tijd nodig had om na te denken over de vervolgstap in mijn carrière. Ik stel het op prijs dat je me de ruimte gaf toen ik in de slotfase van mijn onderzoek was en ik wekelijks moest pendelen tussen Leiden en Groningen. Last but not the least, bedankt voor het oppoetsen van mijn Nederlands samenvatting.

Onze levens werden verrijkt door hechte vriendschappen en hier begin ik met onze dierbare vrienden Cor en Loes Hoefsloot. Van jullie hebben we niet alleen de Nederlandse taal en cultuur geleerd, maar jullie zijn ook onze familie geworden. De fiets- en autotochten die we met zijn allen hebben gemaakt, staan in onze geheugens gegrift. Daarnaast Cor, leerde je me klussen en dat heb ik nu bij onze woning in Groningen toegepast. Jullie stonden voor ons altijd klaar en we missen jullie gezelschap enorm.

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Toen ik naar Groningen verhuisde, had ik feitelijk twee banen. Ik moest mijn proefschrift met spoed afronden en de draad van mijn nieuwe aanstelling oppakken. Daarnaast moest ik me nog in een onbekende stad aanpassen. Dit betekende lange dagen op het werk om het bij te kunnen houden. Na een lange dag kwam ik meteen tot rust op de bovenste verdieping van de warme en comfortabele woning op de Kraneweg waar Maria en Jan wonen. Door jullie gastvrijheid en zorgzaamheid, gedurende de tijd dat ik bij jullie verbleef, voelde ik me altijd thuis.

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A handwritten signature in black ink, appearing to read 'Amit', with a stylized flourish at the end.

Amit

LIST OF PUBLICATIONS/PRESENTATIONS

1. **Taneja A**, Di Iorio VL, Danhof M, Della Pasqua O, Translation of Drug Effects from Experimental Models of neuropathic pain and analgesia to humans. *Drug Discovery Today*. 2012 (15-16):837-49.
2. **Taneja A**, Nyberg J, de Lange E, Danhof M, Della Pasqua O. Application of ED-optimality to Screening Experiments for Analgesic Compounds. *Journal of Pharmacokinetics and Pharmacodynamics*. 2012;39(6:673-81).
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4. **Taneja A**, Troconiz IF, Danhof M, Della Pasqua O. Semi-Mechanistic Modelling Of The Analgesic Effect Of Gabapentin In The Formalin-Induced Rat Model Of Experimental Pain. Published online. 5th October 2013. *Pharm Res*.

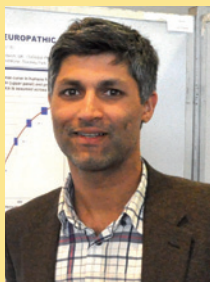
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5. **Taneja A**, Danhof M, Della Pasqua O, From Behaviour to Target: Evaluating the putative correlation between clinical pain scales and biomarkers. PAGE 2013, June 2013, Glasgow UK. *Nominated for the best poster prize at the Spring meeting of the Top Institute Pharma, Jaarbeurs Utrecht, 1 May 2013.*
6. **Taneja A**, Nyberg J, Danhof M, Della Pasqua O, Pain Project Members of the TI Pharma mechanism-based PKPD modelling platform, Optimisation of Screening Experiments for the Assessment of Analgesic Effects, PAGE June 2011, Athens Greece. <http://www.page-meeting.org/default.asp?abstract=2132>
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*Free from attachment, free from egoism, full of a fixed (impersonal) resolution
and a calm rectitude of zeal, unelated by success, undepressed by failure,
that doer is called sattwic.*

-Bhagwad Gita

CURRICULUM VITAE



Amit Taneja was born in Namkum, India on the 25th of June 1970. He graduated in medicine followed by a specialization in Pharmacology from the B.J. Medical college and Sassoon group of hospitals, Pune India which he completed in June 1997.

Thereafter he was employed as a research officer and then as lecturer in the department of Clinical Pharmacology and the department of Pharmacology and Therapeutics, respectively at the Seth GS Medical college and KEM hospital Mumbai, up to 1999.

He then moved to the pharmaceutical industry where he worked in various capacities within the departments of medical affairs and clinical research, clinical development and regulatory affairs, up to 2008. During this period he was involved in the design, conduct and analysis of clinical trials ranging from Phase 1 to Phase 4 studies for both new chemical entities, and generic drugs and their combinations. For the majority of his industry career, he worked for the Indian pharma major Dr. Reddy's laboratories Ltd. Motivated by emerging literature on quantitative and model based drug development he decided to seek new frontiers which could also provide solutions to some of the challenges faced by drug developers world-wide. In June 2008 he moved to Leiden the Netherlands, to do his PhD in model based analysis of neuropathic and chronic pain, at the division of Pharmacology of the Leiden Academic Center for Drug Research, under the supervision of Dr Oscar Della Pasqua, which culminated in this thesis.

He is presently working as a research fellow at the Department of Pharmacokinetics and drug delivery, the University of Groningen, the Netherlands. His current research focus is the translational modeling of prolactin response.

