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

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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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## 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 are 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.

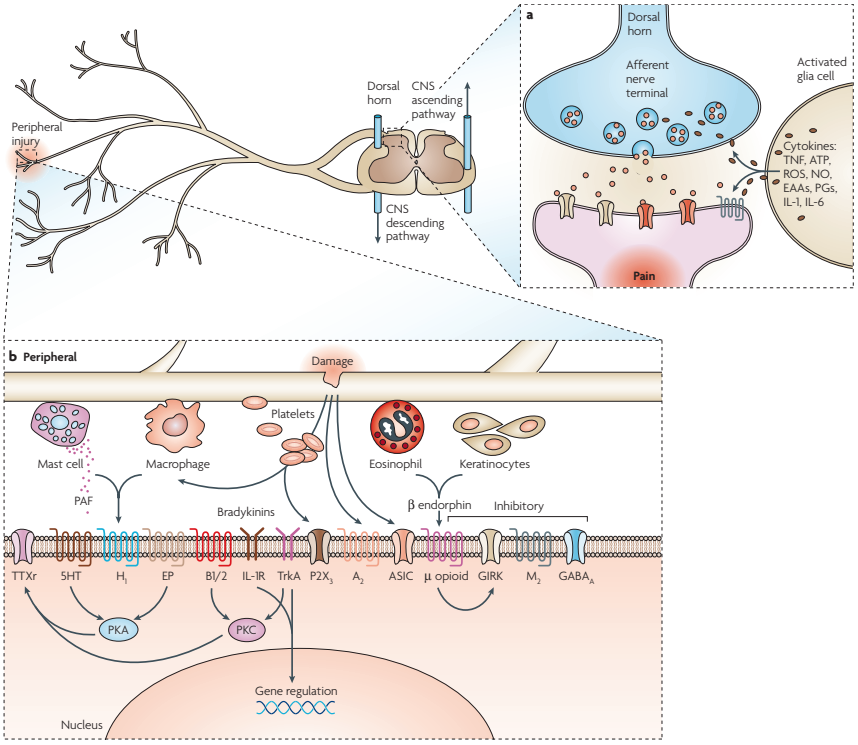


**Figure 1.1 :** a) Upper panel: Following nerve injury, neurochemical modulation of synaptic transmission occurs in the dorsal horn, post-synaptic receptors and ion channels are activated by excitatory amino acids released pre-synaptically and further sensitised by cytokines from activated glial cells. 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+; $H_1$= histamine receptor;5HT=5 hydroxy-tryptamine; IL 1/2=interleukins 1/2; $M_2$= muscarinic 2 receptor; NO= nitric oxide; $P_2X_3$= purinergic receptor $X_3$;PAF= platelet activating factor; PGs= prostaglandins; ROS= reactive oxygen species; TNF= tumour necrosis factor; TTXr= tetrodoxin receptor; TrkA= tyrosine receptor 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.

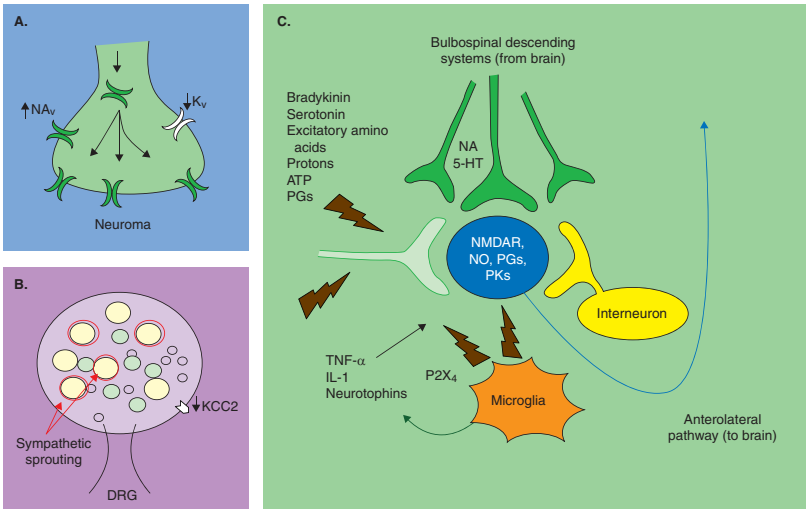


**Figure 1.2:** NP arises following nerve injury or dysfunction. a): Following nerve damage, transcription and axonal trafficking of $Na^+$ channels to the site of injury is increased, with concomitant attenuation of $K^+$ channels. The altered expression of ion channels results in hyperexcitable neurons 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 $P_2X_4$ 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. Aß=A beta neuron, Aδ=A delta neuron, C=C nociceptor, 5 HT=serotonin, $KCl_2$=chloride transporter, NA=noradrenaline, Nav=sodium channel, NO=nitric oxide, Kv=potassium channel, PGs=prostaglandin, PKs=protein kinases, $P_2X_4$=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 $P2X_4$ 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.

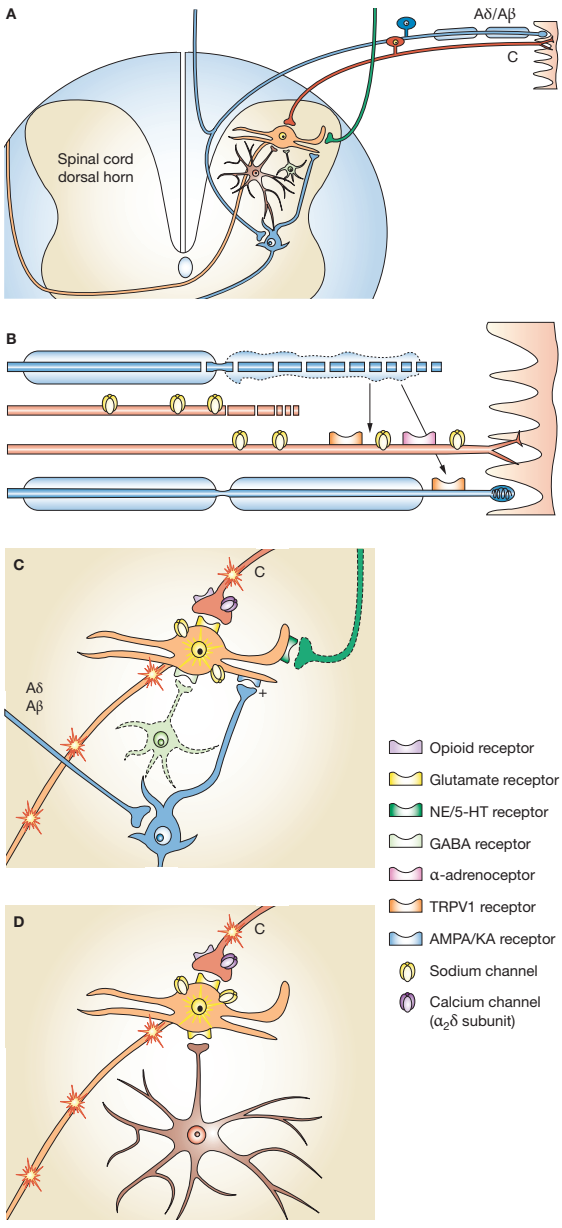


**Figure 1.3:** Mechanisms of peripheral and central sensitisation in NP a) Primary afferent pathways and their connections in the dorsal horn of the spinal cord. Nociceptive fibres terminate at the spinothalamic projection neurons in the superficial laminae whereas non- nociceptive myelinated A fibres 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, $TRV_1$ 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 pathophysiologial 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, $PGE_2$, 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-1α, 1ß, 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 Aß 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, Omoiqui 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 cyclo-oxygenase 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 in up-regulated, cyclo-oxygenase 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 $H_2$ ($PGH_2$), 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].

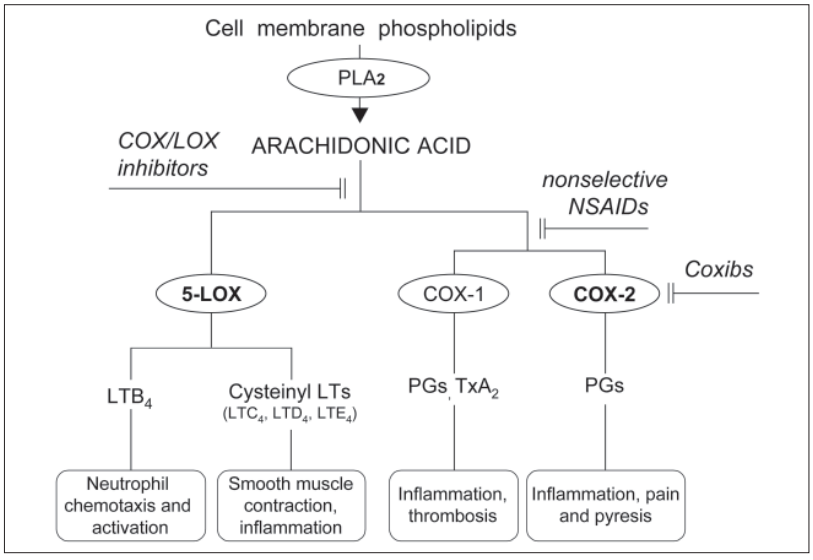


**Figure 1.4:** Main metabolic pathways associated with arachidonic acid degradation during the inflammatory response. Known targets for anti-inflammatory drugs are also shown.COX=cyclo-oxygenase,5-LOX=5-lipooxygenase,LTs=leukotrienes, PGs= prostaglandins, $PLA_2$=phospholipase $A_2$, $TXA_2$= thromboxane $A_2$ . *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.

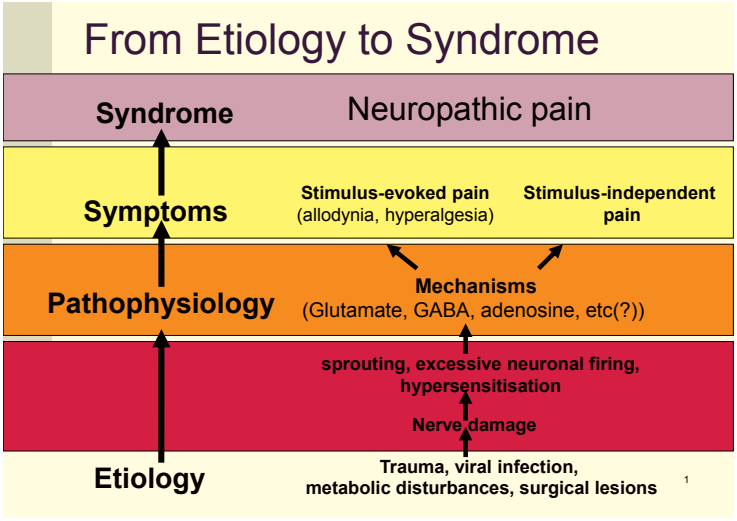


**Figure 1.5:** A flow diagram showing the progression of an underlying aetiology to the ultimate clinical/outward manifestations of NP. *Adapted with permission from*[25].

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 pathophysiolgical 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.

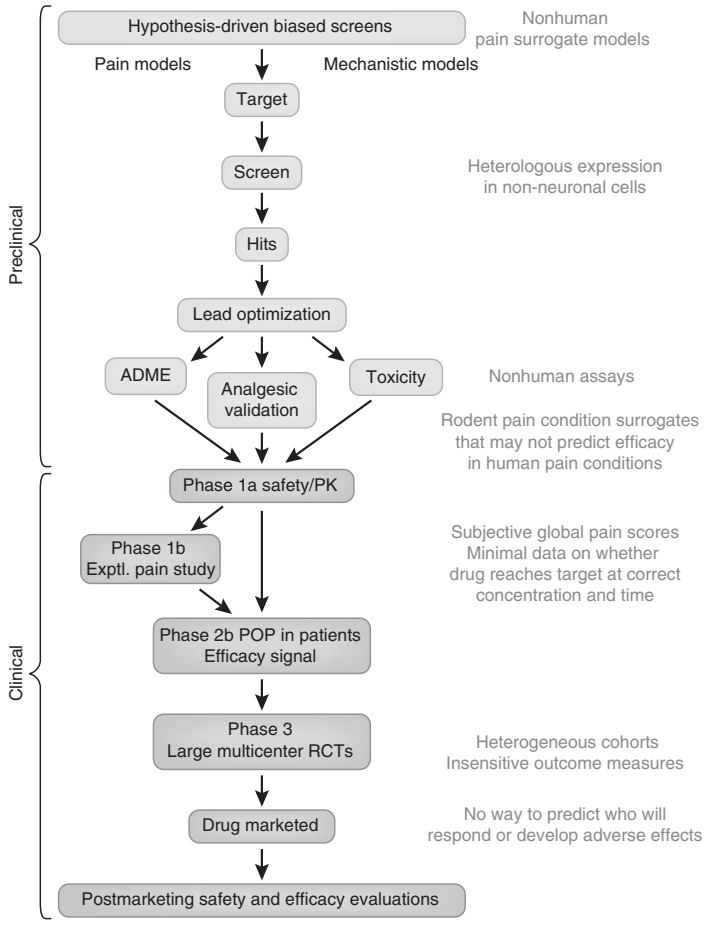


**Figure 1.6:**Sequential steps used in the discovery and development of analgesic drugs. Typically, R&D efforts start with target selection and end with regulatory approval for the indication in the target patient group. Clearly, failures in phases 2 or 3 are a major cause of attrition, and represent 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 $NK_1$ 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]

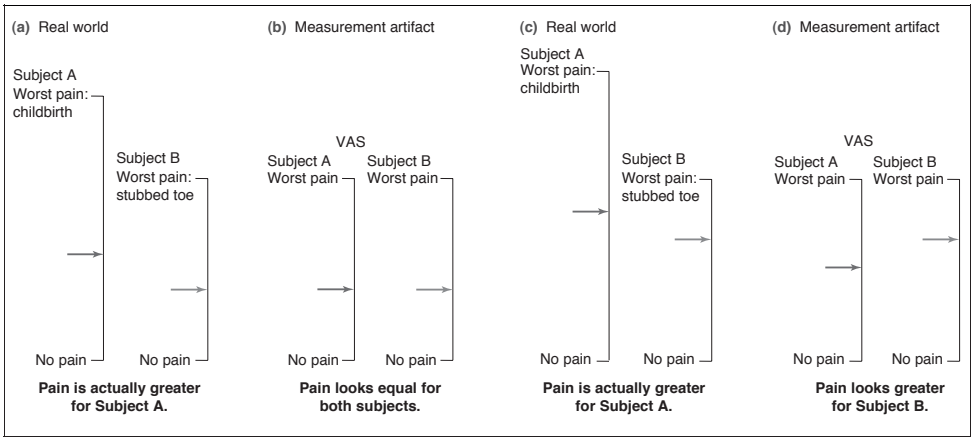


**Figure 1.7:** Fallacies of pain comparisons using the VAS. If one subject's worst pain is childbirth and another's is a stubbed toe, rating the same point on a scale would result in a discrepancy between the actual magnitude of pain experienced and that reported on a conventional VAS. Thus, as depicted 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].

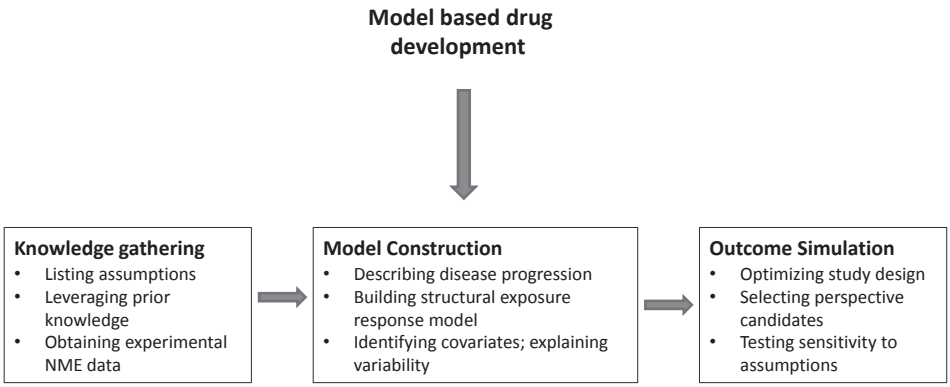


**Figure 1.8:** Main steps for the implementation of model-based approaches in drug development. NME=New molecular entity. *Adapted with permission from* [51].

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 completed 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 |
|---|---|---|---|---|
| *Pain signalling/peripheral sensitisation at primary afferent neurons* | | | | |
| -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 |
|---|---|---|---|---|
| | LOX pathway<br>Leukotrienes (receptors on smooth muscle) | | LT-Induction of platelet activation and constriction of smooth muscle<br>Increased vascular permeability and leukocyte attraction | |
| -Release of the interleukins | Others<br>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 PGE2, COX-2, matrix metallo-proteases (MMP)<br>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. |
| *Pain processing* | | | | |
| Central sensitisation (spinal cord) | Glutamate (Presynaptic:μ, glutamate receptors) | Unknown | Dynamic mechanical allodynia | Spread of spinal hyper-excitability |
| | Substance P (Calcium channels-($\alpha_2$-δ))<br>Protein kinase C (NMDA receptors) | | Punctate mechanical allodynia | Expansion of neuronal fields[6, 7] |
| Phenotypical switch<br>Nociceptor peptides normally expressed by A δ and C fibres are expressed by large myelinated Aß fibres | 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)<br>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<br>Decreased supraspinal descending modulation<br>Descending facilitation | Serotonin/NE/DA (α-2 ,5-HT Dorsal horn inhibitory interneurons)<br>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 |
| *Pain perception/plasticity in the brain* | | | | |
| | | | Maintain synaptic plasticity.<br>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.<br>Dramatic alterations in cortical spatial maps can be detected after nerve injury that may contribute to phantom pains[9] |

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