Characterization and re-evaluation of experimental pain models in healthy subjects

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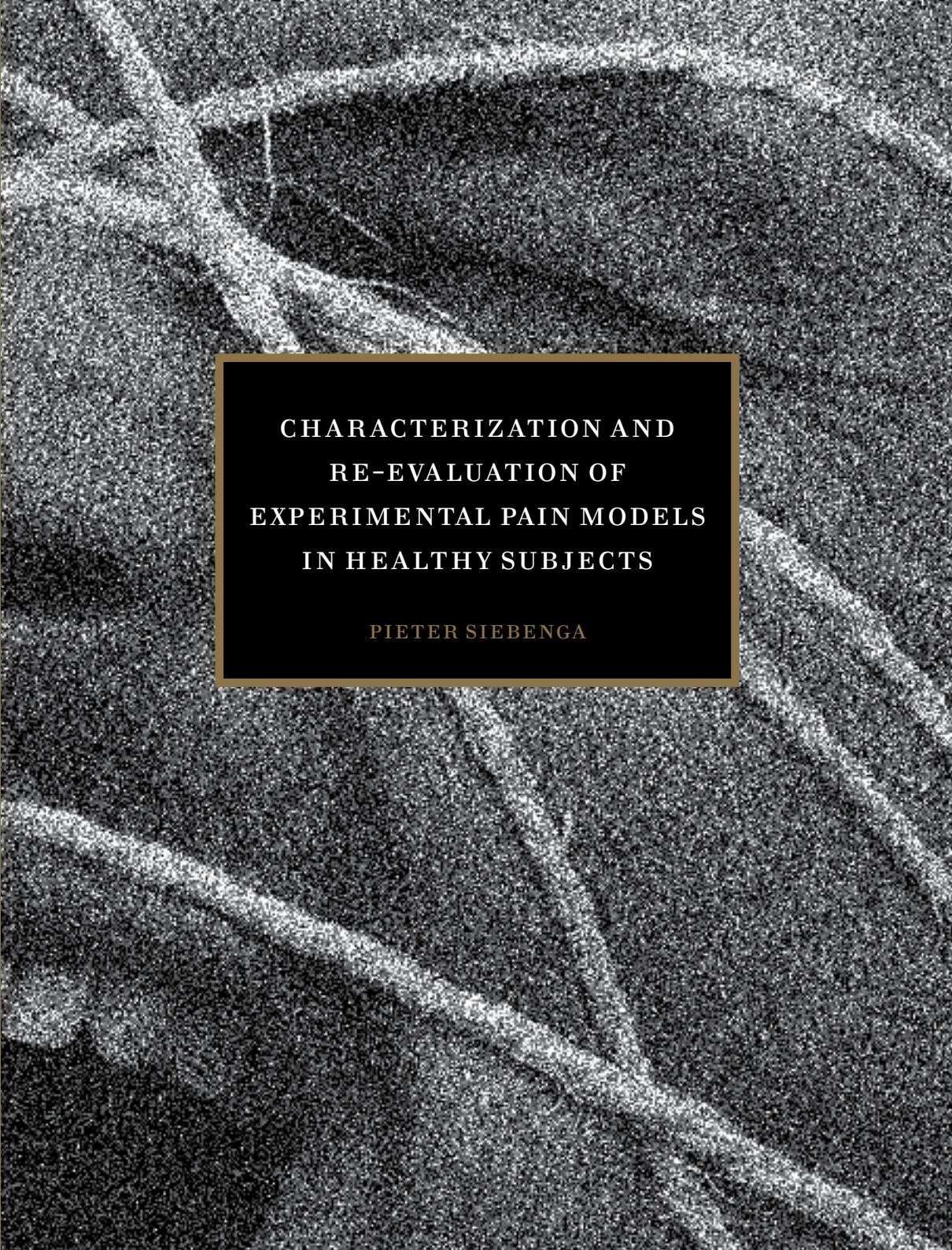
CHARACTERIZATION AND
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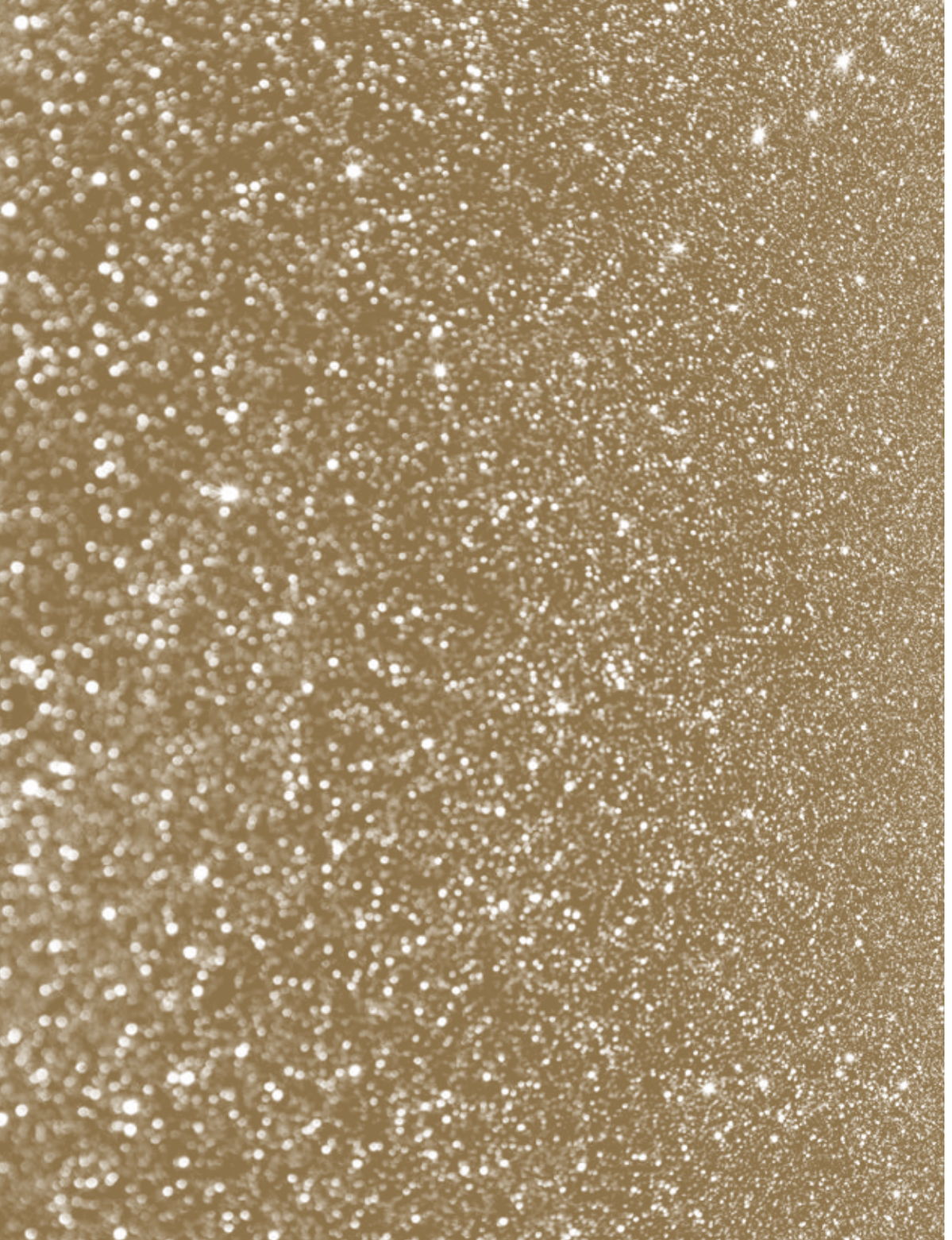
IN HEALTHY SUBJECTS
PIETER SIEBENGA



CHARACTERIZATION AND RE-EVALUATION OF EXPERIMENTAL PAIN MODELS IN HEALTHY SUBJECTS

CHARACTERIZATION AND RE-EVALUATION OF EXPERIMENTAL PAIN MODELS IN HEALTHY SUBJECTS

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1 Pharmacodynamic Evaluation: Pain Methodologies — **7**

SECTION I The efficacy of different (novel) analgesics by using the PainCart

2 Analgesic potential of PF-06372865, an α2/α3/α5 subtype selective GABAA partial agonist, demonstrated using a battery of evoked pain tasks in humans — **53**

3 Demonstration of an anti-hyperalgesic effect of a novel pan-Trk inhibitor PF-06273340 in a battery of human evoked pain models — **73**

4 Lack of detection of the analgesic properties of PF-05089771, a selective Nav1.7 inhibitor, using a battery of pain models in healthy subjects — **93**

SECTION II Validation and improvement of human evoked pain models

5 Reproducibility of a battery of human evoked pain models to detect pharmacological effects of analgesic drugs — **109**

6 Effect profile of paracetamol, D9-THC and promethazine using an evoked pain test battery in healthy subjects — **127**

7 The ultraviolet-B heat pain model: postinflammatory hyperpigmentation and validation of a lower exposure paradigm to induce hyperalgesia in healthy subjects — **147**

8 The capsaicin-induced hyperalgesia model: validation and incorporation in a multi-modal nociceptive and hyperalgesia pain test battery to detect analgesic effects of drugs in healthy subjects — **163**

9 General discussion — **185**

Nederlandse samenvatting — **201**
Appendices — **211**

Chapter 1

PHARMACODYNAMIC EVALUATION: PAIN METHODOLOGIES

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ABSTRACT

Despite many advances in the last decades in understanding pain, the development of new analgesic compounds has not followed at the same pace. The development of more targeted analgesic compounds with fewer side effects is therefore essential. With an increased demand to demonstrate pharmacodynamic effects of new analgesic compounds, the importance of human evoked pain models is now higher than ever.

Pharmacodynamic evaluation with human evoked pain models offers the possibility to determine the dose ranges at which new analgesics exert their pharmacological effect. Pain models may also aid in the choice of target population, determine which modality of pain a new drug is expected to be most suitable, help to differentiate between a central or more peripheral mode of action of new drugs, and help determine which other effects contribute to its mode of action *e.g.*, sedation.

Human evoked pain models are conducted in standardized laboratories where factors like stimulus intensity, frequency, duration and location can be controlled. Using pain models in healthy volunteers has important advantages over assessing the effects of new drugs in patients with pain; the pain elicited in human pain models is predictable in its intensity while clinical pain will naturally fluctuate. Analgesic properties can be investigated with pain models without the influence of accompanying symptoms that are often seen in patients with pain.

GENERAL INTRODUCTION

Pain is intended as a warning to the body that a noxious stimulus can (potentially) harm the body. The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[22] Prevalence studies show that in Western Countries 19-31% of the adult population suffer from a form of chronic pain.[110,123] Despite the availability of potent analgesics such as opioids, chronic pain remains a high unmet medical need as many effective analgesics have important side effects and chronic treatment with opioids leads to tolerance and addiction. The development of better and more specific analgesic compounds therefore remains essential. With an increased demand to demonstrate pharmacodynamic effects of new compounds as early as possible in clinical drug studies, the importance of human evoked pain models is now more than ever.

In a pure neurophysiological sense, nociceptive pain occurs when nociceptors are stimulated by noxious stimuli (*e.g.*, mechanical, thermal, electrical or chemical stimuli). After a threshold has been reached, the nociceptive nerve fibre transmits the pain signal to the spinal cord. The signal is modulated at several locations along ascending pathways through the dorsal horn and spinal cord. From the spinal cord, the pain signal is projected to supraspinal centres where the brain can modulate the excitatory activity via descending control.[137] Perception of pain is even more complex where more than one sensory system is responsible for transmission of the painful stimulus.[2]

From a more neuropsychosocial point of view, pain is a complex experience influenced by many factors such as emotion, fear, anxiety, but also cultural background, sex, genetics and educational background. Due to its complexity, it can be difficult to assess the effects of analgesic drugs on pain in patients and animal pain models demonstrate low predictability for clinical efficacy in humans. Several explanations are receptor dissimilarity between species, differences in pharmacokinetics and morphological and functional differences between the brains of animals and humans.[137] Human evoked pain models can control some of these influencing factors. Therefore, these models are an important step in the translation of animal research to pain patients.

Pharmacodynamic evaluation though human evoked pain models offers the possibility to differentiate between a centrally or peripherally acting drug, for which modality of pain a new drug will be most suitable (nociceptive, neuropathic or inflammatory), and which other effects contribute to its mode of action (*e.g.*, sedation, tolerance).[133,135,137,173] This can be done in early clinical trials with healthy volunteers, which is not only cost-reducing but also time saving. Other advantages of using human evoked pain models are (1) stimulus intensity, duration and modality are controlled and do not vary over time; (2) differentiated responses to different standardized stimulus modalities; (3) the response can be assessed quantitatively and compared over time; (4) pain sensitivity can be compared quantitatively between various normal/affected/treated regions; (5) models of pathological conditions can be studied and the effects of drugs on such mechanisms quantified; and (6) pain models can be easily performed in healthy subjects, who are easier to recruit into clinical studies.[10]

Evoked pain is mostly short-lasting, with most stimuli being applied exogenously and are generally focused on cutaneous nociceptor activation. Arguably, evoked pain models are limited in intensity due to ethical constraints related to the risk of tissue damage. In contrast to natural occurring pain which is mostly caused by endogenous factors, longer lasting and influenced by complex emotions.[122] Since clinical pain is a complex sensation involving psychological, physiological and cognitive factors, no single pain model is able to replicate all aspects of clinical pain.[135]

Several methods exist for evoking pain in humans, such as mechanical, thermal, electrical and chemical stimulation. A stimulus can be either phasic or tonic. Stimuli can be applied to different tissue types for instance skin, muscles or viscera.[10] This chapter focuses on the different pain models that are used to induce evoked pain in humans and the means to assess the evoked pain. Human evoked pain models are divided into the energy domain (*i.e.*, mechanical, thermal, electrical and chemical induction) and are further subdivided into area of stimulation (*i.e.*, skin, muscle and viscera) that is stimulated.

PAIN ASSESSMENT TECHNIQUES

An evoked pain model consists of two elements; a stimulus needs to be applied to evoke pain and the related pain response needs to be measured.[57] Possible assessment techniques for evoked pain responses can be divided into several categories: psychophysical, electrophysiological and imaging.

Psychophysical methods

Psychophysical methods aim to describe the relationship between physical stimuli (Section 3) and corresponding subjective responses in a quantitative manner.

These methods are widely used to study stimulus processing in various research fields, including pain processing. The methods to quantify the relationship between stimulus and response can be subdivided into unidimensional methods and multidimensional methods.

Unidimensional measures

PURPOSE AND RATIONALE

Unidimensional questionnaires such as the Numeric Rating Scale (NRS), the Verbal Rating Scale (VRS), or the Visual Analogue Scale (VAS) provide a single subjective measure to the intensity of a stimulus. More quantitative unidimensional measures are thresholds: the intensity where a stimulus is first perceived as painful (pain detection threshold), and the intensity where the stimulus is no longer tolerable (pain tolerance threshold).

PROCEDURE AND EVALUATION

The NRS consists of a horizontal line with evenly spaced perpendicular lines. The patient or subject is asked to rate his or her pain from 0 to 10 (11 point scale) or from 0 to 100 (101 point scale) where 0 equals 'no pain' and 10 or 100 equals 'worst possible pain'. The VRS consists of a list of descriptive terms to express the different levels of pain. It at least should include the extremes, in this case 'no pain' and 'worst possible pain'. Additional term usually used are 'very mild pain', 'mild pain', 'moderate pain', 'severe pain, and 'very severe pain'. The VAS is a horizontal line consisting of the two extremes and subjects are asked to indicate on the horizontal line indicating the perceived intensity.

For the determination of pain thresholds, the stimulus intensity increases until a certain pain threshold is reached.[57] The pain detection threshold (PDT) is reached when a change in sensation from non-painful to painful is felt by the subject. The pain tolerance threshold (PTT) is the stimulus intensity at which the pain is no longer tolerable. Depending on the method used, the stimulus is ceased before or when reaching the pain tolerance threshold.

Several stimulus modalities (such as thermal, mechanical or electrical) can be used to quantify pain perception and to assess sensory function.[12] These can then also be used as an indicator of the current state of the pain system. For example, it can be used to detect hyperalgesia, which is a condition in which an enhanced pain response to noxious stimuli is observed. Hyperalgesia is indicated by a decrease in the pain thresholds and an increase in pain to suprathreshold stimuli.

CRITICAL ASSESSMENT OF THE METHOD

The main disadvantage of NRS, VRS and VAS scales is that they measure a single qualitative aspect of pain, namely intensity or unpleasantness, while pain consists of more qualities (Section "Multidimensional measures"). Moreover, even though these (subjective) unidimensional scales can be used as a coarse measure of the level of pain a subject experiences, they cannot be used to distinguish individual contributions of nociceptive and pain related mechanisms.

MODIFICATION OF THE METHOD

To obtain a more detailed description of pain perception and qualities, multidimensional questionnaires can be used (Section "Multidimensional measures").

More advanced psychophysical procedures are being developed to relate stimulus properties (*e.g.*, pulse width, number of pulses, and inter-pulse interval) and perceptions to nociceptive processes.[39,193] Also, combining unidimensional measures with neurophysiological measures may provide more information on underlying processes.

Multidimensional measures

PURPOSE AND RATIONALE

Unidimensional questionnaires are often found to be limited in their capabilities in describing pain perceptions. Therefore, multidimensional measures provide means to describe perception using multiple sensory and affective qualities of pain.

PROCEDURE

In contrast to the unidimensional measures, subjects are asked to fill in several answers to various questions, or have to choose from a large range of adjectives to describe their pain perception. One of the most widely used multidimensional tool is the McGill Pain Questionnaire.[114] This questionnaire not only measures the pain intensity but also measures the sensory and affective qualities of pain. The McGill Pain Questionnaire has been used in a large number of studies, and has been translated and validated in multiple languages.[115,116]

CRITICAL ASSESSMENT OF THE METHOD

Multidimensional questionnaires often take more time to complete than simpler unidimensional questionnaires. Particularly in a clinical setting, multidimensional questionnaires either need to be compressed (such as the short-form

McGill Pain Questionnaire) or replaced by unidimensional questionnaires. Additionally, in the experimental setting of evoked pain models not all components of a questionnaire may be applicable, *e.g.*, items related to affective aspects of pain sensation.

MODIFICATION OF THE METHOD

Numerous pain questionnaires exist that measure different qualities of neuropathic and non-neuropathic pain. These include the Pain Quality Assessment Scale (PQAS), Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) and PAINDETECT. Moreover, questionnaires targeting specific patients groups exist as well; the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Knee Society Score (KSS) assign pain, stiffness, and functional scores to patients suffering osteoarthritis.

Electrophysiological and imaging methods

Electrophysiological readouts include evoked potentials via electroencephalography (EEG). Imaging readouts include functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Electrophysiological and imaging readouts provide a more objective measurement of pain. However, they have a larger variation in outcome measurements, are more expensive and are technically more difficult to perform in a large group of subjects.[10,87,188]

Functional magnetic resonance imaging

PURPOSE AND RATIONALE

Neuroimaging has identified several cortical regions in the brain that are typically active when a painful stimulus is applied to the body. With fMRI these pain responses in the brain can be observed. Structures that are active during pain perception are the primary and secondary somatosensory, the cingulate and the insular cortices which together are called the pain matrix.[81,142,149,151,181] Activation of the pain matrix due to nociceptive stimuli is the functional imaging analogue of conscious pain perception. Measuring the activities in the pain matrix during a painful stimulus can be used as an objective measure for pain perception.[23]

PROCEDURE AND EVALUATION

A subject is given a painful stimulus while being scanned in the MRI. fMRI measures brain activity by detecting changes in blood flow (hemodynamic response) associated with neuronal activation.[76] It uses the relative abundance of deoxyhemoglobin in blood that changes the proton signal from water molecules surrounding a blood vessel, producing blood oxygenation level-dependent contrasts (BOLD).[134] Via the BOLD signal an indirect index of neural activity is provided. Several fMRI methods are used in pain research, which reveal the neural correlation of pain perception and modulation by characterizing the brain response to evoked stimuli (*e.g.*, pain, allodynia), task-driven responses, or drugs (phMRI).[23]

CRITICAL ASSESSMENT OF THE METHOD

This non-invasive method of measuring pain can provide a measure of cerebral perfusion that correlates with an acute painful stimulus in healthy volunteers. Detecting chronic pain is more complex due to confounding factors like disease and treatment. Brain systems like emotion, memory and motivation are also active during measurement in these patients.[23] These systems can also be triggered by placebo analgesia, which adds to the complexity.[124] Additionally, the resolution of fMRI is inferior compared to the EEG, which means that it is not suitable to investigate the primary neuronal activity directly related to the pain stimulus and less suitable to investigate the deeper structures of the brain (*e.g.*, brainstem and thalamus).[137]

MODIFICATIONS OF THE METHOD

When including fMRI in a study, a distinction can be made in the type of pain model that will be used during the study. Mechanical,[14] thermal,[100,166] electrical[95] and chemical[14] induced pain models can be used during an fMRI scan, with the exception of models that entail the use of water and metal. Modifications can be made in the type of MRI, protocol used for scanning and analyzing protocols.

Electroencephalography and Evoked Potentials

PURPOSE AND RATIONALE

EEG is a non-invasive technique which records (spontaneous) synchronized postsynaptic neuronal activity of the human cortex. In contrast to brain imaging techniques, EEG has a high temporal resolution. This high resolution makes EEG an effective method of observing (rapid) changes in brain activity. Additionally, EEG is a suitable method for recording evoked potentials (EP) to painful stimuli (*e.g.*, thermal, mechanical, or electrical) and may provide important information on (central) pain processing.[126]

PROCEDURE AND EVALUATION

To record EEG, several electrodes are placed on the scalp, either using individual Ag/AgCl electrodes or specialized caps. The impedance should be similar among all electrodes and is advised to be held under 50 kΩ. Quantifying resting state EEG can be done by means of spectral analysis. Due to its nonstationary behavior, recorded data is divided in short epochs ranging between 2-10 seconds.[83] Each epoch is then transformed into the frequency domain and after removing or correcting epochs affected by artefacts (*e.g.*, ocular or muscular activity) averaged. The frequency range is then subdivided into bands (*i.e.*, delta, theta, alpha, beta, and gamma), and then integrated over frequency bands to obtain the total power per frequency band.

EPs are monophasic deflections of spontaneous EEG and are time and phase locked on the onset of the stimuli.[126] These waveforms are typically characterized by their polarity, latency, amplitude, and position on the scalp. As the signal-to-noise ratio (SNR) is relatively low when recording EPs, repeatedly stimulating and recording cortical responses is required. This allows improving the SNR by means of averaging and allows the characterization of the evoked response in terms of the amplitudes and latencies. The data collected generally includes the peaks and latencies of the N1, N2, P2, and P3, or the top-top amplitude between N2 and P2.[182] Moreover, the reaction time after the presentation can also be recorded. The N1 is most prominent at the contralateral temporal side (*i.e.*, T_3 or T_4) referenced to the frontal Fz. The N2 and P2 are most prominently visible at the vertex Cz referenced to the (linked) earlobes A1 and A2. The P_3, which is not thought to be nociceptive specific has a relatively long latency and is best observed at Pz referenced to the earlobes.

CRITICAL ASSESSMENT OF THE METHOD

It is important to know that EEG recordings will be contaminated with artefacts. Common sources of artefacts are ocular movements and blinks, muscle contraction and cardiac activity. Each of these artefacts have their own characteristics in both time domain and frequency domain and must be dealt with prior to analyzing EEG. Possibilities for dealing with these artefacts are either excluding parts of the recording for analysis, or correcting for them (*e.g.*, by using regression techniques, filtering, or blind source separation techniques).

EPs have been shown to be sensitive to various changes in pain pathways. EPs are sensitive to changes induced by analgesics.[161] It must be taken into account however that the amplitudes of the EPs are dependent on the attention of the subject; reduced attention results in significantly lower peak amplitudes. Moreover, the across trial variability is relatively high making comparisons between groups and trials complicating.

MODIFICATION OF THE METHOD

When several electrodes are recorded during a resting state EEG, multichannel topography allows observing the activity recorded at several locations. Time-dependent changes in power spectra can therefore also be visualized. Even though EEG has a relatively low spatial distribution in contrast to techniques such as fMRI, source localization techniques are used to find brain sources of the recorded potentials.[63] However, high-density electrode placement is required for more reliable source localization.[170]

As a result of averaging EPs, non-phase locked information is lost. Time-frequency analysis of epochs does provide the means to study non-phase-locked information.[75,126]

Nociceptive spinal flexion reflex

PURPOSE AND RATIONALE

The nociceptive spinal flexion reflex (NFR), also called the RIII reflex, is a physiological, polysynaptic reflex allowing for painful stimuli to activate an appropriate withdrawal response.[168] It is one of the available tools for objective quantification of spinal nociception in humans. The NFR reflex can be elicited in all four limbs. Here, the most standard procedure, with stimulation of the lower limb (sural nerve), is described.

PROCEDURE AND EVALUATION

Electrical constant current stimulation is delivered to the retromalleolar pathway of the sural nerve. Each stimulus consists of five pulses of 1 ms duration, separated by 4 ms, resulting in a total duration of 21 ms. Electromyographic responses are recorded from the ipsilateral biceps femoris (short head) via surface electrodes placed 4-5 cm apart over the muscle belly. The RIII reflex is identified as a polyphasic muscle response appearing with an onset latency between 90 and 130 ms after stimulation.[192] Following stimulation of the sural nerve, three responses are sequentially recorded: the tactile reflex (also known as RII), the nociceptive flexion reflex (RIII), and an involuntary movement signal.[168] For quantification of the RIII reflex response, the reflex area is obtained by integrating the rectified signal within a 50 ms analysis window starting between 90 and 120 ms after stimulation. Stimulus-response curves are recorded by increasing

stimulation intensity in 0.5 mA steps starting from 0.5 mA. Participants can rate the pain intensity of each stimulus using an NRS or VAS. The pain threshold is determined as the stimulus intensity that first evokes a painful sensation (defined as an NRS rating ≥1 or VAS > 0). The RIII threshold is defined as the stimulus intensity that first evokes a reflex response exceeding a raw area of 100 µV×ms.[157]

CRITICAL ASSESSMENT OF THE METHOD

Reduction of the NFR by a pharmaceutical compound does not necessarily imply reduction of ascending nociception, but may also indicate modulation of other components that play a role in the RIII reflex, such as deep dorsal horn interneurons[162] or motor neurons.[157]

MODIFICATIONS OF THE METHOD

NFR is affected by demographic factors. It is indicated that female subjects and children have lower NFR thresholds.[139,159] Other factor affecting the outcome include cardiac cycle, baroreceptors, stimulation site and even diurnal rhythm.[168] All should be addressed before the start of the trial.

Conditioned Pain Modulation

PURPOSE AND RATIONALE

Conditioned pain modulation (CPM) (also known as descending noxious inhibitory control (DNIC) for animal studies or heterotopic noxious counter-stimulation) is a paradigm that uses a conditioning stimulus to influence a test stimulus and can be used to assess the endogenous analgesic capacity of both healthy subjects and patients.[130] The assessment of CPM provides an indication of the balance between descending facilitation and inhibition. It is hypothesized that malfunction of pain modulation network may be the cause rather than the effect, of chronic pain development.[194]

The endogenous network is mediated via descending serotonergic, noradrenergic, and dopaminergic pathways, with the conditioning stimulus activating decreasing the activity of on-cells in the rostral ventromedial medulla (descending noxious inhibitory control).[73]

PROCEDURE AND EVALUATION

CPM is assessed by using a conditioning stimulus which is generally a tonic nociceptive stimulus, however non-painful conditioning stimuli have also been reported.[24,103] Various methods can be used as the conditioning stimulus. The method most commonly used is the cold pressor test.[150] However, other stimuli have also been reported including hot water, ischemic pain, heat, chemically induced pain, electrical induced pain, physically induced muscle pain.[148]

Various methods are used as the test stimulus including nociceptive flexion reflex, electrical, heat and pressure stimulation amongst others. CPM is considered to be a systemic experience, and as such, heterotopic stimulation is used for the assessment of CPM. CPM is assessed by comparing the endpoint from the test stimulus before and after administration of the conditioning stimulus. The duration of effect from the conditioned stimuli may be assessed in parallel or soon after administration of the conditioning stimulation however the duration of effect is paradigm-dependent with reports of CPM effects lasting 5 minutes, 30 minutes and up to 60 minutes after application of the conditioning stimulus.[53,60,183]

CRITICAL ASSESSMENT OF THE METHOD

CPM paradigms are reported to have both reasonable to high intra- and inter-individual variability especially due to the endpoint being derived from subjective pain reports.[130] Furthermore, divergent terminology used in the literature for the same phenomena complicate comparisons. Factors affecting CPM are use of medication, psychological state of subjects (anxiety, depression, emotional status and attention span), and even ethnic origin.[55]

MODIFICATIONS OF THE METHOD

As highlighted above and as with other pain models, there are numerous ways to assess CPM including different methods for the conditioning and test stimulus including using different application area. Furthermore, the endpoints vary considerably between research groups, with some groups assessing change in the test stimuli endpoint before and after the conditioning stimuli while others reporting changes in the endpoint during administration of the conditioning stimulus.[38]

PAIN STIMULATION TECHNIQUES

Mechanical Stimulation

Human evoked pain models date back to the late 19th century, in which mechanical pressure was used to induce pain.[68] Over time, mechanical stimulation techniques became more accurate and are used to stimulate the skin, muscle or viscera. The skin is the most used organ, because of its practical

implementation. However, muscles can also be targeted both endogenous (post-exercise or ischemic) or exogenous (saline injection). Balloon distention in the viscera can be used to investigate new analgesic compounds in healthy volunteers but also as a diagnostic tool in patients (*e.g.*, gut disorders). Mechanical stimulation can be divided into touch/pinprick, pressure or pinching methods. This chapter focusses on the different techniques related to mechanical stimulation on the skin, muscles and viscera using touch/pinprick, pressure or pinching methods.

Mechanical skin stimulation

Touch and pinprick

PURPOSE AND RATIONALE

Mechanical stimulation via touch can be done with a cotton swab or a brushstroke (light mechanical stimulation) or pinprick. Light mechanical stimulation will not induce pain, but can be used to assess allodynia (lowered activation threshold for a non-painful stimuli) by other pain stimuli.

A common method of applying pinprick stimulation is the use of von Frey filaments. These filaments are used to quantify touch as well as the PDT and the PTT. In animal and patient experiments, it is mostly used to determine functional recovery of Aδ- or Aβ-fibers. In human pain models it is also used to determine hyperalgesia effects (*e.g.*, in the capsaicin/UVB model).

PROCEDURE AND EVALUATION

Von Frey filaments are calibrated filaments, originally made of human or animal hair, and later with acrylic or synthetic fibres or optical glass fibres. The filaments bend at a certain designated force.[52] The applied force is dependent on the stiffness, which is in turn dependent on the diameter of each filament. During stimulation, the filament is placed perpendicular to the skin and pressed down with a constant increase in force until it bends for 1 second. Subsequently, the filament is removed with a constant decrease in force and the subject is given some time to evaluate the stimulation.

CRITICAL ASSESSMENT OF THE METHOD

The von Frey test is characterized by its simplicity, thereby showing advantages in clinical settings for rough quantification of functional nerve regeneration. In addition, it can be used to classify responders in the evaluation of hyperalgesia.

Although the von Frey method is still commonly used to evaluate peripheral nerve function in patients, it has several disadvantages as a human pain model. Pinprick stimulation always co-activates non-nociceptive Aβ-fibers. This may contaminate the signal and influence the results affecting the specificity. Additionally, intra- and inter-observer variability of this method is high. The skill of the observer will have an important impact on the results. Furthermore, each filament is produced for one constant standardized level of pressure, which means that many filaments are needed to determine sensory or pain thresholds with a high precision. Environmental changes may affect the calibration of the filaments. Lastly, it is difficult to combine von Frey stimulation with quantification methods that require time-locked responses, such as evoked potentials or time-frequency analyses in EEG.

MODIFICATIONS OF THE METHOD

Von Frey filaments are inherently limited for method modification, because each filament is calibrated for one designated force application. Depending on the calibrated force, the filaments activate mainly Aβ-fibers (0.5-128 mN) or Aδ-fibers (128-512 mN).[35] Both conventional as electronic pinprick paradigms have been described.[121] To eliminate the intra- and inter-observer variation one can consider electronic pinprick devices.

Impactometers / Pinch interdigital web / joint

PURPOSE AND RATIONALE

Pressure algometers are usually applied to the muscle or bone, but other sites including or the interdigital web, skinflap, earlobe, or a finger or toe joint can be used as an area of investigation.[27,34,172] Handheld or computer controlled pressure algometers are clinically similar to palpation.[137] For methods using a pinch methodology, the pain is due to a combination of mechanical stimulation and local ischemia, while when pressure is applied to muscle the pain is related to muscle strain.[137]

PROCEDURE AND EVALUATION

A pressure algometer is applied to the area of interest. In a controlled manner the pressure increases at a constant rate until a psychophysical endpoint of interest is attained. Many commercial handheld pressure algometers provide user feedback to ensure pressure is applied at a constant rate. For repeated applications, the pressure algometer should be applied to the same area to ensure

intra-individual variability is minimized as variation in location can lead to different thresholds. PDT and PTT are the usual primary outcome measures.

CRITICAL ASSESSMENT OF THE METHOD

For handheld pressure algometers, control of the rate of onset, muscle contraction and examiner expectations are the primary limitations.[190] Differences in the size and shape of the probe limit comparisons between research groups.

MODIFICATIONS OF THE METHOD

The model can be used in combination with other methods that induce hyperalgesia or sensitization. Stimulus-response curves can be compared with non-sensitized locations.

Rather than using a punctate pressure algometer, cuff algometry can be used with the advantage that the model is generally computer-controlled thereby controlling the rate of application of the pressure) pain.[147] Pain induced by cuff algometry is primarily related to muscle pain with minimal contribution from skin nociceptors.

Mechanical muscle stimulation

Post-exercise muscle soreness

PURPOSE AND RATIONALE

Delayed onset muscle soreness (DOMS) is believed to be mediated by a combination of lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation, and endogenous substances (*e.g.*, bradykinin and prostaglandines).[129] This pain model is thought to mimic clinical pain by inducing central sensitization while having no spontaneous pain at rest compared with exogenous induced pain models.[137] Central sensitization is an increase in general excitability of the dorsal horn neurons which can be caused by nerve injury.[99] Secondary hyperalgesia is thought of as a transient state of central sensitization.[180]

PROCEDURE AND EVALUATION

Subjects perform an eccentric exercise that they are unaccustomed to with insufficient rest periods. Peak allodynia/hyperalgesia occurs 24-48 hours post-exercise. The affected muscles are assessed with a VAS to evaluate pain intensity and pressure algometry to evaluate allodynia/hyperalgesia.

CRITICAL ASSESSMENT OF THE METHOD

The method is somewhat non-specific, with an inflammatory component but these may be site specific.[171]

MODIFICATIONS OF THE METHOD

The method can be modified by using various locations/muscle groups with development of hyperalgesia dependent on the size of the muscle.[176]

Ischemic tourniquet

PURPOSE AND RATIONALE

The tourniquet model is a tonic pain model with nociceptive contributions from the muscle, skin and periosteum (the vascular connective tissue enveloping the bones). Clinically, tourniquets are used to perform intravenous regional anaesthesia or to provide a bloodless operating field. The tourniquet leads to metabolic changes, primarily acidosis, and compression which leads to the release of prostaglandins. Neuropathic pain induced by nerve compression may also contribute to the pain felt.[98] The method can also be used as a conditioning stimuli for the CPM paradigm (Section "Conditioned Pain Modulation").

The pain is thought to be mediated by the unmyelinated, slow conducting C-fibers that are usually inhibited by the Aδ-fibers.[98] The Aδ-fibers are blocked by mechanical compression after about 30 min, while the C-fibers continue to function.[31] Tourniquet compression leads to release of prostaglandins by the injured cells.[98] These prostaglandins increase pain perception by sensitizing and exciting pain receptors. Also, limb ischemia causes central sensitization.[98]

PROCEDURE AND EVALUATION

A pneumatic tourniquet is applied to an extremity, generally the thigh, following exsanguination of the leg. The cuff is inflated above the systolic blood pressure with ranges of 100-600 mmHg above the systolic pressure having been reported.[169]

CRITICAL ASSESSMENT OF THE METHOD

For healthy subjects, the tourniquet can be left for up to 2 hours, however pain is non-specific with pain being felt under the tourniquet and/or in the lower limb. The method can also lead to temporary hypoaesthesia and lower limb paralysis. Following reperfusion, the subject may experience hyperalgesia/allodynia and muscle cramps in the affected limb.

MODIFICATIONS OF THE METHOD

The method can also be used with combination with voluntary muscle contractions or exsanguination of the leg by gravity or esmarch bandage.[65,137] The width and type of tourniquet and the maximum inflation pressure can be used to modify the method.

Hypertonic saline injection

PURPOSE AND RATIONALE

Intramuscular injection of hypertonic sodium chloride (4-6%), results in a tonic, deep, diffuse pain. The pain usually lasts for several minutes following infection and leads to both local and referred pain. The pain is primarily mediated via direct excitation of C-fibers than caused by saline-induced tissue injury.[163,175] The procedure can also be used to induce local, cutaneous hypoesthesia.[59]

PROCEDURE AND EVALUATION

A bolus saline solution is injected intramuscularly using computer-controlled infusion pump. Earlier models used manual bolus injections.[61] Pain intensity is measured by using a VAS with peak pain or area under the curve (AUC) being the primary outcome measures. Referred pain patterns and changes in the pressure pain thresholds of the local and referred pain areas can also be assessed.[54]

CRITICAL ASSESSMENT OF THE METHOD

A limitation of the model is that hypertonic injection may elicit excitation from both non-nociceptive and nociceptive nerve fibers.[96]

MODIFICATIONS OF THE METHOD

Potassium chloride is occasionally used instead of sodium chloride. Various sites can be used for injection with the most common being the musculus trapezius and the musculus tibialis anterior.[54,163]

Mechanical visceral stimulation

Barostat / Oesophagal distention / Bladder distention

PURPOSE AND RATIONALE

Induction of pain in viscera is difficult to perform due to the location of the organs associated with visceral pain as pain originates from the internal thoracic, pelvic or abdominal organs.[84] Evoked pain models assessing mechanical visceral pain are generally limited to different accessible areas of the gastrointestinal (GI) tract, the urinary tract[111] and the uterine cervix.[42]

PROCEDURE AND EVALUATION

Mechanical stimulation of the viscera is generally performed using a balloon placed in the GI tract with the preferred locations being the oesophagus or rectum. The most common method used is the barostat method whereby changes in air volume within a balloon while maintaining constant pressure are measured.[41] Generally the balloon/bag is inflated until moderate pain is reported (up to approximately 7 on a VAS) and the corresponding balloon volume is reported.[172]

CRITICAL ASSESSMENT OF THE METHOD

One of the main limitations of organ distention is the distortion of the balloon. However, calculation of strain by impedance planimetry or calculation of balloon radius may overcome these limitations.[43,172] Assessment of visceral pain is difficult due to the diffuse, referred, vague and deep nature of the pain associated. Furthermore, autonomic reactions and the risk of perforation may limit the use of visceral pain models.[128]

MODIFICATIONS OF THE METHOD

Perfusion of the GI tract with chemical substances (*e.g.*, acid or capsaicin) can be used to sensitize the organs and nervous system and generally mimics the clinical situation where the organs are hypersensitive.[84]

Thermal Stimulation

Thermal stimuli can be used to induce pain. These can be roughly subdivided in cold and heat stimuli. Cold stimulation can be induced by emerging a body part in a cold water bath (cold pressor test), by using a cooling thermode, by inducing a freezing lesion or by applying ice directly to the skin. Heat pain tasks are among the most widely used pain models in human volunteers to investigate nociception, due to the relative ease of application and robustness. Nociceptive nerve fibers are activated by changes in temperature detected by a range of thermal receptors, of which Transient Receptor Potential ion channel subfamily vanilloid (TRPV) is most responsible. When a sharp increase in temperature (>43°) is detected, Aδ-fibers are activated, whereas C-fibers are activated by slower or more

dull temperature changes. Different clinical pain states can lead to sensitization to heat pain stimuli and consequently lower activation thresholds, which can be replicated using hyperalgesia models.

Thermal skin stimulation

Cold stimulation: Cold pressor

PURPOSE AND RATIONALE

The cold pressor test can be used to investigate nociception, but also cardiovascular responses (sympathetic functions) and can be used as to induce CPM. The cold sensation and pain induced by this method is mediated by activity of Aδ-fibers (cold sensation) and C-fibers (cold pain).[137]

PROCEDURE AND EVALUATION

One of the methods of induction of cold pressor pain is based on methods previously described by Eckhardt et al. and Jones et al..[47,85] Here, subjects place their non-dominant hand into a warm water bath for 2 minutes. At 1 minute 45 seconds a blood pressure cuff on the upper-arm is inflated to 20 mmHg below resting diastolic pressure. At 2 minutes the subject moves their hand from the warm water bath, directly placing their hand into a cold water bath. The baths are two thermostat-controlled, circulating water baths set at 35.0 ± 0.5°C and 1.0 ± 0.5°C. The subject rates their pain intensity using a rating scale (*e.g.*, VAS or NRS). When pain tolerance is reached, or when a time limit is reached, subjects are instructed to remove their arm from the water, at which point the blood pressure cuff is deflated. Typically PTT expressed in seconds after immersion in the cold water is recorded as primary outcome measure.

CRITICAL ASSESSMENT OF THE METHOD

Many different methodologies have been described which negatively influences the extent to which different studies can be compared. Small variations in water temperature can result in significant changes in pain intensity and tolerance times.[120] Therefore, it is important to use water baths that are able to circulate that water to prevent warming of the water around the hand.

MODIFICATION OF THE METHOD

Different temperature settings of the water baths can be used and different body parts can be immersed in the baths. Instead of a cold water bath, also a cool gel substance has been reported to induce pain which makes it suitable for fMRI testing.[100] Also a blood pressure cuff can be used to prevent compensatory blood flow to the hand.

Cold stimulation: Cooling thermode

PROCEDURE AND EVALUATION

The cold pressor test (Section "Cold pressor") is the most commonly used method to induce cold pain. However a cooling thermode can also be used to induce cold pain. A contact thermode is attached to a part of the human body. The method consists of administering a temperature with an intensity that gradually decreases at a constant rate, usually 1°C per second. The subject halts the stimulus when the cold pain threshold is reached or when a set lower cut off temperature is reached. Most studies using this methodology only report the cold pain detection threshold; the temperature at which the sensation has 'just become painful'.

CRITICAL ASSESSMENT OF THE METHOD

For none of these compounds this methodology provided evidence for analgesic efficacy, while other pain induction methods were able to provide this evidence.[173,174] The added value of this methodology in clinical pharmacology studies is limited.

MODIFICATION OF THE METHOD

Several adjustments can be made to this paradigm. The temperature at which the test starts, the rate at which the temperature decreases and the temperature at which the tests ends can be modified. Cold hyperalgesia can be induced by applying menthol to the skin prior to testing.[7] A mean threshold of multiple measurements can be taken to minimize subject variability.

Cold stimulation: Thermal grill

PURPOSE AND RATIONALE

The thermal grill is based on the phenomenon in which simultaneous application of innocuous cutaneous warm and cold stimuli can induce a sensation of burning pain, the so-called 'thermal grill illusion'. The illusion is thought to be caused by the central integration of ascending pain and temperature sensory channels, where the inhibition that is usually exerted by the cold afferents on the nociceptive system is reduced.[25,33]

PROCEDURE AND EVALUATION

The thermal grill consists of a number of juxtaposed bars of cold and warm non-painful temperatures (*e.g.*, 18°C and 42°C) on which the subject places a body part for a certain period of time. Possible outcome measures, during and after the test, can be cold and hot sensation, pain intensity and sensation of unpleasantness.

CRITICAL ASSESSMENT OF THE METHOD

Studies in which the thermal grill has been used applied a range of combinations of warm and cold stimuli to assess relationships between painful and non-painful sensations.[1,88,135] The occurrence of paradoxical pain elicited by the thermal grill illusion can be variable. A study by Bouhassira and colleagues reported a large subpopulation of subjects who only reported paradoxical pain when large cold-warm differentials were applied.[25] Due to the apparent necessity to tailor this method to each individual subject, it is difficult to standardize this method.

MODIFICATION OF THE METHOD

For the induction of the thermal grill illusion different temperature combinations of the cold and warm bars can be used. Also a different number and width of the bars and a different distance between the bars can be used.

Cold stimulation: Skin freezing

PURPOSE AND RATIONALE

Skin freezing is an induction method of hyperalgesia. Hyperalgesia can be experimentally induced with chemical and electrical stimulation or by injuring tissue using UVB or freeze lesions.[89,107]

PROCEDURE AND EVALUATION

A copper cylinder is cooled to -28°C and placed to a part of the skin for a brief period of time. For better thermal contact, a filter paper soaked with saline can be placed between the skin and the copper cylinder. This freezing induces cutaneous inflammation and hyperalgesia. Approximately 24 hours after induction sensory testing can be performed. After induction of the freeze lesion, pain and sensation testing can be performed via mechanical stimulation with stroking brushes (subjects have to indicate if a stroke with a certain load is painful), von Frey filaments (with increasing strength, subject have to indicate when the punctuation becomes painful) and blunt pressure using a pressure algometer (threshold in N/cm^2), or electrical stimulation using a constant current device (thresholds in mA).

CRITICAL ASSESSMENT OF THE METHOD

There are only a handful of studies reporting using freeze lesions. An advantage of this method is the extent to which this methodology can be standardized. The temperature, pressure and exposure time for induction of the lesion can be controlled. Furthermore, the lesion provides stable test conditions 1 day after induction. The lesion ceases over a period of days.[107] The freeze lesion may cause a hyperpigmentation, which can be visible for several months.[89]

MODIFICATION OF THE METHOD

The location, temperature, pressure and exposure time for induction of the lesion can be varied. Furthermore, the time window between induction of the lesion and testing can be changed.

Heat stimulation: Heating thermode

PURPOSE AND RATIONALE

Heat pain thresholds can be determined by applying a peltier element to the skin, where the increase in temperature activates nociceptors via TRPV and TRPM channels.[32,106]

PROCEDURE AND EVALUATION

A contact heat thermode probe, typically with a surface of 9.0-12.5 cm^2, is placed on the skin at a standardized non-painful baseline temperature between 30-39°C. Temperature is subsequently increased in a tonic or phasic fashion at a predetermined rate up to a temperature of 50-52°C. After a subject has indicated its pain detection or tolerance threshold, the probe is rapidly cooled to the baseline temperature. To reduce variability, the test can be repeated consecutively three times, and the average of these measurements is considered the pain threshold.[21] Outcome measures consists of pain thresholds as well as subjective pain scores (NRS, VAS).

CRITICAL ASSESSMENT OF THE METHOD

Heat pain thresholds are considered to be robust and reproducible endpoints, due to their clear physiological relationship with nociceptor activation thresholds. This method is widely used and contact heat thermodes are commercially available. Limitations to using a contact heating thermode is the relatively slow heating and cooling rate of the thermode, and the fact that the thermode touches the skin compared to for example laser, making it less suitable for investigating temporal summation or specific activation of Aδ-fibers.

MODIFICATIONS OF THE METHOD

In addition to investigating pain sensation in healthy skin, this method is often used to quantify sensitization, by comparing pain sensation of normal skin to an area of sensitized (UVB, capsaicin, menthol, cinnamaldehyde) skin.[154,161] The contact heat thermode can be used in conjunction with an EEG or fMRI modality, together known as CHEPs (Contact Heat Evoked Potentials).[153,154]

Heat stimulation: UVB erythema

PURPOSE AND RATIONALE

Inflammation is the biological response to any type of bodily injury and is recognized by increased blood flow, elevated cellular metabolism, vasodilatation and the release of soluble mediators, extravasation of fluids and cellular influx. Many different neuro-active factors are released during inflammation which stimulate nociceptors itself or by lowering the depolarization threshold of afferent nerves. The UVB (or 'sunburn') model is regarded as a model for inflammatory pain and as such it is most sensitive to the effects of NSAIDs[21,184]; in this model, hyperalgesia is evoked by exposing an area of skin to an individualized dose of UVB on the skin.

PROCEDURE AND EVALUATION

Prior to the start of the study, the minimal erythema dose (MED) for a subject is determined.[160] Subsequently, a one-, two- or threefold multiple of this dose is applied to the skin. Over the course of 2-96 hours, a clearly discernible dose-related area of erythema becomes apparent, where allodynia and hyperalgesia is observed. Maximum hyperalgesia is reached at 24 hours after irradiation. Typically no background pain is observed. UVB induced hyperalgesia or allodynia can be quantified using a thermal (heat or cold) or mechanical (stroking, pinprick, pressure algometry) challenge. Pain thresholds or a subjective pain score can be used as endpoints. Mechanical allodynia to pinpricks or a pressure algometer can be expressed as a PDT, when ascending strengths of von Frey filaments are used. Moreover, the area of allodynia is measured using a fixed von Frey filament or brush.

CRITICAL ASSESSMENT OF THE METHOD

The UVB model has been proven to be valuable tool to induce hyperalgesia and allodynia associated with inflammatory pain. One caveat however, is the risk of post inflammatory hyperpigmentation (PIH).[26] PIH is a harmless condition in which areas of skin become darker in color compared to the surrounding skin. PIH can occur at any age and any skin type, however it is more common in patients with darker skin (Fitzpatrick skin type 4-6).[50]

MODIFICATIONS OF THE METHOD

In general there are three degrees of freedom to modify the UVB method: (1) the dose can be altered between estimated 1 to 3 MED[16,64,80]; (2) the location can be varied between leg, arm, back; (3) the time between UVB exposure and hyperalgesia assessment may vary between 12-36 hours.

Heat stimulation: Heat burn model

PURPOSE AND RATIONALE

A first degree burn, comparable to a slight sunburn, resulting from a heat stimulus is used to initiate a local inflammatory response which results in reduced pain sensation thresholds.[179] Additionally, the intense nociceptive excitation is thought to induce central sensitization,[140,189] rendering the burn model a model for both peripheral and central neuronal sensitization.

PROCEDURE AND EVALUATION

A superficial cutaneous burn is induced using a thermode at a fixed temperature of 45-47°C, for a period of 2-7 minutes, which is applied at a standardized pressure on the skin. The leg is predominantly selected as the location, but the arm is also used. The acceptable timeframe for detectable hyperalgesia and allodynia is typically up to 4 hours after exposure to the heat stimulus. A distinction in sensitization can be made when investigating responses in the primary (exposed) area and the secondary (adjacent, non-exposed) area. Hyperalgesia resulting from the heat burn model is most distinctly quantified using a thermal or mechanical stimulus, due to locally reduced pain sensation thresholds in the primary area.[184] The PDT is predominantly selected as an outcome measure. Furthermore, the area of secondary hyperalgesia can be quantified using mechanical (pinprick, stroking) stimuli.

CRITICAL ASSESSMENT OF THE METHOD

The heat burn model in combination with a mechanical (pinprick) assessment of sensitization is moderately sensitive to the effects of NMDA receptor antagonists.[79,119] Analgesic effects of other treatments are less conclusive. As an evoked pain model, its principle is founded in controlled tissue damage, by inducing a first degree burn, with reports of blistering in up to 20% of the studies conducted with this paradigm.[184] This may be considered to be an advantage in terms

of external validity. However, from an ethical perspective a more short-lasting model without actual tissue damage may be preferred.

MODIFICATIONS OF THE METHOD

The execution can vary from using a contact heat thermode with a short and intense stimulus (100 seconds at 50°C), to the more commonly used prolonged exposure at lower temperature (7 minutes at 47°C). Other heat sources, including laser stimulation or heat radiation can be used. The arm or leg can be used as location of exposure.

Heat stimulation: Lasers

PURPOSE AND RATIONALE

Laser stimulation (LS) uses the energy to heat up the epidermis and parts of the dermis with very brief (range of ms) and powerful (8-200 mJ/mm^2) stimuli directed at the skin.[144] This type of stimulation causes a characteristic double pain sensation, consisting of an initial sharp pinpricking-like pain (Aδ-fibers) and a second longer burning pain (C-fibers).[145,146] Most commonly used laser stimulators are based on CO2, Argon and the YAG (yttrium-aluminium-garnet).

PROCEDURE AND EVALUATION

LS can stimulate the skin in a well reproducible manner making it useful as a tool to elicit evoked potentials. Evoked potentials via EEG can easily be registered due to the brief nature of the stimulus of which the timing can be controlled as well. EEG has been used in combination with laser stimulation to distinguish between Aδ- and C-nociceptive activities.[125]

CRITICAL ASSESSMENT OF THE METHOD

Importantly, during LS no (Aβ-fibers) tactile mechanoreceptors are activated, making LS a useful tool for investigating the nociceptive system without the interference of non-nociceptive input. However, due to the fast rise in temperature of the skin, overstimulation may cause nociceptors to become fatigued over repetitive stimuli.[77] This in turn has an unwanted effect on quantification of the nociceptive system, as laser evoked potential habituation may occur.[77,182] It is therefore advised to vary the stimulation location slightly after each stimulus and use a randomized inter-stimulus interval. Additionally to habituation, precise settings are necessary to prevent damage to the skin. Hence, power, duration and surface area must be properly set up.

MODIFICATIONS OF THE METHOD

LS stimulates both Aδ- and C-fibers by thermal activation. However, adjusting the stimulation method may shift the preferential activation of either nociceptor. Preferential C-fiber stimulation is based on a characteristic difference in heat threshold (Aδ-fiber ± 46°C, C-fiber ± 40°C) and distribution density in the upper skin.[132] Shifting between Aδ- and C-fiber activation using LS is possible by choosing the right pulse width, stimulation area in combination with keeping track of the skin temperature and reaction time.

Thermal muscle stimulation

Heated saline

PURPOSE AND RATIONALE

Thermosensitive receptors located on muscle tissue afferents are thought to be involved in thermoregulation.[74] As such, these have been identified as potential targets to investigate nociception of deep muscle tissue. This is investigated by exposing muscular tissue to a high intensity thermal stimulation.[62] Only a single study was found using this method.

PROCEDURE AND EVALUATION

An intramuscular injection of sterile isotonic (1.5 mL) heated saline is injected over 20 seconds (270 mL/hr) into the musculus tibialis anterior. Hyperalgesia can quantified using a thermal and mechanical stimulus.

CRITICAL ASSESSMENT OF THE METHOD

Compared to hypertonic saline in the same study, peak pain score resulting from intramuscular injection of isotonic saline at different temperatures was significantly lower. Mechanical sensitization appeared to be largest after injection at the highest temperature (48°C). To avoid cutaneous sensations, the injection site was anaesthetized with intradermal injections of 0.2 mL lidocaine before the intramuscular injection.[62]

MODIFICATIONS OF THE METHOD

In the single study using this method, intramuscular injections of isotonic saline at different temperatures were investigated, ranging from 8°C to 48°C. Different muscles can be used for injection.

Thermal visceral stimulation

Oesophageal

PURPOSE AND RATIONALE

Thermal stimulation of the GI tract activates specific nociceptive afferents selectively through TRPV1. This in contrast to mechanical and electrical stimulation, which activate afferents both superficial and deeper in the layers of the viscera.[164] This makes thermal stimulation of the GI tract a useful technique for specific activation of non-myelinated afferents in the mucosa.

PROCEDURE AND EVALUATION

In several studies, a model was used to thermally stimulate the oesophagus.[13,41,43,97] In these experiments thermal stimuli were performed by changing the temperature (5-60°C) of re-circulating water in a bag that was placed in the lower part of the oesophagus. Temperatures were continuously measured inside the bag to control the thermal stimulation in the oesophagus. Both for cold as for heat pain, a linear stimulus-response (°C-VAS) can be observed.

CRITICAL ASSESSMENT OF THE METHOD

The upper GI tract (oesophagus) is able to differentiate between thermal stimuli in the temperature range that can be used without chronic damage. Quantification of the visceral pain is more difficult to distinguish.

MODIFICATIONS OF THE METHOD

It has been demonstrated that fast increases in temperature (1.5°C/min) affect the precision of the response.[136] Therefore, the experimental oesophageal model can be modified by using slower temperature increases to ascertain better results in pain assessments.

Electrical Stimulation

Electrical stimulation is used extensively for testing the sensitivity of the pain system in studies activating cutaneous structures, muscular structures, and in visceral structures.[4,8,102] Electrical stimulation initiates activity in nerve fibers directly without activating receptors. The stimulus intensity determines the size of the current field in the tissue and thereby the number of fibers activated.[5] In case a rectangular pulse is applied to the skin, thick fibers mediating mechanoreceptive input are activated at the lowest stimulus intensities. Increasing. Increasing the stimulus intensity leads to concurrent activation of thin myelinated fibers (Aδ-fibers), and eventually C-fibers.

Electrical skin stimulation

Stimulation can be done cutaneous or intracutaneous with various stimulation paradigms with diverse waveforms, frequencies, and durations are used to selectively activate different afferents and nervous structures and thereby evoke various pain sensations. In addition, summated neural activity, as a result of the stimuli, can activate central mechanisms,[94] which is described further in paragraph “Electrical Single Stimulation”.

Electrical single stimulation

PURPOSE AND RATIONALE

This electrical stimulation paradigm leads to a nociceptive, Aδ- and C-fiber mediated type of pain, which is well controllable. The electrical current stimulates nerve fibers directly because the intensity is far below that required to stimulate the actual receptors in the skin.[40]

PROCEDURE AND EVALUATION

For cutaneous electrical pain, two electrodes (Ag/AgCl) are placed on clean (scrubbed) skin, *e.g.*, the skin overlying the tibial bone. Electrical resistance between electrodes should be less than 2 kΩ. Each stimulus (10 Hz tetanic pulse with a duration of 0.2 ms) is controlled by a computer-controlled constant current stimulator. Current intensity increases from 0 mA in steps of 0.5 mA/sec (cutoff 50 mA). The pain intensity after each stimulation is measured using an (electronic) VAS, until pain tolerance level is reached or a maximum of 50 mA is reached.[138]

CRITICAL ASSESSMENT OF THE METHOD

Electrical stimulation is easily controlled. Electrical stimulation of the skin to induce pain has several shortcomings: (1) they excite the afferent pathways in an unnatural synchronized manner; (2) they excite the full spectrum of peripheral nerve fibers (Aβ-, Aδ- and C-fibers); and (3) stimulation bypasses the receptors on the sensory nerve endings, and therefore, all information on specific activation and transduction processes is lost.[67]

MODIFICATIONS OF THE METHOD

Aδ- and C-fibers are activated at different stimulus intensities where C-fibers have a higher activation threshold. Modeling approach can be useful for certain drug trials.[67,105] The non-specificity towards nociceptive specific stimulation is thought to be overcome by using small specialized needle-like electrodes. These electrodes slightly protrude through the epidermis and can preferentially stimulate nociceptive Aδ-fibers.[28,82,127] Intracutaneous stimulation can be chosen to mimic more a stinging/burning sensation and less throbbing.[29]

Electrical burst (temporal summation)

PURPOSE AND RATIONALE

Increasing pain in response to a series of stimuli (temporal summation) reflects the first phase of "wind-up" in animal studies. Temporal summation can be induced with mechanical, thermal and electrical stimulation.[9,58,112,129] Temporal and spatial summation evoked in the skin reflects a central nervous system modulation of the response and it is believed to mimic neuropathic pain conditions because a likely contribution of central sensitization to neuropathic pain has been demonstrated.[191] Application of transcutaneous electrical stimuli, with variation in electrical burst frequency, has been shown to be a reliable model to induce temporal summation in human subjects.[9]

PROCEDURE AND EVALUATION

For burst stimulus, each single stimulus is repeated 5 times with a frequency of 2 Hz. Pain threshold is taken as the value (mA) whereby a subject indicates either: all 5 stimuli are painful, or the train of 5 stimuli started feeling non-painful but ends feeling painful (VAS > 0).[9,71]

CRITICAL ASSESSMENT OF THE METHOD

As facilitated temporal summation is a feature in neuropathic pain patients, it has been hypothesized that induction of temporal summation using electrical stimulation can be used as a biomarker of drug effects on neuropathic pain.[11] In a recent study, drug effects of analgesic compounds, including several used in the treatment of neuropathic pain, could not be established using this evoked pain paradigm, while other evoked pain paradigms manage to demonstrate pharmacological effects convincingly. This appeared to be related to a higher intra-subject variability that may necessitate larger subject groups.[135]

High-frequency electrical stimulation

PURPOSE AND RATIONALE

High-frequency electrical stimulation (HFS) of the human skin induces increased pain sensitivity in the surrounding unconditioned skin.[185] It has been shown that sustained nociceptive input can induce activity-dependent changes in synaptic strength within nociceptive pathways, leading to an amplification of nociceptive signals.[78] This is thought to play a key role in the development and maintenance of chronic pain and in particular some forms of hyperalgesia.[101,158] HFS-induced hyperalgesia within the surrounding unconditioned skin mimics the phenomenon of secondary hyperalgesia.[118] As such, it constitutes a suitable model to study the mechanisms underlying central sensitization of nociceptive pathways.[92]

PROCEDURE AND EVALUATION

HFS is delivered to the test site, *e.g.*, the volar forearm, and consists of 5 trains of 100 Hz pulses lasting 1 second, (10-s interstimulus interval; 2-ms single pulse duration) at 10 times the detection threshold.[143] The electrical stimulation is generated by a constant-current electrical stimulator and delivered to the skin using a specifically designed electrode that has been demonstrated to activate peptidergic nociceptive afferents in the skin.[91] The heterotopical effect of HFS is usually characterized using mechanical punctate stimuli. The test stimuli are applied to the skin surrounding the area onto which HFS is applied as well as to the same skin area on the contralateral arm, which serves as control to take into account a possible time-dependent habituation.[185]

The intensity of perception elicited by the three types of test stimuli is assessed using a numerical rating scale (NRS). After approximately 1 hour, the level of heterotopical hyperalgesia starts to diminish, however is still measurable and significant from baseline up to 8 hours after HFS.[143]

CRITICAL ASSESSMENT OF THE METHOD

HFS offers an Alternative to other models that lead to secondary hyperalgesia, such as the capsaicin model or the UVB model with some important advantages. The major advantage versus the UVB model is that the mechanism underlying the secondary hyperalgesia is thought to involve heterosynaptic facilitation and, hence, to constitute a suitable model of central sensitization of nociceptive pathways,[92] while the secondary hyperalgesia in the UVB model is thought to be due to a more peripheral sensitization of nociceptors, induced by inflammation.[21] The

interval during which the secondary hyperalgesia is measurable can be carefully regulated and is relatively stable over the first hour after application of HFS.[143] The major disadvantage of this method is that is has not been used to demonstrate pharmacodynamic effects of analgesic drugs, Although one recent study did show that the model can be combined with the assessment of drug effects.[187]

MODIFICATIONS OF THE METHOD

Modifications of the method are primarily related to the type of sensory stimulus to determine the heterotopical hyperalgesic effect and to the quantification. Heterotopical hyperalgesia can be demonstrated for mechanical punctate stimuli, but also for thermonociceptive stimuli induced by heat probes or laser stimulation. Van den Broeke et al. used the model in conjunction to event related potentials to objectively demonstrate the hyperalgesic phenomena.[185]

Electrical muscle stimulation

PURPOSE AND RATIONALE

Electrical stimulation of muscle tissue can be used to elicit both local and referred muscle pain. It possesses the ability to generate referred muscle pain in an 'on and off' manner, and it is capable of maintaining referred pain for at least 10 minutes.[102] Intramuscular electrical stimulation appears to be used more often to study the nature of muscle pain than as a model to determine the pharmacodynamic effects of new analgesic compounds.

PROCEDURE AND EVALUATION

In the intramuscular electrical stimulation paradigm two needle electrodes with uninsulated tips are inserted into a muscle (*e.g.*, the musculus tibialis anterior). A computer-controlled constant current stimulator is used to induce referred pain in the ventral part of the ankle by stimulating the muscle.[102] Each stimulation consists of five constant current rectangular pulses (1 ms) delivered at 200 Hz. The referred pain threshold is defined as the lowest stimulus intensity required for the subject to notice a 'just barely painful' sensation in the referred pain area. Referred pain thresholds are determined by a staircase regime consisting of five ascending and four descending series of stimuli.[56,102]

CRITICAL ASSESSMENT OF THE METHOD

Electrical muscle and skin stimulation can use the same modalities which makes it possible to compare both models. A disadvantage of the model is that referred pain due to intramuscular electrical stimulation does not occur in all subjects; approximately three quarters of patients experience it.[102] The referred pain typically arises approximately 40 seconds after the onset of electrical stimulation, which may mean that temporal summation is involved.[102]

MODIFICATIONS OF THE METHOD

Modifications can be made with the stimulation settings. Pulse range of 100-200 Hz have been described, as well as a pulse width of 1-2 seconds.[102,185]

Electrical visceral stimulation

In the viscera it is difficult to determine the pain threshold to a single stimulus, whereas the pain threshold is easily determined if a train of stimuli is used. Furthermore, the referred pain area gradually expands if stimulation is continued for 120 seconds.[8]

Chemical stimulation

Administration of algonenic substances to the skin, muscle or viscera is believed to be a close resemblance of clinical inflammation. Various substances have been used to induce cutaneous hyperalgesia. The most commonly used are capsaicin, Nerve Growth Factor (NGF), glutamate, mustard oil and menthol, but other chemical stimulation models exist as well. Intramuscular injection of chemical substances is less common and harder to control in a clinical trial. The oesophagus is the target organ when it comes to chemical viscera stimulation because of its easy access.

Chemical skin stimulation

Capsaicin

PURPOSE AND RATIONALE

Capsaicin is a highly selective agonist for TRPV1, notorious for its pungent property in red chili peppers. TRPV1 channels are major transducers of physically and chemically evoked sensations.[70] The vanilloid 1 subtype is activated by noxious heat (≥ 43°C)[51] and is expressed on C-fibers, and on a subset of Aδ-fibers.[104] The direct effects of applying topical capsaicin are burning sensations, hyperalgesia, allodynia and erythema. In addition, it triggers the release of pro-inflammatory agents at peripheral terminals, such as substance P and calcitonin gene-related peptide (CGRP).[86,194]

PROCEDURE AND EVALUATION

Capsaicin can be administered topically and intradermal. Intradermal injection with capsaicin 0.1 mg can cause hyperalgesia, but a dose of 100 mg or higher is needed to produce hyperalgesia for an hour.[167] A dose of 100 mg is most frequently used.[14,165,180]

Topical administration of capsaicin in low concentrations (up to 3%) can cause temporary mechanical and heat hyperalgesia. Sensitization can be induced by preheating the skin to 45°C for 5 minutes with a thermode directly before capsaicin application. Sensitization can be rekindled throughout a study by reheating the skin up to 40°C for 5 minutes. Application of the capsaicin is most commonly done on the forearm or the back, but can be done on any area of the skin. Topical application of capsaicin can induce peripheral and central sensitization shown respectively by primary mechanical/thermal hyperalgesia and by secondary mechanical hyperalgesia/allodynia. This pain model can therefore be used to study novel analgesic compounds targeting these typical symptoms of neuropathic pain. Peripheral sensitization is caused by modulation of peripheral afferents and is therefore restricted to the site of injury, *i.e.*, primary hyperalgesia. Central sensitization is caused by modulation of the nociceptive processing in the central nervous system. To quantify the effects of this pain model, laser stimulation can be used in combination with electro-encephalogram.

CRITICAL ASSESSMENT OF THE METHOD

Peripheral sensitization is closely linked to primary hyperalgesia and central sensitization is partly explained by hyperalgesia in the surrounding area, *i.e.*, secondary hyperalgesia. Moreover, nociceptive integration at spinal cord level may include non-nociceptive mechanoreceptors. Therefore, central sensitization may also cause Aβ-fiber mediated pain (allodynia). Higher concentrations (capsaicin 8%) initially causes increased sensitivity but is then followed by a decrease in sensitivity due to a reduced TRPV1 expression.[117,184] High concentration capsaicin is indicated in postherpetic neuralgia. Besides, capsaicin may also have a neurolytic property, where it (partly) eliminates epidermal nerve fibers (ENFs) in treated areas over time.[45] Re-innervation occurs over time.[77]

MODIFICATIONS OF THE METHOD

There are several variations that need to be addressed when designing a study utilizing capsaicin, *e.g.*, concentration of the capsaicin, dose administration (intradermal or topical), vehicle of the capsaicin (alcohol or cream), duration of the application, location of administration and pre-/rekindling.

Nerve growth factor injection

PURPOSE AND RATIONALE

NGF is a member of the neurotrophin family, which also includes brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5). NGF binds to both a high affinity tyrosine kinase receptor TrkA and a low affinity receptor p75. NGF can sensitize nociceptors so that they show an increased response to thermal and chemical stimuli.[17] Administration of NGF to human skin evokes mechanical sensitization and profound hyperalgesia to thermal stimuli that develops within 3 hours post injection and peaks between day 1 and 7.[46] Sensitization to heat and hyperalgesia to cold develops within days after injection and lasts up to 21 days, while hypersensitivity to mechanical impact stimuli develops over a longer period and persists for at least 49 days.[156] Intradermal NGF administration provokes a pattern of sensitization that can be used as experimental model for neuropathic pain.[156]

PROCEDURE AND EVALUATION

One microgram of human recombinant lyophilized NGF is dissolved in 50 µL saline and injected intradermally into the central volar forearm. The same volume of saline is administered into the contra-lateral site as vehicle control.[156] Vasodilatation upon NGF- and saline-injection can be recorded by laser Doppler imaging. Nociceptor sensitization can be explored to mechanical (touch, pinprick, pressure), thermal (cold, heat), and electrical (current pulses) stimuli. Stimuli for investigating static and dynamic allodynia and pinprick hyperalgesia are administered 5-7 cm distal from the injection site and continued in steps of 1 cm until the subject reports a definite increase of pinprick pain or switch from touch to an aversive sensation.[156] The point where this starts is marked on the skin and the distance to the injection site measured. Pain thresholds and subjective scores with NRS/VAS can be used to evaluate the mechanical, thermal or electrical stimulation.

CRITICAL ASSESSMENT OF THE METHOD

Increased levels of NGF have been reported in human painful disorders including arthritis.[177] Injection of NGF therefore appears to mimic processes found in clinical disease.[137] Even though NGF may also be upregulated in the UVB burn, anti-NGF has been shown to only partially reduce UVB induced hyperalgesia.[20] Apparently, the NGF induced mechanism of mechanical sensitization is different to UVB evoked primary hyperalgesia. NGF induces a particularly long lasting

mechanical sensitization including static allodynia and cold hyperalgesia without any visible signs of inflammation and therefore adds to the spectrum of human evoked pain models.[156] The long lasting local allodynia and hyperalgesia after subcutaneous or intradermal injection, up to 49 days after injection, form the most important disadvantage of the model. Even though considered a model for neuropathic pain, it is unlikely that central sensitization plays a role.

MODIFICATIONS OF THE METHOD

Systemic administration of NGF 1µg/kg I.V. has been shown to lead to mild to moderate muscle pain mainly in the bulbar and truncal musculature that lasted 2-8 days.[141]

NGF has been injected into the musculus masseter to induce allodynia and hyperalgesia and as a model of myofascial temporomandibular disorder pain.[177]

Other chemical mediated models

MUSTARD OIL

Mustard oil is a plant-derived irritant. The noxious effects of mustard oil are currently ascribed to specific activation of the cation channel transient receptor potential, subfamily A, member 1 (TRPA1) in nociceptive neurons.[137] Topical administration leads to a burning pain in the area exposed to mustard oil as well as secondary allodynia and hyperalgesia in the surrounding unaffected area, similar to the topical capsaicin model.[93]

MENTHOL

Menthol acts as an agonist on the transient receptor potential cation channel subfamily M member 8 (TRPM8) receptor. The topical application of high concentration (40%) menthol, is thought to activate and sensitize cold-sensitive TRPM8-expressing C-nociceptors and activates cold-specific Aδ-fibers.[19] Topical application has been used as an evoked pain model of cold hyperalgesia, which is a clinical symptom that occurs frequently in patients with peripheral or central nervous system lesions.[69] In addition to cold hyperalgesia, the model elicits primary and secondary mechanical (pinprick) hyperalgesia combined with the sensation of burning.[19] The menthol model has been shown to be sensitive to a range of analgesics.[3]

Chemical muscle stimulation

Nerve growth factor

Intramuscular injection with NGF is most commonly done in the musculus tibialis anterior or musculus masseter.[6,178] It induced a long-lasting hyperalgesia and lower pressure pain threshold can be observed, lasting up to 4 days in the musculus tibialis anterior and up to 14 days in the musculus masseter.[6,178] An advantage of the intramuscular NGF paradigm is the long-lasting hyperalgesia which can simulate clinical pain more than most other paradigms, but this is also the disadvantage where ethical consideration may play a role. The paradigm is difficult to control where hyperalgesia is dependent on the dose and the size of the muscle.[6]

Chemical visceral stimulation

Oesophagal (gut) perfusion with acid, alcohol, glycerol, capsaicin and hypertonic saline

PURPOSE AND RATIONALE

Chemical stimulation of the GI tract may be used to stimulate C-fibers selectively via TRPV1 receptors and modulate the visceral pain system due to their sensitization effects. Having a model of central sensitization of the viscera can be helpful in the development of new analgesics, as this is thought to be an important element of chronic visceral pain.

PROCEDURE AND EVALUATION

Using acid to stimulate the oesophagus is the most used method to sensitize the gut[18,37,44,152] However, other chemicals such as alcohol, glycerol, capsaicin and hypertonic saline are used to stimulate the gut as well.[42,43,109] The chemical compound is usually infused into a container/bag residing in the oesophagus with a small perfusion hole to release the compound into the oesophagus. Chemical stimulation is able to modulate the visceral pain system by selectively activating non-myelinated C-fibers for a longer amount of time. This tonic activation may result in central sensitization effects, which can be quantified by subsequent thermal, electrical or mechanical stimulation.

CRITICAL ASSESSMENT OF THE METHOD

A high variation in the outcome measures is seen with this model. The reproducibility is challenging because several factors are hard to control, like exposure time to the chemical stimulus, size of the treated area and latency time to onset of effects. Furthermore, tissue injury results in the release of multiple molecules working together, and to mimic this situation it may be necessary to use a mixture of chemical substances.[109] Blinding this procedure is difficult, since subjects are able to taste the compound. Therefore, the experimental setup requires that both subject and assessor are ignorant of the possible influence of the compound on the pain threshold.[43]

MODIFICATIONS OF THE METHOD

Each chemical substance will have an impact on the results. For example, the motility may interfere with the results when glycerol is used. Other stimuli, such as injection of hypertonic saline and application of capsaicin, the pain is elicited shortly after the chemical comes into contact with the mucosa, and the motility has minor impact on the results.

DISCUSSION

Healthy subjects versus patients

Despite many advances in the last decades in understanding pain, the development of new analgesic compounds lacked behind. In almost 60 years, only 59 compounds were registered for the treatment of pain, of which two-thirds were specifically developed as analgesics.[90] Historically, pain states have been classified and investigated on the basis of a disease state. Based on preclinical animal models, target patient populations were selected. In patient studies, efficacy is then reported as change in the patient's response to pain.[113] Unfortunately, several promising compounds have failed in this late-stage development where pharmacotherapy only provides meaningful pain relief in less than 50% of patients with neuropathic pain.[48,49] But a negative outcome does not automatically mean inefficacy of the compound. Pathophysiological mechanisms of pain vary between individuals with the disease state. Selecting and clustering the patients in groups of pathophysiology rather than disease might be necessary to obtain meaningful results. The use of human evoked pain models can provide more information.

Multi-modal testing in healthy volunteers can provide information about the analgesic activity of the compound and possibly find the active dose level range. In a way, by using different pain modalities, the results will create a certain pain profile of the compounds.[135] These results may reflect effects of analgesic drugs on mechanisms involved in clinical pain. Thus, multi-modal pain testing may aid in determining the optimal target population for new analgesic compounds based on their profile of effects on a diversity of pain mechanisms and depending on the contribution of each of these mechanisms in clinical pain phenotypes. In several chronic pain populations, such as chronic whiplash, rheumatoid arthritis, vulvodynia and fibromyalgia, changes in pain tolerance levels, pain modulation and augmented brain responses and altered responses to analgesics have been found.[36,66,186] Using evoked pain in these patients can provide insight into the analgesic mechanisms -or lack thereof- in these altered pain states.[137] In patients with chronic (neuropathic) pain, different sensory profiles exist. These profiles possibly match with different neurobiological mechanism of pain.[15]

Predictive value of models for drug development

Human evoked pain models in healthy volunteers can be conducted in standardized laboratories. Factors like stimulus intensity, frequency, duration and location can be controlled and when a model is stable and reproducible, it can be regarded as suitable for pharmacodynamic evaluation of new analgesic drugs. Using pain models in healthy volunteers has important advantages over assessing the effects of new drugs in patients with pain; the pain elicited in human pain models is predictable in its intensity while clinical pain will naturally fluctuate and in pain models analgesic properties can be investigated without the influence of accompanying symptoms that are often seen in patients with pain. However, it should always be asked whether a pain model at all resembles naturally occurring pain. Clinical pain is a subjective perception, influenced by cognitive processes, by emotions, social context and even cultural background, while pain models are solely based on the infliction of a noxious stimulus and its response. An important question is whether or not a positive result in a certain evoked model is also predictive of clinical efficacy.

Two approaches have been used to investigate this. Moore and colleagues investigated which naturally occurring pain was physiologically most in agreement with evoking a pain response causing the same type of pain. For instance, they concluded that intramuscular electrical stimulation closely matched clinical acute musculoskeletal pain.[122] Oertel and Lötsch evaluated the differences between human pain models and clinical efficacy. First they looked at which drugs were effective in different pain conditions (*e.g.*, NSAIDs were effective for inflammatory

arthritis), then they investigated which drugs were effective in which pain model (*e.g.*, NSAIDs influence pain response in laser evoked pain). If a certain drug was effective both in the model and in the particular clinical setting, the model was concluded to possibly be predictive for the type of clinical pain. Some level of agreement could be observed for a large number of pain models with many different clinical forms of pain.[133] In another review, the mutual agreement between pain models and clinical efficacy was statistically assessed. It was observed that a small set of pain models seemed predictive for efficacy in the clinic, for example capsaicin induced hyperalgesia with mechanical stimulation is associated with trigeminal neuralgia and renal colic, and UVB induced hyperalgesia in combination with heat stimulation can be linked to burn injuries or postoperative pain.[108]

Several reviews investigated which evoked pain models were sensitive to the analgesic effects of different classes of analgesics in healthy subjects.[133,135,173,174] With the aid of these studies, well-considered decisions can be made on which evoked pain models to include in studies investigating potentially analgesic compounds.

Multi-model assessment of pain

Pain comes in various types and can originate in many different tissues. It is obvious that different analgesics will influence different types of pain according to their respective mechanism of action. If an analgesic drug with a novel mechanism of action is studied, it can occur that a single pain model, thought to relate to a specific clinical pain syndrome, demonstrates lack of efficacy of the new compound. In these cases, a combination of human evoked pain models can be used to screen for possible analgesic effects of these compounds. For instance, a combination of a mechanical, thermal and electrical pain models: pressure stimulation assesses the nociception generated from within the muscle,[147] cold pain induced by the cold pressor test mainly activates C-fibers in the skin,[137] heat stimulation initially activates A-fibers in the skin, followed by C-fiber activation, induction of inflammation via sunburn or UVB, induces the production of cytokines that lead to sensitisation of cutaneous nociceptors,[21] and electrical stimulation directly stimulates sensory nerve endings of both Ad and C-fibers in the skin.[67] This multimodal testing with a battery of different pain models has been performed by multiple study groups.[135,136,172] The batteries have in common that they induce pain via different modalities and in different tissues and mimics clinical pain better than a single pain model can. The multimodal batteries can be used to profile the analgesic effects of new drugs, to obtain the optimal dose of new analgesics and to benchmark new drugs against profiles of well-known analgesics.[135]

This thesis is composed of two sections. The first section focusses on the efficacy of different (novel) analgesics using a battery of human evoked pain models (PainCart). Validation or improvement of human evoked pain models are discussed in section 2.

Section 1

THE EFFICACY OF DIFFERENT (NOVEL) ANALGESICS BY USING THE PAINCART

Chapter 2
An overview of the performance of two dose levels of a novel α2/α3/α5 $GABA_A$ subunit selective partial positive allosteric modulator, PF-06372865, was summarized after utilizing the PainCart. PF-06372865 has the potential to provide analgesia but with less sedation or cognitive effects than non-selective benzodiazepines.

Chapter 3
PF-06273340 is a small molecule inhibitor of Trks A, B and C that reduced chronic pain in nonclinical models where there has been some sensitization, such as ultraviolet B. The trial aimed to investigate the pharmacodynamics of this first-in-class molecule in healthy subjects to translate nonclinical effects in to men. Pharmacodynamic assessment was done with the PainCart.

Chapter 4
PF-05089771 is a potent selective $Na_V1.7$ sodium channel blocker, developed for the treatment of acute and chronic pain. The study was performed to demonstrate analgesic properties of PF-05089771, alone and in conjunction with pregabalin, using the PainCart on healthy subjects.

Section 2

VALIDATION AND IMPROVEMENT OF HUMAN EVOKED PAIN MODELS

Chapter 5
The reproducibility of the PainCart was measured by demonstrating the variability of the analgesic effects of ibuprofen and pregabalin. Results were extracted from three (ibuprofen) or four (pregabalin) studies previously completed with the PainCart.

Chapter 6

A better understanding of the analgesic profile of existing analgesics by using the pain models of the PainCart can help in a more detailed prediction of nociceptive activity of a new analgesic compound. Chapter 6 describes the pharmacodynamic results of a classic (paracetamol) and a non-classic (Δ9-THC) analgesic compound to expand knowledge of different classes being tested with the PainCart.

Chapter 7

Unexpected long-term side effects of the 3x minimal erythema dose (MED) UVB pain model were the base for a retrospective evaluation of this model. Postinflammatory hyperpigmentation (PIH) is a usually harmless condition but has impact on the subject's self-consciousness, social well-being and personal relationships. Based on finding from this study a second study was conducted to improve the UVB model to a 2x MED model with minimal risk to develop PIH.

Chapter 8

Chapter 8 describes the results of the capsaicin induced hyperalgesia model. Laser evoked potentials were included to obtain an objective outcome parameter. The first part of the study was to demonstrate the validity of the model. Analgesic properties of tramadol and duloxetine were evaluated in the second part. This was done in a multimodal test setting where the capsaicin induced hyperalgesia model was incorporated in to the PainCart.

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SECTION I

Chapter 2

ANALGESIC POTENTIAL OF PF-06372865, AN α2/α3/α5 SUBTYPE SELECTIVE $GABA_A$ PARTIAL AGONIST, DEMONSTRATED USING A BATTERY OF EVOKED PAIN TASKS IN HUMANS

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ABSTRACT

This study investigated the analgesic effects of two dose levels (15 and 65 mg) of a novel α2/α3/α5 $GABA_A$ subunit selective partial positive allosteric modulator (PAM), PF-06372865, compared to placebo and pregabalin (300 mg) as a positive control.

We performed a randomised placebo-controlled crossover study (NCT02238717) in 20 healthy subjects, utilizing a battery of pain tasks (electrical, pressure, heat, cold- and inflammatory pain, including a paradigm of conditioned pain modulation). Pharmacodynamic measurements were performed at baseline and up to 10 hours post-dose.

A dose of 15 mg PF-06372865 increased Pain Tolerance Thresholds (PTT) for pressure pain at a ratio of 1.11 (90% CI: 1.02, 1.22) compared to placebo. A dose of 65 mg PF-06372865 led to an increase in PTT for the cold pressor at a ratio of 1.17 (90% CI: 1.03, 1.32), and pressure pain task: 1.11 (90% CI: 1.01, 1.21). Pregabalin showed an increase in PTT for pressure pain at a ratio of 1.15 (95% CI: 1.06, 1.26) and cold pressor task: 1.31 (90% CI: 1.16, 1.48).

We conclude that PF-06372865 has analgesic potential at dose levels that do not induce significant sedation or other intolerable adverse events limiting its clinical use. Additionally, the present study further established the potential role for this battery of pain tasks as a tool in the development of analgesics with a novel mechanism of action, for the treatment of various pain states including neuropathic pain and to establish proof-of-concept.

INTRODUCTION

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS) and in that capacity it is involved in a myriad of functions and behaviours.[1] $GABA_A$ receptors are heteropentameric ligand-gated chloride ion channels that mainly contain two α, two β, and one γ subunit.[2] Conventional, non-selective benzodiazepines are positive allosteric modulators (PAMs) of the $GABA_A$ receptors.[3] Animal studies have shown that $GABA_A$ α1 activity is responsible for the sedative effects[4] and studies in healthy human subjects with α1-sparing, α2/α3 PAMs have confirmed these findings in humans[5-7] $GABA_A$ α2 and α3 subunits have been associated with anxiolysis,[8] whereas the α5 subunits are believed to be involved in cognitive and memory performance.[9-11]

Since Melzack and Wall's Gate control theory in 1965,[12] it is widely accepted that a central modulatory mechanism that regulates pain perception is present in the mammalian nervous system. However, only recently the putative role for $GABA_A$ and glycine receptors in this modulatory processing of nociceptive input and its role in the development of neuropathic pain has been confirmed.[13-15] This potential pharmacological target for the treatment of chronic pain was first established by a preclinical study investigating the subtype selective α2/α3 $GABA_A$ receptor ligand, L-838,417. This treatment clearly impaired the nociceptive response, both in a reduction in nociceptive input to the brain as well as reduced brain activity in associative-emotional components of pain, as shown by functional magnetic resonance imaging (fMRI) in rats.[16] Further pharmacological evidence was provided in a preclinical investigation where the novel subtype-selective $GABA_A$ receptor-positive modulator NS11394, which possesses a functional efficacy selectivity profile of α5 > α3 > α2 > α1 at $GABA_A$ α subunit-containing receptors, showed analgesia at doses a 20-to 40-fold lower than the dosages that induced sedation or reduced motor function.[17] Likewise, the α2/α3 $GABA_A$ receptor ligand, HZ166, demonstrated a dose-dependent antihyperalgesic effect in mouse models of neuropathic and inflammatory pain. These effects were observed at dose levels that not exhibited reduced motion or sedation.[18] The effects of both NS11396 and HZ166 were reversed by the benzodiazepine antagonist flumazenil, indicating that the observed analgesia was indeed mediated via the benzodiazepine binding site of the $GABA_A$ receptors. A preclinical experiment in four lines of point-mutated mice, in which only one of the receptor subtypes α1/α2/α3/α5 at $GABA_A$ receptors remained benzodiazepine sensitive, has elegantly shown that targeting specifically α2 $GABA_A$ receptors achieves strong antihyperalgesic effects. In these mice, diazepam and midazolam produced α2-mediated analgesia, in the absence of sedation, reduced locomotion or development of tolerance, after treatment with diazepam and midazolam, both non-selective benzodiazepines. Reversal of hyperalgesia was also observed, albeit to a lesser extent, in those mice expressing α3 or α5 $GABA_A$ receptors.[19]

The role for GABAergic pain modulation in humans is supported by two clinical studies in healthy subjects using evoked acute and hyperalgesic pain models to investigate the analgesic potential of two non-selective benzodiazepines, clobazam and clonazepam.[20,21] Additionally, several clinical studies in specific pain populations have shown their efficacy in treating pain, Although it can not be excluded that these effects were due to their myorelaxing properties, rather than genuine analgesia.[22,23] However, Although conventional non-selective benzodiazepines are considered safe and reasonably well-tolerated, clinical use for the treatment of pain is precluded by adverse effects (AEs) including sedation, postural instability and memory disturbance.[24,25]

PF-06372865 (IUPAC name: 7-ethyl-4-(4'- ethylsulfonyl)-6-flouro- 2'methoxybiphenyl-3- yl)-7H-imidazo[4,5-2]-pyridazine) is a potent ligand of the allosteric benzodiazepine site of the $GABA_A$ receptor, which exhibits functional selectivity for receptors containing α2, α3 or α5 over those containing α1.[26] Therefore, PF-06372865 has the potential to provide analgesia but with less sedation than non-selective benzodiazepines.

The present study aims to explore the analgesic effect profile of PF-06372865. This was performed by investigating the effects of two different dose levels in a comparison to placebo and a positive control (pregabalin) using a validated test battery of human evoked pain models.[27]. Since its approval, pregabalin plays a prominent role in the treatment of acute- and postoperative pain,[28-30] and neuropathic pain.[31] Furthermore, its analgesic properties have been quantified before, using this pain test battery, where a dose of 300 mg pregabalin demonstrated a distinct analgesic effect profile.[32-33] As such, the present study was to provide information on the analgesic potential of an α2/α3/α5 subtype selective $GABA_A$ partial agonist, at dose levels that were previously shown to exert a more favourable neurocognitive pharmacodynamic effect profile compared to a non-selective benzodiazepine.[26]

METHODS

Subjects and study design

The study was a double blind, double dummy, single dose, randomised, placebo-controlled, 4-period cross-over study in which the effects of two dose levels of PF-06372865 were compared to placebo and pregabalin (300 mg) was included as a positive control. The study was conducted at the Centre for Human Drug Research in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. This study was registered in the public registry of Clinicaltrials.gov under registration number: NCT02238717.
Each subject provided written informed consent before any screening procedures were performed. A total of 20 healthy male subjects between 18 and 55 years of age with a body mass index of 17.5 to 30.5 kg/m^2 were enrolled. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and haematology, urinalysis, electrocardiogram (ECG) and assessment of the minimal erythema dose (MED) for UVB light to assess eligibility. Subjects with a clinically significant known medical condition, in particular any existing condition that would affect sensitivity to cold or pain were excluded. Subjects with Fitzpatrick skin type V or VI, widespread acne, tattoos or scarring on the back were excluded due to the inability to accurately assess MED. Also, any subject who was a regular user of any illicit drugs, had a history of drug abuse or a positive drug screen at screening was excluded. Smoking and the use of xanthine-containing products was not allowed during dosing days. Alcohol was not allowed at least 24 hours before each scheduled visit and during the stay in the research unit.

Two dose levels of PF-06372865 (15 mg and 65 mg) were selected based on the safety and tolerability data from the previous single dose and multiple dose studies as well as the anticipated receptor occupancy (RO) predictions based on a previous PET study.[26] 15 mg dose was predicted to give ~50% RO at α2 and 65 mg dose was predicted to give ~ 80% RO at α2. A dose of 300 mg pregabalin has been investigated in previous human evoked pain model studies[33-34] and was well-tolerated and lies within the labelled dose range in the European Union (EU).

Safety

All observed or volunteered Adverse Events (AEs) regardless of treatment group or suspected causal relationship to the investigational product are recorded. An AE is considered any untoward medical occurrence in a subject participating in the clinical investigation. The following anchors for severity assessment by a qualified medical doctor were deployed: Mild (Does not interfere with subject's usual function; Moderate (Interferes to some extent with subject's usual function); and Severe (Interferes significantly with subject's usual function). All directly observed AEs and all AEs spontaneously reported by the study subject are recorded. In addition, each study subject is questioned about AEs, using non-probing questions, following local Standard Operating Procedures.

Pharmacodynamic assessments

Pain thresholds were measured using a battery of human evoked pain models, as described previously.[27,32,33] The battery consists of an integrated range of pain tasks for measuring different modalities of pain, which takes approximately 30 minutes to complete. Assessments were conducted twice pre-dose (double baseline) and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose by trained personnel. A training session was included as part of the screening examination to exclude non- or extreme responders. To reduce variability from affects associated with fear of pain, the subjects themselves were responsible for starting and ending each pain task. To eliminate the risk of tissue damage, all pain tasks had a maximum safety cut-off.

The utilised battery of evoked pain tasks consists of the following tasks for nociception: the electrical stimulation task, pressure stimulation task, heat pain and the cold pressor task. Furthermore, the test battery includes a model for inflammatory pain, the UVB model and a paradigm to quantify Conditioned Pain Modulation (CPM).

For the electrical stimulation task, the pressure stimulation task and the cold pressor task, pain intensity was measured continuously (beginning from when the first stimulus was applied until the end of the test) using an electronic Visual Analogue Scale (VAS) scale ranging from 0 (no pain) to 100 (most intense pain tolerable). For the abovementioned pain tasks, the Pain Detection Threshold (PDT), Pain Tolerance Threshold (PTT), Area Under the Curve (AUC) and a post-test Visual Analogue Scale (VAS) score were determined. For the thermal pain tasks (normal skin and UVB exposed skin) only the (average of triplicate) PDT was determined, since assessment of heat PTT is prone to induce tissue damage.

Pharmacokinetic assessments

During all study periods, blood samples (3 ML) to provide a minimum of 1.5 mL plasma for pharmacokinetic (PK) analysis PF-06372865 were collected pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 hours after study drug administration for PK analysis. Pharmacokinetic parameters were maximum observed plasma concentration (C_{max}), area under the plasma concentration-time profile from time 0 to the time of last quantifiable concentration (AUC_{last}) and time for C_{max} (T_{max}) were calculated for each subject using non-compartmental analysis of plasma concentration-time data.

Statistics

The sample size and decision criteria were based on the mean effect over the first 6 hours post-dosing for the primary endpoints: PTT for the pain tasks cold pressor, pressure pain and electrical pain, and the PDT for the thermal pain tasks (normal skin and UVB skin). A sample size of 20 subjects was selected to ensure balance in the design and to provide acceptable operating characteristics for decision-making based on conservative estimates of within-subject standard deviations (SD) from two previous studies.[32,35] Consequently, not each drop-out had to be replaced. The criterion used for each primary comparison was having at least 95% confidence that the effect of either dose of PF-06372865 was better than that of placebo. This is equivalent to a one-sided test for statistical significance using an alpha of 0.05. No adjustment was made for multiplicity as this was an early-phase clinical study designed to explore the pharmacodynamics of PF-06372865. The Williams design (balanced for first-order carry-over effects) randomization code was generated by an independent team. Randomisation numbers were sequentially allocated by the study physician, and blinded study treatments were prepared and dispensed by an independent operating pharmacy.

A mixed effect repeated measures model was fitted for each endpoint, using data collected during the first 6 hours post treatment. This time window was selected based on the pharmacokinetic and pharmacodynamic profile as observed in the First-in-Human study.[26] The fixed effects included in the model were baseline, period, time, treatment and treatment by time interaction, with baseline as a covariate. Subject was fitted as a random effect and time point was repeated within each subject by period as a repeated effect. Baseline was included as 2 separate variables.[36] The PTT and PDT endpoints for the electrical-, pressure-, and cold pressor task were log-transformed prior to analysis. The treatment effects and comparisons to placebo for the log-transformed outcome measures were back transformed and are reported as geometric Least Square Means (LSMeans) and ratios, respectively along with corresponding 90% confidence intervals. For the thermal pain tasks (normal skin and UVB exposed skin) PDT and secondary parameters for each pain task (AUC and VAS), no log-transformation was performed and the contrasts are presented as absolute mean differences in LSMeans versus placebo with 90% confidence intervals. Conditioned pain post-VAS was conducted post-hoc utilizing the same approach as that from the other analyses.

RESULTS

A total of 20 subjects were randomised, of which 19 subjects completed the study. One subject was excluded due to a positive drug screen prior to the second study period. The majority of the subjects were white (90%). A summary of the baseline demographics is provided in Table 1.

Pharmacodynamics

The pharmacodynamic effect profiles for each treatment on the primary endpoints are graphically summarised in Figure 1. The results of the analyses for the PTT for all pain tasks, except for thermal pain (PDT for Normal Heat and UVB Heat) are presented. Time profiles for each treatment on the primary endpoints are shown in Figure 2.

A detailed overview of the results of the analyses for the pharmacodynamic output variables (PTT, PDT, AUC and VAS) is provided in Table 2. 15 mg PF-06372865 significantly increased Pressure PTT at a ratio of 1.11 (90% CI: 1.02, 1.22) compared to placebo. A dose of 65 mg PF-06372865 significantly increased Cold pressor PTT at a ratio of 1.17 (90% CI: 1.03, 1.32), as well as VAS for Electrical stimulation compared to placebo. Over the 6-hour period, a statistically significant increase at a ratio of 1.11 (90% CI: 1.01, 1.21) on Pressure PTT was also observed for PF-06372865 65 mg versus placebo. However, Pressure PDT was statistically significant decreased, indicating an increased sensitivity in pain detection. The positive control, pregabalin (300 mg) significantly affected pain sensation for Cold pressor PTT by 1.31 (90% CI: 1.16, 1.48) compared to placebo as well as Cold pressor AUC. Additionally, Pressure PTT was significantly increased at a ratio of 1.15 (95% CI: 1.06, 1.26), as well as Pressure AUC. No statistically significant effects

were detected for any of the three treatments versus placebo for any of the endpoints related to the thermal pain tasks (normal skin and UVB skin). As a result of the conditioning stimulus, the PTT for electrical pain was on average decreased by 1.16 mA in the placebo group. None of the CPM parameters were statistically significant different between the three active treatments and placebo.

Pharmacokinetics

Median plasma PF-06372865 concentration-time profiles are presented in Figure 3 and pharmacokinetic (PK) parameters are summarised descriptively in Table 3. Following administration of single oral doses of PF-06372865 15 mg and 65 mg, median T_{max} was observed at 2 and 3 hours, respectively. Both plasma PF-06372865 AUC_{last} and C_{max} appeared to increase proportionally with dose from 15 mg to 65 mg. Summary statistics for AUC_{inf} and $T_{½}$ were not reported because <50% of the subjects had reportable parameter values; PK was sampled to 10 hours and thus terminal phase was not characterised. No active metabolites were identified.

Safety

The majority of subjects reported AEs in the system organ class (SOC) of nervous system disorders, general disorders and administration site conditions, almost all of which were considered treatment-related. The three most frequently reported adverse events after treatment with PF-06372865 15 mg were dizziness (39%), fatigue (33%) and bradyphrenia (28%). For treatment with PF-06372865 65 mg the six most frequently observed adverse events were dizziness (53%), somnolence (32%), bradyphrenia (32%), fatigue (26%), balance disorder (26%) and feeling abnormal (26%). For treatment with the positive control pregabalin 300 mg the three most frequently observed adverse events: somnolence (55%), fatigue (40%) and dizziness (35%), which was in line with what is previously reported for single dosing of pregabalin (300 mg).[37] In the placebo treated arm, nasopharyngitis (16%), headache (16%) and bradyphrenia (16%) were most frequently reported. All recorded AEs were mild in severity.

DISCUSSION

In this clinical study investigating the effects of PF-06372865, the two dose levels (15 mg and 65 mg) were safe and well tolerated. The doses were selected to achieve ~50% and ~80% receptor occupancy at α2 $GABA_A$ receptors, respectively. The observed adverse events were mild and confirm previous observations.[26]

PF-06372865 demonstrated an analgesic effect on Pressure pain and the Cold pressor task. The magnitude of effect was greater for a dose of 65 mg compared with a dose of 15 mg for the Cold pressor but the effects were similar for the Pressure pain task. Pregabalin 300 mg attenuated pain induced using the Cold pressor and the Pressure pain task.

At present, to our knowledge few studies have previously reported the effects of GABAergic modulating compounds utilising evoked pain models in healthy subjects in a comparable setting as the current study. What is more, no studies using this type of multimodal methodology to investigate the analgesic potential for α2/α3/α5 subtype selective $GABA_A$ agonists in healthy subjects were found. One study investigating the effects of two non-selective benzodiazepines, clonazepam and clobazam[21] was identified. In this study, an antihyperalgesic effect was demonstrated using the capsaicin model in combination with a cuff algometry challenge, in addition to other methods. Even though the deployed methodology is different from the present study, some parallels can be drawn. For example, PF-06372865 also attenuated pressure PTT, of which similar findings suggestive of analgesia were previously reported. Furthermore, the earlier observed lack of treatment effects on cutaneous electrical pain and CPM, were also seen here.

Absence of analgesia against UV induced inflammatory pain of PF-06372865 is perhaps surprising, as spinal $GABA_A$ α2 receptors have been identified as potential targets to exert antihyperalgesic effects in preclinical research.[13,16,18,38] $GABA_A$ α2 receptors are located and act at a spinal level.[39] Hyperalgesia resulting from cutaneous (UVB induced) inflammation, is thought (albeit debatable) to be of peripheral origin,[40] resulting from decreased activation thresholds for local nociceptive and non-nociceptive neurons alike. This is a response to damaged DNA as a result of exposure to UVB radiation, which results in the induction of NFκB that leads to local production of cytokines IL-1, IL-6 and TNF-α.[41] This specific type of inflammation and associated inflammatory hyperalgesia is distinct from other types of experimentally induced inflammation typically used in preclinical research in its origin and underlying pathophysiology.[42,43] UVB-induced hyperalgesia is most effectively counteracted by inhibition of cyclooxygenase (COX), thus preventing the formation of prostaglandins and thromboxane, which are responsible for the lowered activation thresholds.[32,44,45] PF-06372865 exerts its effects via GABAergic modulation of the central nociceptive system, which is known to demonstrate mixed effects on UVB induced inflammatory pain in human evoked pain studies.[46] The absence of antihyperalgesic effects is at odds with previously

reported antihyperalgesic effects of clobazam on UVB induced heat perception thresholds.[47] A potential explanation could be the less profound $GABA_A$ receptor subtype selectivity of clobazam compared to PF-06372865, as its metabolite N-desmethyl clobazam is proposed to demonstrate more selectivity for α2 $GABA_A$ receptors over α1 $GABA_A$ receptors, compared to the parent.[48] Similar to the findings in the present study, it was shown that CPM was not affected by treatment with the nonselective benzodiazepine lorazepam.[49] The role for GABAergic interneurons in descending modulation and their role in chronification of pain has been recognised previously.[50-52] The underlying mechanism has been described as the GABA disinhibition hypothesis of analgesia.[53] This hypothesis describes a particular descending periaqueductal gray - rostroventricular medulla (PAG-RVM) pathway that mediates the phenomenon of stress-induced analgesia. This pathway exerts analgesia via suppression of inhibitory GABAergic inputs onto output neurons that constitute the descending analgesic pathway. However, the CPM paradigm based on electrical pain, which was used in the current study, may not be the most suitable paradigm to determine the potential effects of GABAergic intervention, as it has been shown that spinal enkephalins and GABA presynaptically modulate mechanonociception via A-delta fibres, whereas electrical stimulation is generally thought to activate both A-delta and C-fibres.[54-55] Finally, as also suggested previously[49] the lack of a modulating effect on CPM by $GABA_A$ agonism in the present study, may be explained by the fact that in the healthy state the role of GABAergic interneurons in CPM is optimised for endogenous GABA agonism and thus not susceptible for external influence in the absence of true stressors.

In contrast, patients suffering from chronic pain, where it is known that GABAergic dysregulation may be a large contributing factor,[16] may be susceptible for pain alleviation through GABA modulation. This hypothesis is moderately substantiated by the overlap observed in effect profiles of pregabalin and PF-06372865. This overlap in clinical application may suggest that PF-06372865 has a potential role in the treatment of different types of neuropathic pain, similar to pregabalin.[56] Alternatively, variability of CPM response is higher than the variability of the other pain tasks and perhaps the study was underpowered to detect an effect on CPM response.

Even though the clinical application of PF-06372865 and pregabalin may demonstrate a slight overlap, their mechanism of action is different. Pregabalin is a calcium channel antagonist that shows specific binding affinity for the α2-δ auxiliary subunits of voltage-gated calcium channels (VGCC). There have been a number of studies showing an up-regulation of VGCC's in dorsal root ganglion and dorsal horn in neuropathic pain.[57] Administration of pregabalin is shown to partially reverse the up-regulated calcium α2-δ-1 at the pre-synaptic nerve terminals in the dorsal horn.[58] Even though there is a structural resemblance with GABA, different studies have shown that pregabalin does not appear to mimic GABA or pharmacologically enhance its actions.[59] As such, pregabalin is not considered a positive control from a mechanistic perspective, but rather from a clinical perspective. More importantly its analgesic effects and the reproducibility thereof on the pain test battery that was used in the current study have been demonstrated multiple times before.

The population included in the study was considered to be very homogeneous, which improves the internal validity but may impact generalisability of the study results to other populations. The study included only male subjects, of which 90% was white. The existence of sex differences in terms of sensitivity to clinical and experimental pain is widely known.[60,61] It has been suggested that sex-specific differences in $GABA_A$ receptors may play a role in this differentiation.[62] However, it is also known that the menstrual cycle influences pain perception,[63,64] which would interfere significantly with the crossover design of the study, therefore it was decided to include only male subjects were in the present study. Furthermore, since the UVB model was a primary endpoint of the study, subjects with Fitzpatrick skin type V or VI were excluded due to the inability to accurately assess MED and to safely induce UVB induced inflammation, leading to a predominantly white study population. Research has identified differences in pain sensitivity between different ethnic or racial populations in experimental pain research.[65,66] Whether those findings can be extrapolated to the Dutch population and what is the exact cause of the differences in pain perception is unknown.

The present study is an early-phase hypothesis-generating clinical study designed to guide decision-making and explore the pharmacodynamics and pharmacokinetics of PF-06372865 in healthy subjects using a multi-modal battery of pain tasks. As such, the study was powered to detect a potential analgesic effect on the primary endpoints. However, given the novelty of the pharmacological mechanism of action, secondary parameters for which no formal power calculation was performed are taken into account when reviewing the generated results. Consequently, interpretation of these findings requires more caution and is potentially more prone to type I or type II error, since no correction for multiple testing was performed.

The findings in the present study are indicative of the analgesic potential for PF-06372865 at the doses tested, in addition to the neurocognitive anxiolytic potential that has been identified previously.[26] The observed effect profile does not appear to result from sedation alone for various reasons. In both this study

and a previous study, PF-06372865 was shown to exert only mild sedative effects. Second, in a recently performed study,[67] we showed that the test battery of evoked pain tasks was not sensitive to the effects of sedation, by investigating the effects of a sedative H1 antihistaminergic agent, promethazine.

Translation of the findings of a human evoked pain model utilised in healthy subjects to clinical pain remains elusive, but the present study has demonstrated clearly that PF-06372865 has analgesic potential, at dose levels that do not induce significant sedation or other intolerable adverse events limiting its clinical use. This analgesic potential however, was not found in a recent clinical study where the effects of 2.5 mg (one week) followed by 7.5 mg (three week treatment period) PF-06372865 on chronic low back pain were investigated.[68] These discrepant findings may result from several factors, but the difference in dosing regimen and consequently lower receptor occupancy in the patient study could be the putative cause.

Finally, the present study further established the potential role for this battery of pain tasks as a tool in the development of analgesics with a novel mechanism of action, for the treatment of various pain states including neuropathic pain and to determine proof-of-concept.

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Table 1 – Summary of subject characteristics (n=20).

Age (years)		
	Mean (SD)	30.3 (10.8)
	Range	18-50
RACE (N)		
	White	18
	Other	2
WEIGHT (KG)		
	Mean (SD)	78.7 (10.5)
	Range	60.1-100.7
BMI (KG M^{-2})		
	Mean (SD)	23.3 (2.9)
	Range	18.5- 27.2

SD = standard deviation

Table 2 – Summary of least square means analyses for pain test battery.

Endpoint	Placebo	PF-06372865 15mg			PF-06372865 65mg			Pregabalin 300mg		
	LSMean (90%CI)§†	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)
COLD PRESSOR (S)										
PTT	18.37 (16.83, 20.06)	19.55 (17.86, 21.40)		1.06 (0.94, 1.21)	21.45 (19.64, 23.43)		1.17 (1.03, 1.32)*	24.02 (22.05, 26.17)		1.31 (1.16, 1.48)*
PDT	4.54 (3.85, 5.35)	4.24 (3.58, 5.03)		0.94 (0.74, 1.19)	4.18 (3.55, 4.93)		0.92 (0.73, 1.16)	5.35 (4.56, 6.29)		1.18 (0.94, 1.49)
AUC	10541 (10326, 10756)	10342 (10122, 10563)	-198.54 (-506.69, 109.60),		10330 (10115, 10545)	-210.61 (-514.63, 93.41)		10025 (9815, 10234)	-516.30 (-816.19, -216.41)*	
VAS	76.15 (73.84, 78.46)	78.21 (75.71, 80.70)	2.06 (-1.28, 5.39)		74.94 (72.65, 77.24)	-1.21 (-4.47, 2.06)		74.06 (71.64, 76.47)	-2.09 (-5.50, 1.32)	
ELECTRICAL STIMULATION (MA)										
PTT	19.28 (18.07, 20.58)	20.27 (18.96, 21.66)		1.05 (0.96, 1.15)	21.08 (19.74, 22.50)		1.09 (1.00, 1.20)	20.25 (19.01, 21.58)		1.05 (0.96, 1.15)
PDT	6.10 (5.28, 7.05)	6.22 (5.37, 7.21)		1.02 (0.83, 1.25)	6.85 (5.93, 7.92)		1.12 (0.92, 1.38)	6.63 (5.75, 7.63)		1.09 (0.89, 1.33)
AUC	3360 (3214, 3506)	3331 (3181, 3480)	-29.30 (-238.66, 180.06)		3196 (3049, 3344)	-163.52 (-372.25, 45.22)		3278 (3134, 3422)	-81.86 (-285.64, 121.93)	
VAS	74.99 (72.45, 77.54)	74.29 (71.57, 77.01)	-0.70 (-4.51, 3.10)		70.34 (67.80, 72.88)	-4.65 (-8.28, -1.02)*		72.19 (69.63, 74.76)	-2.80 (-6.34, 0.74)	
CPM: ELECTRICAL STIMULATION (DIFFERENCE PRE-POST COLD PRESSOR) (MA)										
PTT	1.16 (0.77, 1.55)	1.16 (0.78, 1.55)	0.00 (-0.55, 0.55)		1.37 (1.00, 1.74)	0.21 (-0.33, 0.75)		1.04 (0.68, 1.41)	-0.12 (-0.65, 0.42)	
PDT	0.51 (-0.24, 1.25)	0.28 (-0.49, 1.04)	-0.23 (-1.29, 0.84)		-0.07 (-0.79, 0.66)	-0.57 (-1.61, 0.47)		0.36 (-0.37, 1.09)	-0.15 (-1.19, 0.90)	
AUC	-88.41 (-145.30, -31.51)	-89.22 (-146.76, -31.68)	-0.82 (-81.82, 80.19)		-78.91 (-135.14, -22.68)	9.50 (-70.85, 89.84)		-79.57 (-134.15, -25.00)	8.83 (-69.83, 87.50)	
VAS	0.41 (-0.77, 1.59)	1.08 (-0.10, 2.25)	0.67 (-1.02, 2.35)		1.59 (0.46, 2.72)	1.18 (-0.47, 2.83)		0.53 (-0.57, 1.64)	0.12 (-1.49, 1.74)	

Table 2 – continued

PRESSURE STIMULATION (KPA)										
PTT	40.20 (37.75, 42.82)	44.74 (41.95, 47.71)		1.11 (1.02, 1.22)*	44.57 (41.86, 47.46)		1.11 (1.01, 1.21)*	46.36 (43.62, 49.27)		1.15 (1.06, 1.26)*
PDT	11.15 (8.76, 14.18)	9.03 (7.04, 11.59)		0.81 (0.57, 1.15)	7.14 (5.61, 9.08)		0.64 (0.46, 0.90)*	9.34 (7.38, 11.84)		0.84 (0.60, 1.17)
AUC	6982 (6633, 7332)	6841 (6483, 7198)	-141.48 (-646.97, 364.00)		6699 (6352, 7046)	-283.02 (-780.61, 214.57)		6439 (6102, 6775)	-543.23 (-1025.52, -60.94)*	
VAS	68.83 (66.24, 71.42)	70.11 (67.44, 72.77)	1.27 (-2.45, 5.00)		64.80 (65.81, 70.99)	-0.43 (-4.10, 3.24)		66.49 (63.97, 69.01)	-2.34 (-5.95, 1.27)	
NORMAL HEAT (°C)										
PDT	47.077 (46.631, 47.523)	46.934 (46.478, 47.391)	-0.143 (-0.780, 0.495)		46.780 (46.336, 47.223)	-0.297 (-0.928, 0.333)		47.291 (46.856, 47.726)	0.214 (-0.412, 0.840)	
UVB HEAT (°C)										
PDT	41.063 (40.465, 41.661)	41.206 (40.600, 41.811)	0.143 (-0.700, 0.985)		41.406 (40.806, 42.006)	0.343 (-0.523, 1.209)		41.445 (40.872, 42.019)	0.383 (-0.454, 1.219)	

Contrasts presented are over 0-6 hours post dose.*90% confidence intervals (CI) that do not overlap with zero (for differences to placebo) or one (for ratios to placebo) indicate that the pre-defined criterion was met (marked in bold). Contrasts presented are over 0–6 hours after dose. LSMeans, least square mean; PTT = pain tolerance threshold; PDT = pain detection threshold; AUC = area under the curve; VAS = visual analogue scale; CPM = conditioned pain modulation.

Table 3 – Summary of Pharmacokinetic parameters for single administration of 15 and 65 mg PF-06372865

Treatment	PF-06372865	
Dose level	15 mg (N=18)	65 mg (N=19)
C_{max} (ng mL^{-1})[a]	59.04 (37)	304.8 (33)
T_{max} (h)[b]	2.00 (0.500-4.00)	3.00 (0.500-5.00)
AUC_{last} (ng•h mL^{-1})[b]	342.4 (57)	1837 (41)

a Geometric mean (% Geometric CV); b Median (range)

Figure 1 – Spiderplot summary of Pharmacodynamic response profile for pain test battery normalised to placebo. Dashed placebo line (green) represents a value of 1 to which other treatment effects are normalised. Distal from the centre beyond the placebo line indicates Least Square Mean PTT/PDT greater than placebo, towards the centre and within the placebo line indicates Least Square mean PTT/PDT lower than placebo. A closed circle (•) indicates meeting pre-specified decision criteria relative to placebo for treatment on pain task.

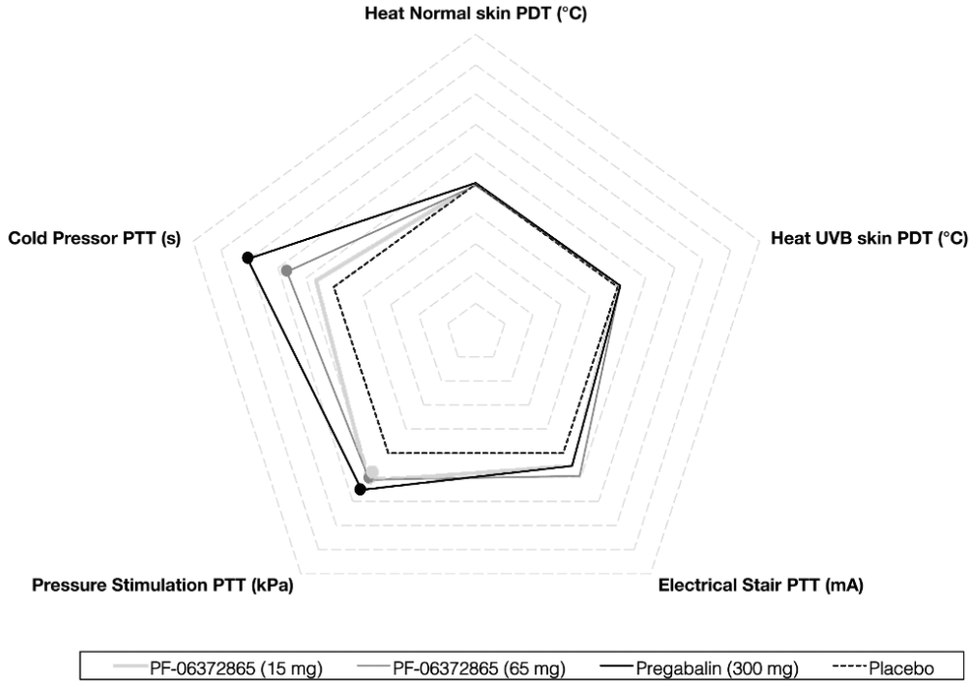
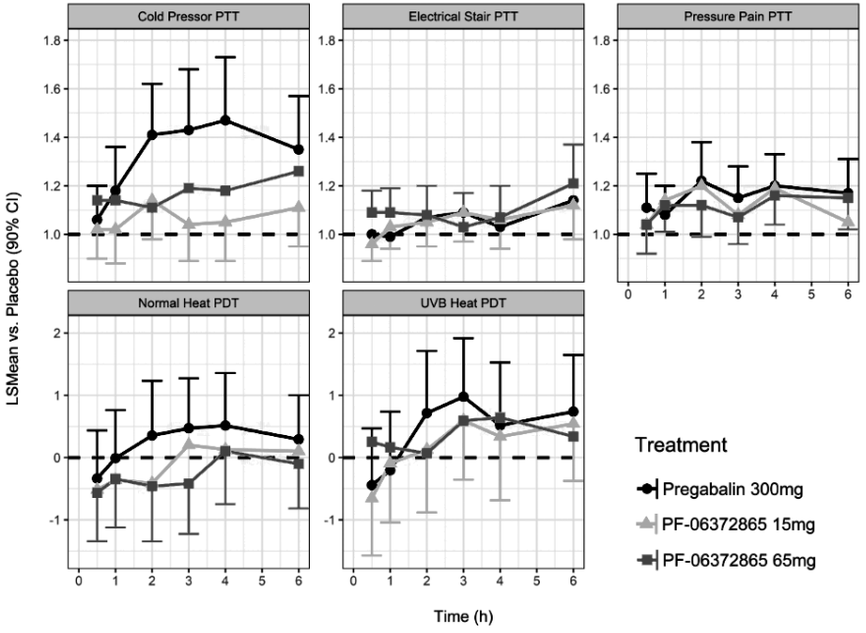
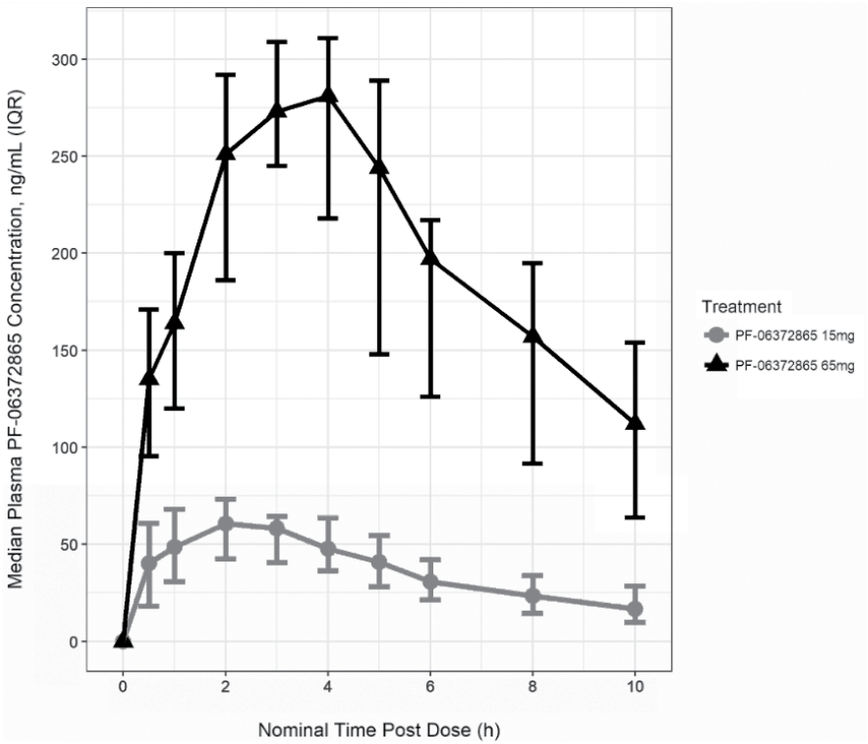


Figure 2 – Graphical overview of Pain Thresholds Time profiles for pressure pain task



CI = confidence interval; H = hours; PTT = Pain Tolerance Threshold; PDT = Pain Detection Threshold

Figure 3 – Pharmacokinetic profile for single administration of 15 and 65 mg PF-06372865 median plasma PF-06372865 concentration over time.



H = hour; IQR = inter-quartile range / ● = 15 mg PF-06372865 / ▲ = 65 mg PF-06372865

Chapter 3

DEMONSTRATION OF AN ANTI-HYPERALGESIC EFFECT OF A NOVEL PAN TRK INHIBITOR PF-06273340 IN A BATTERY OF HUMAN EVOKED PAIN MODELS

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ABSTRACT

Inhibitors of nerve growth factor (NGF) reduce pain in several chronic pain indications. NGF signals through tyrosine kinase receptors of the tropomyosin-related kinase (Trk) family and the unrelated p75 receptor. PF-06273340 is a small molecule inhibitor of Trks A, B and C that reduces pain in nonclinical models and this study aimed to investigate the pharmacodynamics of this first in class molecule in humans.

A randomized, double blind, single dose, placebo and active-controlled 5 period cross-over study was conducted in healthy human subjects (NCT02260947). Subjects received 5 treatments: PF-06273340 50 mg, PF-06273340 400 mg, pregabalin 300 mg, ibuprofen 600 mg and placebo. The 5 primary endpoints were the pain detection threshold for the thermal pain tests (normal and UVB skin) and the pain tolerance threshold for the cold pressor, electrical stair and pressure pain tests. The trial had pre-defined decision rules based on 95% confidence that the PF-06273340 effect was better than placebo.

20 subjects entered the study, with 18 completing all 5 periods. The high dose of PF-06273340 met the decision rules on the UVB skin thermal pain endpoint (LSMeans versus placebo: 1.13, 95% confidence interval: 0.64-1.61), but not on the other 4 primary endpoints. The low dose did not meet the decision criteria for any of the 5 primary endpoints. Pregabalin (cold pressor and electrical stair) and ibuprofen (UVB thermal pain) showed significant analgesic effects on expected endpoints.

This study demonstrated for the first time translation of nonclinical effects into man in an inflammatory pain analgesic pharmacodynamic endpoint using a pan-Trk inhibitor.

INTRODUCTION

Nerve growth factor (NGF) is a key mediator of chronic pain. Administration of NGF to animals or to human subjects causes pain,[1] and studies with anti-NGF scavenging monoclonal antibodies such as tanezumab have demonstrated efficacy in Phase 3 trials in several chronic pain indications.[2,3] NGF is a member of the neurotrophin family that signal through both tyrosine kinase receptors of the tropomyosin-related kinase (Trk) family and the unrelated p75 receptor. The neurotrophins comprise NGF which signals preferentially through TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT4) which signal through TrkB, and neurotrophin 3 (NT3) which signals through TrkC. The neurotrophins are equipotent at the p75 receptor. NGF signaling through TrkA is known to induce both acute and chronic regulation of pain signaling, through phosphorylation dependent regulation of ion channels involved in pain transmission and upregulation of pain related genes respectively.[4] BDNF was also found to be implicated in nonclinical pain signaling and was found to be upregulated in clinical pain states in human subjects.[5]

PF-06273340 is a peripherally restricted small molecule inhibitor of Trks A, B and C, where the structural formula has been published previously.[6] It is equipotent at the three Trk receptors, but is otherwise broadly selective. PF-06273340 and other molecules in this class reverse chronic pain in nonclinical models where there has been some sensitization such as UVB sensitization to the skin[6] or carrageenan irritation of the joint.[7] To date there are no data on whether these analgesic effects of small molecule pan Trk inhibitors translate to human subjects.

In the current study the analgesic effects of PF-06273340 were assessed using a battery of human evoked pain models. These models have been shown to provide robust evidence of analgesia in healthy human subjects using a number of positive controls assessed against different pain stimuli and endpoints.[8,9] By assessing PF-06273340 using this methodology we intended to establish whether the nonclinical data demonstrating efficacy in the UVB sensitization model would translate to human subjects. The inclusion of other pain models in the study provided a comparison with non-sensitised pain states. Furthermore we would be able to provide the first demonstration of analgesic efficacy for this novel class of compounds in a small, easy to recruit trial prior to investing in larger patient studies.

METHODS

Subjects and study design

The study was a double blind, double dummy, single dose, randomized, placebo-controlled, 5-period cross-over study (NCT02260947). In this study PF-06273340 was the drug under investigation and ibuprofen and pregabalin were used as positive controls. The study was conducted at a single site at the Centre for Human Drug Research in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. Each subject provided written informed consent before any screening procedures were performed.

Approximately 20 healthy male subjects, between 18 to 55 years of age, were to attend the clinic on 7 separate occasions (Screening, Periods 1-5 and follow-up) to examine the effects of PF-06273340 on evoked pain endpoints. Periods 1-5 were spaced apart by at least 7 days, which based on the half-lives of the single doses of treatments would give sufficient time to allow for the wash-out of pharamacokinetic (PK) and potential pharmacodynamic (PD) effects.

Study drugs

The study included placebo, ibuprofen and pregabalin (positive controls) and 2 dose levels of PF-06273340. Subjects were randomised to one of 10 sequences that consisted of two Williams 5x5 design that were balanced for first-order carry-over. At each investigational period, subjects received a total of 4 tablets and 1 capsule: 2 tablets of 200 mg PF-06273340/placebo, 1 tablet of 50 mg PF-06273340/placebo, 1 tablet 600 mg ibuprofen/placebo and 1 capsule 300 mg pregabalin/placebo. The doses of PF-06273340 selected for this study were 50 mg and 400 mg given as single doses. These doses were justified based on margins to toxicology findings and on clinical toleration and safety data from Phase 1 single and multiple ascending dose studies (https://clinicaltrials.gov/ct2/results?term=PF-06273340) in healthy young and elderly subjects. The top dose of 400 mg PF-06273340 was close to the maximum dose given previously to human subjects, whereas the lower dose of 50 mg PF-06372865 allowed examination of the bottom end of the predicted pharmacologically active range.

Doses of 600 mg ibuprofen and 300 mg pregabalin had been used as positive controls in previous human evoked pain model studies.[8,9] These doses were well tolerated and within the labeled dose range for ibuprofen and pregabalin in the European Union (EU).

Pharmacokinetic assessments

During all study periods, blood samples (3 mL) to provide a minimum of 1.5 mL plasma for PK analysis PF-06273340, pregabalin and ibuprofen were collected at pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 hours after study drug administration for PK analysis of PF-06273340, pregabalin and ibuprofen. Plasma PF-06273340 pharmacokinetic parameters: maximum observed plasma concentration (C_{max}), area under the plasma concentration-time profile from time 0 to the time of last quantifiable concentration (AUC_{last}), time for C_{max} (T_{max}) were calculated for each subject using non compartmental analysis of plasma concentration-time data.

Pharmacodynamic assessments

Pain detection and tolerance thresholds were measured using a battery of human pain models that assess a range of modalities of pain using previously described methodology.[8,9] Briefly, the pain models included thermode, electrical stimulation, mechanical pain and cold pressor, which were all performed sequentially at pre-dose (twice) and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours after study drug administration in each period. Thermal (heat) pain was determined on normal skin and on UVB exposed skin. Pain intensity was measured continuously during each test using an electronic visual analogue scale (eVAS) ranging from 0 (no pain) to 100 (most intense pain tolerable). The pain detection threshold (PDT) and pain tolerance threshold (PTT) were of primary interest (see Supplementary data for more details).

Ibuprofen and pregabalin were included as active controls as based on previous studies;[8,9] ibuprofen had shown effects on the UVB Heat PDT endpoint and pregabalin had shown effects on the Cold Pressor PTT, Pressure Pain PTT, Electrical Stair PTT (pre-Cold pressor) and Normal Heat PDT endpoints.

Statistical Analysis

A mixed effects model was fitted to each endpoint, using data collected during the first six hours post treatment. Absolute values were analysed for PDT endpoints and $\log_e$ transformed values for PTT endpoints as the latter had skewed distributions in previous studies.[8,9] The fixed effects included in the model were baseline, period, time, treatment and treatment by time interaction, with baseline as covariate. Subject was fitted as a random effect. Baseline was included as two separate variables, the average baseline for the subject, and the deviation of each period baseline from the average baseline for each subject.[10] The Kenward-Roger approximation was used for estimating degrees of freedom for the model parameters. The primary analysis included all subjects randomised into the study.

The LSMeans together with 90% confidence intervals were obtained for each treatment averaged across time points that cover the peak exposure of each treatment. Based on the known human pharmacokinetics the average across the first 4 hours was obtained from the mixed effects model for PF-06273340 and Ibuprofen whereas the average across the first 6 hours was obtained for pregabalin. Both the average over the first 4 and 6 hours were obtained for placebo. Differences between treatments and placebo were therefore made using the appropriate average (*i.e.*, ibuprofen was compared to the placebo 4 hour average, whereas pregabalin was compared to the placebo 6 hour average). Differences to placebo are presented as absolute differences for PDT endpoints and ratios for PTT endpoints together with corresponding 90% confidence intervals.

As a sensitivity analysis to the primary analysis, a mixed effects model was fitted for the maximum (over 4 or 6 hours post treatment, where appropriate) change from baseline for each primary endpoint. The fixed effects included in the model were baseline, period and treatment. Baseline was similarly included as 2 separate variables. Subject was fitted as a random effect. Additional sensitivity analyses were conducted that applied the primary analysis models to only subjects who completed all 5 treatment periods or applied the models to all subjects but included all time points (*i.e.*, up to and including the 10 hour measurement).

Sample Size

Decision rules were pre-specified to quantify what was required in the primary objective of the study. The criteria were based on a Bayesian interpretation of the results assuming a non-informative prior. The criterion used for each endpoint was: At least 95% confident that either dose of PF-06273340 effect was greater than placebo. This is equivalent to a one-sided test for statistical significance using an alpha of 0.05. No adjustment was made for multiplicity as this was an early phase clinical study designed to explore the pharmacodynamics of PF-06273340 and as such no stringent requirement to control the type 1 error rate was required for internal decision making.

The sample size was based on the mean effect over the first 4 hours after dosing (*i.e.*, average of 0.5, 1, 2, 3 and 4 hour time points) for the 5 primary endpoints: Cold Pressor PTT; Pressure Pain PTT; Electrical Stair PTT; Normal Heat PDT; and UVB Heat PDT. The primary comparison was of either dose of PF-06273340 against placebo. A conservative estimate of within-subject standard deviation was derived from two previous methodology studies.[8,9] yielding estimates of 0.25, 0.21, 0.16, 1.79 and 1.63 for the Cold Pressor PTT, Pressure Pain PTT, Electrical Stair PTT, Normal Heat PDT, and UVB Heat PDT endpoints, respectively. A sample size of 20 subjects was selected to ensure balance in the design and gave at least 80% power to detect differences of 0.20, 0.17, 0.13, 1.42 and 1.30 for the 5 primary endpoints listed previously.

NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[11] and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16.[12]

RESULTS

Subject disposition

Of the 20 subjects that were randomised, 18 completed the study (Figure 1). Two subjects discontinued from the study: one due to failure to meet inclusion/exclusion criteria (ECG abnormalities) and did not proceed to Period 5 (missed PF-06273340 50 mg); and the other subject due to no longer willing to participate in the study and only received treatments assigned to Periods 1 and 3 (only treated with PF-06273340 50 mg and ibuprofen 600 mg, respectively). All 20 subjects were male, with a mean age (standard deviation) of 26.0 (6.9) and body mass index of 23.7 (2.5) (Table 1). Fifteen subjects reported ethnicity as "White" with the 5 remaining as "Other". All 20 subjects were dosed with ibuprofen 600 mg, and nineteen subjects were dosed with placebo, PF-06273340 50 mg, PF-06273340 400 mg or pregabalin 300 mg.

Pharmacodynamics

A summary of the results to the primary analyses are presented in Table 2 and Figure 2. PF-06273340 400 mg met the decision criteria for the UVB Heat PDT endpoint as shown by a statistically significant increase over placebo of 1.13 units (90% CI = 0.64 to 1.61). There were no statistically significant effects of PF-06273340 50 mg relative to placebo on any of the 5 primary endpoints. Ibuprofen showed a statistically significant effect on the UVB Heat PDT endpoint compared to placebo with an increase of 1.39 units (90% CI = 0.91 to 1.87). pregabalin 300 mg had statistically significant effects over placebo on the Cold Pressor PTT (effect versus placebo = 1.22, 90% CI = 1.11 to 1.34) and the Electrical Stair PTT (effect versus placebo = 1.09, 90% CI = 1.01 to 1.19). The time course profiles of the five treatments across the 5 primary endpoints are presented in Figure 3. Sensitivity analyses gave similar results (data not shown).

Safety

Single doses of PF-06273340 400 mg or PF-06273340 50 mg administered to healthy male subjects were generally safe and well tolerated in this study. There were no SAEs or other clinically significant AEs reported.

The most frequently reported all causality treatment related adverse events were dizziness (16 subjects: 4 subjects in PF-06273340 400 mg group, 2 subjects each in PF-06273340 50 mg and placebo group, and 8 subjects in pregabalin group), somnolence (13 subjects: 2 subjects each in PF-06273340 50 mg and ibuprofen group, 1 subjects each in PF-06273340 400 mg and placebo group, and 7 subjects in pregabalin group), and fatigue (11 subjects: 2 subjects each in PF-06273340 400 mg and pregabalin group, 3 subjects each in PF-06273340 50 mg and ibuprofen group, and 1 subject in placebo group). All treatment related adverse events were mild in severity, except 1 subject in PF-06273340 50 mg treatment group had upper abdominal pain, which was moderate in severity and considered treatment-related by the investigator.

Pharmacokinetics (PK)

Median plasma PF-06273340 concentration-time profile is presented in Figure 4 and PK parameters are summarized descriptively in Table 3.

PF-06273340 was rapidly absorbed following oral administration with mean T_{max} of 1 hour for both treatments. C_{max} and AUC_{last} appeared to increase proportionally with doses from 50 mg to 400 mg, and between subject variability in plasma PF-06273340 exposure based on geometric %CV for C_{max} and AUC_{last} ranged from 42% to 57%.

DISCUSSION

This is the first study to test a novel candidate analgesic targeting the NGF pathway using a panel of human evoked pain models. Previous studies had demonstrated that these models provided a reproducible method to assess the effects of analgesic drugs on a battery of evoked pain assessments in healthy human subjects, with consistent results obtained from the ibuprofen and pregabalin positive controls.[8,9] The current study confirmed previous results showing a significant effect of ibuprofen on the UVB Heat PDT assessment, and of pregabalin on the cold pressor test. pregabalin also demonstrated a modest effect in the electrical stair which achieved statistical significance and had demonstrated an effect at this endpoint in some but not all previous studies.[8,9] Overall the results of the positive controls ibuprofen and pregabalin confirm the validity of this methodology for detecting reproducible analgesic signals in healthy human subjects.

The 400 mg dose of pan Trk inhibitor PF-06273340 significantly reduced the hyperalgesia seen in the UVB Heat PDT assessment but did not have an effect on any other endpoint. This is similar to the pattern seen with ibuprofen and is in agreement with expected biology of the mechanism. NGF is upregulated in experimental models of inflammation including UVB sensitisation,[11,12] and anti-NGF monoclonals and Trk inhibitors (including PF-06273340) have shown efficacy in nonclinical models of inflammatory pain such as complete Freund's adjuvant, carrageenan and ultraviolet B radiation,[6,13] NGF has direct and indirect actions in inflammatory pain (reviewed by Mantyh et al).[13] Administration of NGF leads to binding at TrkA on immune cells (including mast cells) and subsequent release of inflammatory mediators which contribute to sensitisation of nociceptors. In addition, NGF binding to TrkA on sensory nerve fibres elicits signalling cascades which result in trafficking of nociceptors to the cell surface and their sensitisation by phosphorylation. One of the receptors that contributes to increased signalling in this manner is the heat sensitive ion channel TRPV1 which is likely an important component of the UV induced hyperalgesia in man;[14] inhibition of TRPV1 signalling may be one component of PF-06273340 efficacy in this model. The indirect effects of NGF involve the retrograde transport of NGF/TrkA complexes to the nucleus where the transcription of nociceptors and peptides involved in pain signalling are upregulated. In vitro data have shown that this transport is inhibited by PF-06273340 (Bilsland personal communication), and this may also contribute to the effect on UVB seen in the present study Although the longer timescale of this process implies it may be more important in chronic pain states.

Given the proven efficacy of anti-NGF monoclonals in nonclinical species and in human clinical studies it is tempting to ascribe the efficacy of PF-06273340 to a blockade of TrkA signalling. However, a role for BDNF (which signals through TrkB) cannot be discounted as it has been shown to play a role in hyperalgesia and pain in some nonclinical systems and has been implicated in some human biology studies in visceral and neuropathic pain states.[5] The effects of NT3 signalling through TrkC and NT4 signalling through TrkB are not as well defined[15] but published data do not suggest a major role in nociception. One conclusion that can be drawn from the effects of PF-06273340 is that blockade of signalling through p75 (which is spared by PF-06273340 but not by anti-NGF monoclonals) is not required for an analgesic effect in man.

The effect size of the 400 mg dose of PF-06273340 versus placebo on the UVB endpoint was similar to that of ibuprofen, however the lower 50 mg dose of PF-06273340 did not have a significant effect. The median exposure of PF-06273340 at the top dose achieved ~ $30\times IC_{50}$ at C_{max} and dropped to ~ $9\times IC_{50}$ at the end of the assessment period (4 hours), whereas the lower dose achieved ~ $4\times IC_{50}$ at C_{max} and dropped to ~ $1\times IC_{50}$ at 4 hours. The differential efficacy of these two doses implies that at least for this endpoint an exposure that achieves a multiple of IC_{50} throughout the assessment period is required for an acute pharmacodynamic effect in inflammatory pain. This conclusion is consistent with the prediction from a systems pharmacology model of the nerve growth factor (NGF) pathway[16] utilizing PF-06273340 data. Further studies are needed to determine how these pharmacodynamic effects in a healthy volunteer study relate to the exposures needed in patients with a chronic pain condition.

The current study has demonstrated for the first time that a pan Trk molecule can reduce hyperalgesia in human subjects. The observed effect in the pre sensitised UVB assessment is consistent with the observed efficacy of anti-NGF monoclonals in chronic pain states with an inflammatory component such as osteoarthritis. We did not see a significant impact on the cold pressor or electrical stair tests where pregabalin was shown to be effective. This may indicate the pan Trk mechanism will be less effective in neuropathic pain states where pregabalin has proven efficacy; however there is uncertainty regarding the translation of studies in healthy human subjects to those with chronic pain conditions and it should be noted that the anti-NGF monoclonals previously showed efficacy in neuropathic pain,[17] albeit at higher doses and exposures than in the inflammatory pain state of OA. Another uncertainty is how to interpret the effect size seen with the top dose of PF-06273340, which was similar to that observed with ibuprofen. We regard the primary role of the human evoked pain models as an early demonstration

of pharmacodynamics for novel molecules and to provide some guidance as to which pain states might be selected for future clinical studies. Optimism than pan Trk inhibitors may be more efficacious that non steroidal anti-inflammatory drugs such as ibuprofen comes from the data with tanezumab showing superior efficacy to naproxen in OA.[2] Given that the methodology in this study was limited by use of single doses in a healthy subject population the translation of effect sizes to long term dosing in chronic pain states is uncertain, and will need to take account of physiological responses such as changes to nocioceptors and signalling pathways brought about by chronic stimulation.[18]

A concern of the anti-NGF monoclonal antibodies is the increased risk of rapidly progressing osteoarthritis (RPOA), which for tanezumab monotherapy ranges from 0 events per 1,000 patient-years at 2.5 mg dose to 11 events per 1,000 patient-years at a 10 mg dose.[19] Small molecule pan Trk inhibitors have the potential advantages of greater flexibility in dosing and that once dosing is stopped the drug will be rapidly eliminated compared to the much slower clearance of a humanised monoclonal antibody. Whether the risk of RPOA is reduced by small molecule pan Trk inhibitors is unknown. Given the low frequency of RPOA in subjects who received anti-NGF monoclonals an assessment of this risk must await larger clinical trials, unless a predictive nonclinical model of RPOA becomes available. We have seen no safety concerns for joint damage to date in our phase 1 programme.

In summary the current study confirms the usefulness of the human evoked pain models to profile novel pain therapeutics in early clinical development. The pan Trk inhibitor PF-06273340 demonstrated a significant effect in the UVB heat PDT assessment providing good evidence of a translation of nonclinical effects into man. This human pain model is easy to execute due to a small sample size in healthy subjects, and we believe it provides a powerful method for demonstrating an analgesic effect for novel pain medications.

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Table 1 – Summary of demographic and baseline characteristics

		All subjects
	Number of subjects	20
SEX		
	Male	20
	Female	0
AGE (YEARS)		
	< 25	11
	25-44	9
	> 45	0
	Mean (SD)	26.0 (6.9)
	Range	18-43
RACE		
	White	15
	Other	5
WEIGHT (KG)		
	Mean (SD)	77.1 (8.1)
	Range	62.7-95.4
BODY MASS INDEX (KG/M^2)		
	Mean (SD)	23.7 (2.5)
	Range	18.2-29.7
HEIGHT (CM)		
	Mean (SD)	180.4 (5.5)
	Range	169.8-190.2

SD, standard deviation

Table 2 – Summary of results for the primary analyses. PTT endpoints were analysed on the log scale, so results are presented as geometric means with %CVs for individual treatment results, and back-transformed LS mean ratios and 95% CIs for treatment comparisons.

Endpoint	Placebo (n=19; 0-4h)	Placebo (n=19; 0-6h)	Ibuprofen 600mg (n=20; 0-4h)		Pregabalin 300mg (n=19; 0-6h)		PF-06273340 50mg (n=19; 0-4h)		PF-06273340 400mg (n=19; 0-4h)	
	LSMean (90% CI)	LSMean (90% CI)	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*
Cold Pressor PTT	28.05 (26.26, 29.97)	27.81 (26.00, 29.76)	29.33 (27.50, 31.28)	1.05 (0.95, 1.15)	33.97 (31.76, 36.34)	**1.22 (1.11, 1.34)**	27.50 (25.74, 29.38)	0.98 (0.89, 1.08)	28.61 (26.78, 30.57)	1.02 (0.93, 1.12)
Electrical Stair PTT	24.36 (23.06, 25.74)	24.60 (23.21, 26.06)	25.00 (23.68, 26.38)	1.03 (0.95, 1.11)	26.92 (25.40, 28.53)	**1.09 (1.01, 1.19)**	23.66 (22.39, 25.01)	0.97 (0.90, 1.05)	22.98 (21.75, 24.28)	0.94 (0.87, 1.02)
Pressure Pain PTT	53.36 (50.66, 56.19)	53.35 (50.59, 56.25)	56.77 (53.98, 59.69)	1.06 (0.99, 1.14)	57.06 (54.11, 60.18)	1.07 (0.99, 1.15)	53.63 (50.94, 56.47)	1.01 (0.93, 1.08)	56.08 (53.27, 59.04)	1.05 (0.98, 1.13)
Normal Heat PDT	46.68 (46.36, 46.99)	46.74 (46.42, 47.06)	46.54 (46.24, 46.85)	-0.13 (-0.57, 0.31)	47.09 (46.76, 47.41)	0.35 (-0.11, 0.81)	46.87 (46.55, 47.18)	0.19 (-0.26, 0.64)	46.83 (46.52, 47.15)	0.16 (-0.29, 0.60)
UVB Heat PDT	40.71 (40.37, 41.05)	40.72 (40.37, 41.06)	42.10 (41.77, 42.43)	**1.39 (0.91, 1.87)**	41.18 (40.84, 41.53)	0.47 (-0.02, 0.95)	40.98 (40.63, 41.33)	0.27 (-0.22, 0.76)	41.84 (41.49, 42.18)	**1.13 (0.64, 1.61)**

CI, confidence interval; CV, coefficient of variation; GM, geometric mean; LSMeans, least squares mean; PDT, pain detection threshold; PTT, pain tolerance threshold; SD, standard deviation; UVB, ultraviolet B Bold text indicates that the effect over placebo met the predefined decision criterion

Table 3 – Descriptive summary of plasma PF-06273340 pharmacokinetic parameter values.

	Parameter summary statisticsa by PF-06273340 treatment	
Parameter (units)	400 mg	50 mg
N	19	19
AUC_{last} (ng•hr/mL)	3630 (43)	483.5 (42)
C_{max} (ng/mL)	1396 (56)	150.4 (57)
T_{max} (hr)	1.00 (0.500-2.00)	1.08 (1.00-3.00)

AUC_{last}, area under the plasma concentration–time curve from time 0 to the time of last quantifiable concentration; C_{max}, maximum observed plasma concentration; %CV, percentage coefficient of variation; T_{max}, time to reach C_{max}
a Geometric mean (geometric %CV) for all except: median (range) for T_{max}.

Figure 1 – Disposition of subjects.

Randomized (n = 20)

Allocation

Allocation to treatment sequence (n = 20)

Received allocated intervention:

Treatment	Period					
	1	2	3	4	5	Total
Placebo	n = 4	n = 4	n = 4	n = 3	n = 4	n = 19
Pregabalin 300 mg	n = 4	n = 4	n = 4	n = 4	n = 3	n = 19
Ibuprofen 600 mg	n = 4	n = 4	n = 4	n = 4	n = 4	n = 20
PF-06273340 50 mg	n = 4	n = 4	n = 4	n = 4	n = 3	n = 19
PF-06273340 400 mg	n = 4	n = 3	n = 4	n = 4	n = 4	n = 19

Follow up

Completed the study (n = 18)

Discontinued:

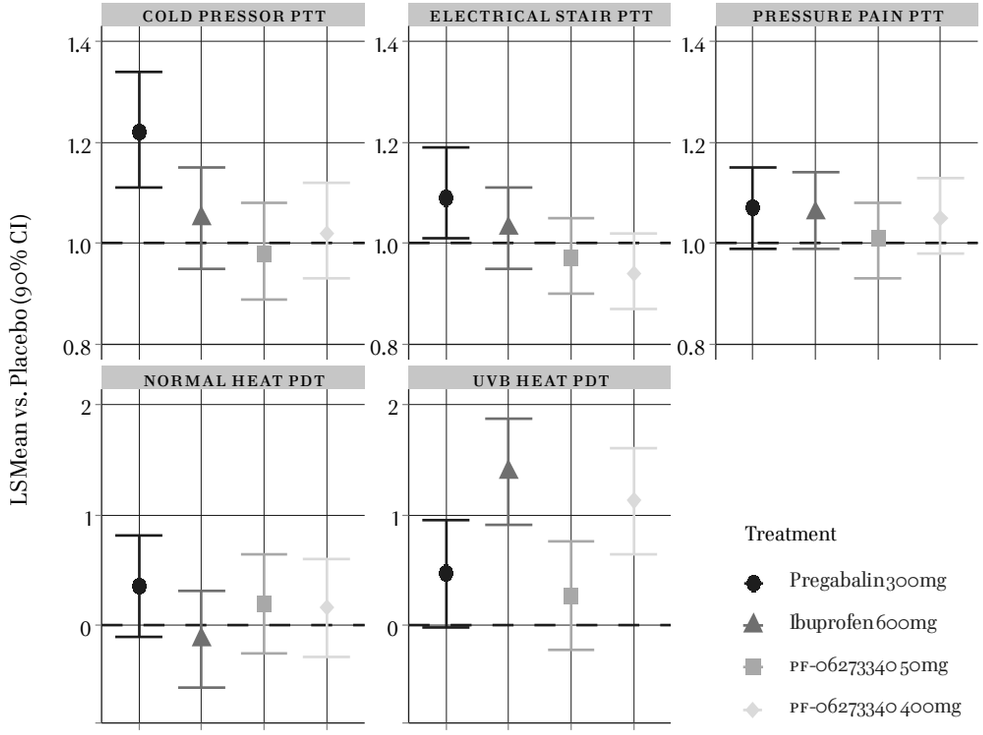
- No longer willing to participate in the study (n = 1)
- Does not meet entrance criteria (n = 1)

Analysis

Analysed (n = 18)

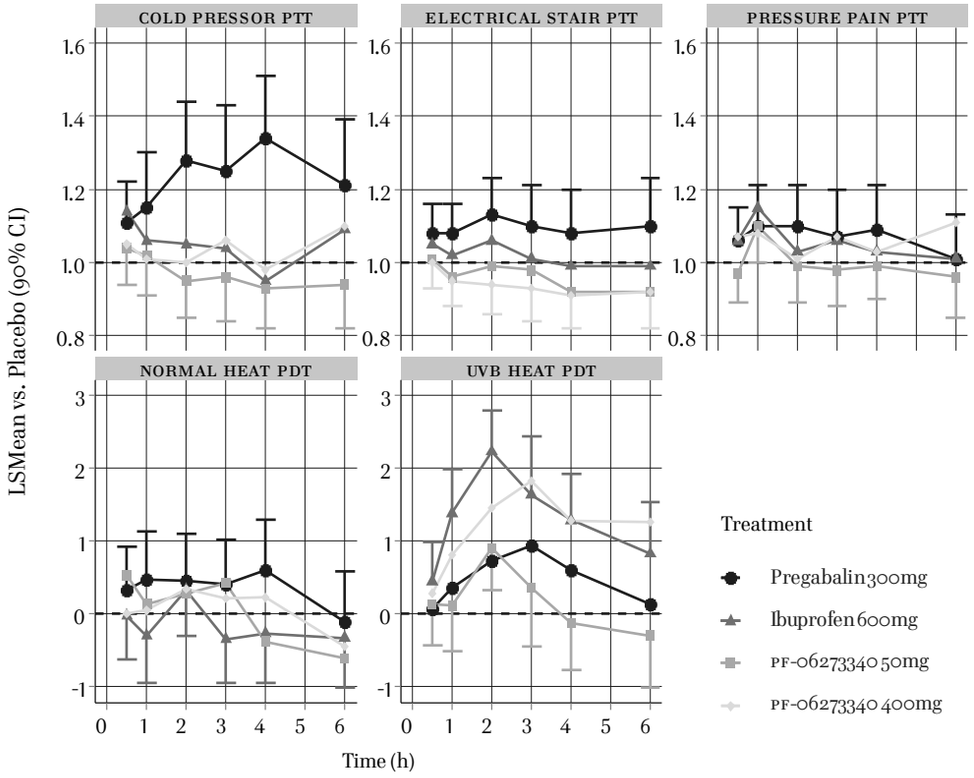
- Excluded from primary analysis (n=0)

Figure 2 – Primary analysis results. The comparisons of PF-06273340 vs. placebo, and ibuprofen vs. placebo were made with LS means averaged over 4 hours. The comparison of pregabalin vs. placebo was made with LS means averaged over 6 hours. The purple horizontal dashed line represents no effect over placebo. PTT endpoints are presented on the fold-change to placebo scale, whereas PDT endpoints are presented on the absolute difference to placebo scale.



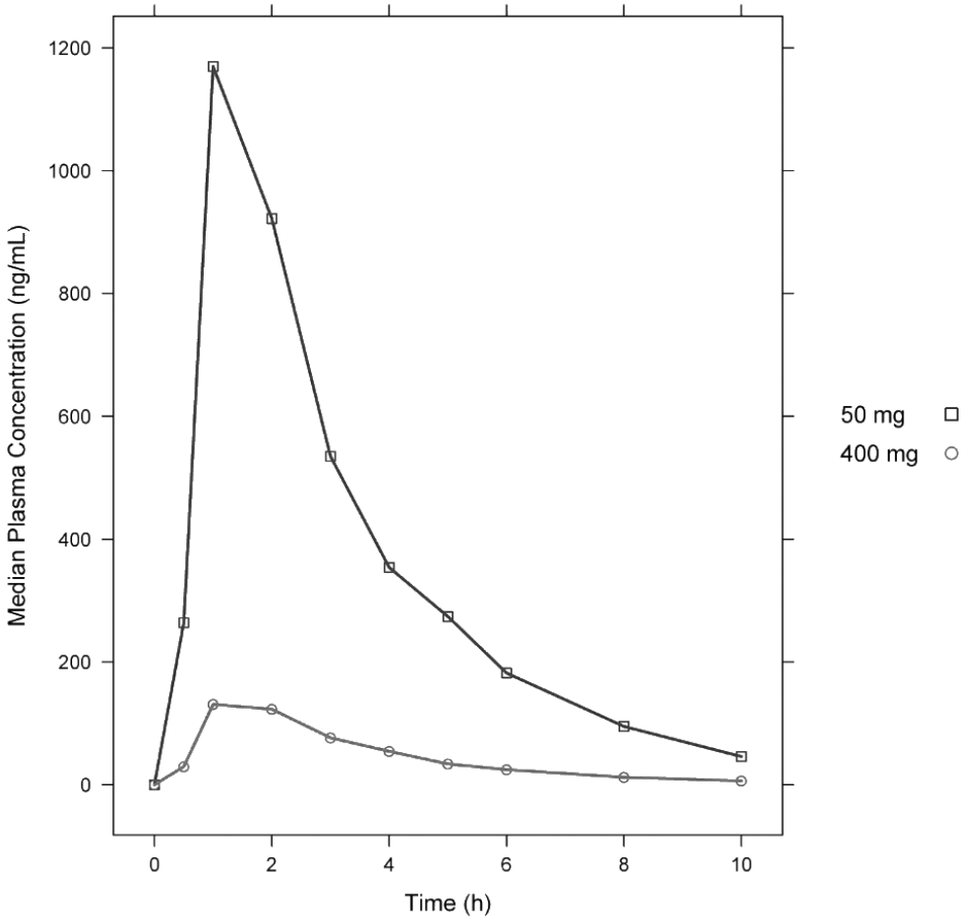
CI, confidence interval; LSMeans, least squares mean; PDT, pain detection threshold; PTT, pain tolerance threshold; UVB, ultraviolet B

Figure 3 – Time course of treatment effects across the 5 Primary Endpoints. The dotted horizontal line represents no effect over placebo. PTT endpoints are presented on the fold-change to placebo scale, whereas PDT endpoints are presented on the absolute difference to placebo scale.



CI = confidence interval; LSMeans = Least Square mean; PDT = pain detection threshold; PTT = pain tolerance threshold.

Figure 4 – Median Plasma PF-06273340 Concentration-Time Profiles.



SUPPLEMENTARY INFORMATION

UVB irradiation was applied at the screening visit in ascending doses (corresponding to different irradiation times) to 6 different 1 × 1 cm areas of skin on the back to determine the individual UVB dose that produced the first clearly discernible erythema. The 3-fold individual minimal erythema dose (MED) of UVB was applied 24 hours prior to dosing to the subject's back to produce local cutaneous inflammation, thereby inducing a homogeneous area of skin erythema and hyperalgesia. The area of skin irradiated was 3 × 3 cm. Subsequently, a 3 × 3 cm thermode was used to measure pain detection thresholds (initially 34°C, ramp 0.5°C/s, average of 3 stimuli) on the normal skin contralateral to the site of UVB irradiation and on the UVB irradiated skin (Cutoff 50°C).

For electrical pain electrodes were placed on skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be less than 2 kΩ. For the single stimulus current intensity increased from 0 mA in steps of 0.5 mA·s-1 to a maximum of 50mA.

For the mechanical pressure pain test, an 11 cm wide tourniquet cuff was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa/s. The pneumatic pressure was increased until the subject indicated maximum pain tolerance using the eVAS slider, or a maximum pressure of 100 kPa was achieved.

For the cold pressor test, subjects placed their non-dominant hand into a water bath at 35 ± 0.5°C for 2 minutes. At 1 minute 45 seconds a blood pressure cuff on the upperarm was inflated to 20 mmHg below resting diastolic pressure. At 2 minutes the subject then moved that hand from the warm water bath, directly into a similar sized water bath at 1.0 ± 0.5°C. The subjects were instructed to indicate when pain detection threshold was reached (first change in sensation from cold non-painful to painful) as well as the pain intensity, by moving the eVAS slider. When pain tolerance or a time limit (120 s) was reached, subjects were instructed to remove their hand from the water, at which point the blood pressure cuff deflated.

In the sinle ascending dose study (B5261001) doses up to 450 mg PF-06273340 as single doses were well tolerated, Although dose was not escalated beyond 450 mg due to 2 events of symptomatic orthostatic hypotension. There were no deaths, serious adverse events (SAEs), severe AEs, dose reductions or discontinuations due to AEs at this dose level. There were no clinically significant changes in laboratory signs, ECGs or physical examination observed at doses up to 450 mg single dose. In a subsequent bioavailability study B5261003, up to 12 subjects received different formulations of 400 mg PF-06273340 on 5 occasions. Dosing was well tolerated with no SAEs, severe AEs, significant abnormalities in laboratory parameters, pulse rate or ECG. All AEs were mild apart from one moderate AE of upper respiratory tract infection. No subject experienced symptomatic orthostatic hypotension, Although several subjects experienced asymptomatic episodes. In multiple dose study B5261002 in healthy young and elderly subjects received doses of 100 mg or 200 mg 3 times a day (TID). The most common AEs were myalgia and orthostatic hypotension. Four subjects discontinued due to AEs: 1 subject on Day 3 due to moderate hypertension, 1 subject withdrew on Day 5 due to moderate orthostatic hypotension, and 2 were withdrawn by the sponsor on Days 10 and 13 due to elevations in ALT. Monitoring continued and ALT returned to normal range by Day 22 in both subjects. There were no notable changes in bilirubin (total, direct or indirect) over this time period, and were no Hy's Law cases. A dose of 100 mg TID was considered maximum tolerated dose and was well tolerated with no changes in Liver Function Tests (LFTS) above ULN. The incidence of symptomatic and asymptomatic orthostatic hypotension was similar to that seen with placebo.

Chapter 4

LACK OF DETECTION OF THE ANALGESIC PROPERTIES OF PF-05089771, A SELECTIVE NAV1.7 INHIBITOR, USING A BATTERY OF PAIN MODELS IN HEALTHY SUBJECTS

Pieter Siebenga, Guido van Amerongen, Justin L. Hay, Aoibhinn McDonnell, Donal Gorman, Richard Butt, Geert Jan Groeneveld

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ABSTRACT

Sodium channel blockers are used for the treatment of pain, but this is limited by the lack of selectivity for different sodium channel subtypes which can result in central nervous system and cardiovascular side effects. As such, there is special interest in the $Na_V1.7$ subtype, which is expressed predominantly in nociceptive and sympathetic neurons. The aim was to demonstrate analgesic properties of a potent selective $Na_V1.7$ sodium channel blocker, PF-05089771, alone and concomitantly with pregabalin in healthy subjects using a battery of human evoked pain models.

This was a double-blind, double-dummy, randomized, placebo-controlled, 5-period cross-over study with PF-05089771 alone and PF-05089771 concomitantly with pregabalin as treatment arms with pregabalin, ibuprofen and placebo as control arms (NCT02349607). A battery of human evoked pain models was used to investigate analgesic properties of PF-05089771.

25 subjects were enrolled in the study of which 23 subjects completed all 5 periods. PF-05089771 alone did not differ from placebo on the primary pain endpoints. The same holds when comparing PF-05089771 concomitantly with pregabalin and pregabalin alone. Pregabalin showed significant effects relative to placebo on thermal pain on the normal skin and UVB skin (least squares means with 90% confidence interval: 0.63(0.32-0.93) and 0.53(0.11-0.96)), pressure stimulation (1.10(1.04-1.18)) and cold pressor (1.22(1.14-1.32)). Ibuprofen demonstrated significant effects on thermal pain UVB skin (1.26(0.82-1.70)) and pressure stimulation assessment (1.08(1.01-1.15)), consistent with historical results.

This study did not demonstrate analgesic properties of PF-05089771, alone or concomitantly with pregabalin in a battery of pain models.

INTRODUCTION

A significant body of evidence implicates sodium channels in mediating the pathophysiological components of both neuropathic and nociceptive pain.[1,2] This is supported by clinical evidence suggesting that drugs with sodium channel blocking properties, such as local anesthetics, certain anticonvulsants and tricyclic antidepressants that block voltage-gated sodium channels (VGSC) have therapeutic utility in managing and treating pain. For example, systemic lidocaine and mexiletine have been used to treat neuropathic pain in humans.[3] The use of these sodium channel blockers has, however, been limited by the lack of selectivity for different sodium channel subtypes which can result in central nervous system (CNS) and cardiovascular side effects.[2] Therefore, a key to improving on the limitations of existing sodium channel blockers is to selectively target those that are involved in pain mechanisms whilst sparing those channels involved in cardiovascular function and in CNS function.[4]

$Na_V1.7$ channels are expressed predominantly in nociceptive and sympathetic neurons. The role of this channel in nociceptive neurons has been characterized by human genetics which indicates an essential and non-redundant role in pain transduction and conduction following noxious stimuli.[5] The importance of $Na_V1.7$ channels has been shown by genetic linkage studies of patients suffering from congenital indifference to pain (CIP), which is the result of a loss-of-function (LOF) mutation in the SCN9A gene encoding the $Na_V1.7$ channel,[6,7,8] and by gain-of-function mutations in the SCN9A gene, which have been implicated in extreme pain disorders such as inherited erythromelalgia (IEM),[9] paroxysmal extreme pain disorder[10] and SCN9A-associated idiopathic small fiber neuropathy.[11]

The physiological role of the $Na_V1.7$ channel is related to the excitability of the sensory afferent terminal.[1] The $Na_V1.7$ channel amplifies small generator potentials and depolarizes the sensory terminal membrane. This causes excitability, facilitating other sodium channels (*e.g.*, $Na_V1.8$) to generate and conduct action potentials. In the disease states genetically linked to a gain-of-function of the $Na_V1.7$ channel, the channel is mutated to increase the sodium influx resulting in a hyperexcitable sensory neuron, and a resultant sensation of pain.

PF-05089771 is a potent and selective, peripherally restricted $Na_V1.7$ channel blocker with an IC50 value of 0.011 µm. It is 11-, 16-, and 59-fold selective over the $Na_V1.2$, $Na_V1.6$, and $Na_V1.1$ channels, respectively. The compound shows ≥909-fold selectivity over $Na_V1.3$, $Na_V1.4$, $Na_V1.5$ and $Na_V1.8$.[12] It has been investigated in nonclinical studies, in healthy subjects and in clinical studies in patients with dental pain due to third molar extraction, diabetic painful neuropathy (DPN) and inherited erythromelalgia.[13,14] A Phase 2 study to investigate the efficacy of PF-05089771 alone, or as an add-on therapy to pregabalin treatment for pain due to DPN has been conducted (NCT02215252). The rationale for the add-on therapy was that the mechanism of action for PF-05089771 is very different from that of pregabalin, the effects of which are mediated via the alpha2-delta subunit of the voltage-gated calcium channels and therefore co-administration of both treatments could be expected to have an additive potential.

It is well known that translation of pain biomarkers to the clinic remains challenging. Insufficient understanding of the pathophysiology of pain in certain diseases and poor predictive value of current human evoked pain models are the major issues to this gap.[15] It is estimated that in 43% of studies it is not possible to conclude whether or not the mechanism of action was investigated.[16] Advances in the field of pain biomarkers could lead to more predictable outcomes and to a more accurate conclusion when it comes to go/no-go decision when a compound fails to show analgesic effects in healthy subjects. From the body of literature on effects of analgesic compounds on evoked pain tests it is clear that certain drugs may show significant results in one pain model, but not show any analgesic efficacy in another pain model.[17,18] For this reason, our study utilized a battery of multimodal pain tests.

The aim of this study was to evaluate the analgesic effects of PF-05089771 alone and concomitantly with pregabalin in a battery of human evoked pain models. These models have demonstrated the ability to detect analgesic properties of compounds/pharmacological effects of analgesic drugs, including pregabalin and ibuprofen, in a robust manner.[19-22]

METHODS

Study Design

The study was a double blind, double dummy, randomized, placebo-controlled, 5 period cross-over study. Subjects were to attend the clinic on 7 separate occasions (screening, 5 study periods and follow up). The 5 study periods were spaced apart by at least 7 days allowing sufficient time for washout of the previous treatment (pharmacokinetic[PK] and pharmacodynamics[PD] effects) based on the half-life of each treatment. PF-05089771 alone and given concomitantly with pregabalin was under investigation. A battery of human evoked pain models was used to demonstrate analgesic properties. The study was conducted at the clinical

research unit of the Centre for Human Drug Research (CHDR) in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki.

Subject Selections

Approximately 25 male subjects, between 18 to 55 years of age, were invited to volunteer for the study. All subjects provided written informed consent prior to undertaking any screening/study-related activities or procedures. Subjects with a Fitzpatrick skin type I-IV, without widespread acne, tattoos or scarring on the back and who were willing and able to comply with all scheduled visits, treatment plan and laboratory tests were included. Subjects were not eligible to participate if they had any existing clinically significant medical or psychiatric condition, or any condition that would affect sensitivity to pain or cold, or had a known hypersensitivity to pregabalin, ibuprofen or any of the excipients. Also excluded were subjects who did not tolerate nociceptive assessment at screening or those who did not achieve tolerance at >80% of maximum input intensity for any nociceptive assessment. Subjects who did not consent to abstain from excessive exposure to sunlight or sunbathing for the duration of the study were excluded.

Study drugs

Subjects received an oral dose of PF-05089771 300 mg (2 × 150 mg tablets), alone and concomitantly with pregabalin 300 mg (1× oral capsule) and matching placebos. The dose was justified based on margins to toxicology findings, on clinical toleration and safety data from a Phase 1 study in healthy volunteers (NCT01259882) and a dental pain study in subjects undergoing third molar extraction where single oral doses of PF-05089771, ranging from 150 mg to 1600 mg, were administered (NCT01529346). Additionally, a dose of 150 mg BID, considered a developable dose of PF-05089771, was used in a clinical trial for the treatment of pain due to DPN. Oral doses of ibuprofen 600 mg and pregabalin 300 mg had been used in previous studies with this battery of pain models.[19,21] These doses were well tolerated and were administered in accordance with European labelling and prescribing information. Pregabalin and ibuprofen were used as positive controls. Previous studies performed at our centre with the same study design demonstrated consistent results, with ibuprofen significantly decreasing the pain threshold for heat pain on UVB exposed skin and pregabalin significantly decreasing the pain thresholds for pressure stimulation and the cold pressor test.[19-22]

Pharmacokinetic assessment

Blood samples (8 mL) were collected from each subject during all study periods to provide a minimum of 4 mL plasma for pharmacokinetic (PK) analysis. Samples were collected at predefined time points: pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 hours post-dose. Plasma concentration over time for PF-05089771, pregabalin and ibuprofen was measured and for each subject, the C_{max}, AUC_{last}, and T_{max} were calculated for PF-05089771. PF-05089771, pregabalin and ibuprofen plasma concentrations were listed and summarized descriptively (results not shown).

Pharmacodynamic assessment

Pharmacodynamic measurements were an integrated range of pain models for measuring different modalities of pain. Nociceptive and inflammatory pain was assessed by means of the heat and inflammatory heat pain assessments, electrical stimulation assessment, cold pressor assessment and the pressure stimulation assessment. Detailed descriptions of the models are described previously.[20] Assessments were conducted pre-dose and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose by trained personnel. Tasks were performed to measure the endpoints pain detection threshold (PDT) and pain tolerance threshold (PTT). Applicable for each pain model except the heat pain assessments, pain intensity was measured continuously with subjects rating their pain intensity using a 100 mm eVAS-slider with 1 as the PDT and 100 defined as the PTT. The intensities of the stimuli were increased until the subjects indicated their PTT, or the maximum stimulus was achieved, whichever came first, at which point the equipment was programmed to cease giving stimuli.

Sample size

Decision rules were pre-specified to quantify what was required in the primary objective of the study. The criteria were based on a Bayesian interpretation of the results, assuming a non-informative prior. The pre-specified decision criteria applied to each primary endpoint were: 1) at least 95% confident that PF-05089771

effect is greater than placebo; and ii) at least 95% confident that PF-05089771 concomitantly with pregabalin effect is greater than pregabalin alone. These are equivalent to one-sided tests for statistical significance using an alpha of 0.05. No adjustment was made for multiplicity as this was an early-phase clinical study designed to explore the PD of PF-05089771 and, as such, no stringent requirement to control the type 1 error rate was required for internal decision making.

The sample size was based on the mean effect over 1 to 10 hours after dosing (*i.e.*, average of 1, 2, 3, 4, 6, 8 and 10 h time points) for the five primary endpoints: cold pressor PTT; pressure pain PTT; electrical stair PTT; normal heat PDT; and UVB heat PDT. A conservative estimate of within-subject standard deviation was derived from two previous methodology studies,[10,11] yielding estimates of 0.279, 0.222, 0.183, 1.86 and 1.80 for the cold pressor PTT, pressure pain PTT, electrical stair PTT, normal heat PDT and UVB heat PDT endpoints, respectively. A sample size of 25 subjects was selected to ensure balance in the design, and gave at least 80% power to detect differences of 0.197, 0.157, 0.13, 1.316 and 1.276 for the five primary endpoints listed previously.

Statistical analysis

The primary endpoints of this study were the PDT for thermal pain (normal skin and UVB skin), and the PTT for electrical stair (pre-cold pressor), pressure pain and cold pressor.

A mixed effects repeated measures model was fitted for each endpoint, using data collected during the first 10 hours post treatment. The fixed effects included in the model were baseline, period, time, treatment and treatment by time interaction, with baseline as covariate. Subject was fitted as a random effect and time repeated within each subject × period as a repeated effect. Baseline was included as 2 separate variables, the average baseline for the subject, and the deviation of each period baseline from the average baseline for each subject.[23] The Kenward-Roger approximation was used for estimating degrees of freedom for the model parameters. The primary analysis included all subjects randomized into the study. The PDT and PTT endpoints for the electrical stimulation, pressure stimulation, and cold pressor tests were log transformed prior to analysis.

The least squares means (LSMeans) together with 90% confidence intervals (CI) were obtained for each treatment averaged across time points that covered the peak exposure of each treatment. The average effect across 1 to 10 hours was obtained for PF-05089771, PF-05089771 + pregabalin and placebo. The average for pregabalin and placebo was obtained from both 1 to 10 hours and the first 6 hours. The average for ibuprofen and placebo was obtained from the first 4 hours. Differences between treatments and placebo or pregabalin were therefore made using the appropriate average (*i.e.*, ibuprofen was compared with the placebo 4 hour average, whereas PF-05089771 was compared with the placebo 1 to 10 hour average). The probabilities for the decision criteria for each of the pain model endpoints were calculated directly from the results of the mixed model, assuming a non-informative prior.

As a sensitivity analysis to the primary analysis, a mixed effects model was also fitted for the maximum (over 10 hours post-treatment) change from baseline for each pain model endpoint. The fixed effects included in the model were baseline, period and treatment. Baseline was included as 2 separate variables, the average baseline for the subject, and the deviation of each period baseline from the average baseline for each subject. Subject was fitted as a random effect. In cases where a subject did not show an increase, the minimum decrease was taken. LSMeans together with 90% CI were obtained for each treatment, and differences in the LSMeans and 90% CI were obtained for the comparisons mentioned above.

RESULTS

Demographics

A total of 25 male subjects were randomized to receive study treatments. Twenty-three (23) subjects each received PF-05089771 300 mg + pregabalin 300 mg, ibuprofen 600 mg and placebo; 24 subjects received PF-05089771 300 mg and pregabalin 300 mg. One subject was discontinued from the study due to a positive urine drug screen for abuse. A second subject discontinued as he was no longer willing to participate in the study. None of the subjects were replaced in this study (Figure S1).

A summary of the demographic characteristics is provided in Table S1.

Pharmacodynamics

A summary of the result for the primary end points are presented in Table 1. PF-05089771 alone did not meet the decision criterion of at least 95% confidence that the effect was greater than placebo for any of the primary endpoints. PF-05089771 concomitantly administered with pregabalin did not differ from pregabalin alone on the primary endpoints. Pregabalin showed evidence (>95%

probability) of effects relative to placebo on thermal pain (normal skin and UVB skin) PDT, pressure stimulation PTT and cold pressor PTT. Ibuprofen showed evidence (>95% probability) of effects relative to placebo on thermal pain (UVB skin) PDT, and pressure stimulation PTT. The overall LSMeans with 90% confidence interval for all pain models is plotted in Figure 1.

Pharmacokinetics

Following oral administration of a 300 mg dose of PF-05089771 administered alone or in conjunction with 300 mg of pregabalin, PF-05089771 absorption was similar for both treatment arms. C_{max} was achieved within a median T_{max} of 2 hours post-dose for PF-05089771 administered alone (individual range 2 to 5 hours) and within a median T_{max} of 3 hours post-dose for PF-05089771 administered in combination with pregabalin (individual range 2 to 5 hours). Overall, PF-05089771 exposure based on geometric mean AUC_{last} and C_{max} values appeared to be similar for both treatments (Table S2).

Safety

Single doses of PF-05089771 were considered safe and well tolerated in this study. None of the subjects experienced a serious adverse event (SAE), dose reduction or temporary or permanent discontinuation due to an AE. The most frequently reported all causality (treatment-related) adverse events were somnolence, dizziness, headache, fatigue and euphoric mood (Table S3). Adverse events were mild in severity, except 1 subject in the pregabalin 300 mg treatment group who experienced somnolence, which was moderate in severity and considered treatment-related. There were no clinically significant changes in safety laboratory assessments, vital signs and ECGs.

DISCUSSION

In the current study, we found no significant analgesic effects of PF-05089771 alone or of PF-05089771 given concomitantly with pregabalin. This is an argument in favor of a strong predictive value of the PainCart with respect to efficacy in patients with clinical pain. A possible explanation for the negative findings could be that the battery of pain models used in the current study is not sufficiently sensitive to detect the analgesic effects of VGSC blockers, however, analgesic effects of another VGSC blocker, phenytoin, a potent sodium channel blocker, were demonstrated using the same battery of pain models.[19,24] In a previous study phenytoin was able to significantly increase the pain detection and pain tolerance thresholds in an electrical stimulation paradigm.[19] In addition, analgesic effects have previously been reported where phenytoin significantly affected pain tolerance threshold in the cold pressor assessment.[25] We cannot rule out that the pain models used in the current pain test battery may not have been sufficiently sensitive, nor have had the dynamic range required to detect analgesic effects of selective $Na_V1.7$ channel blockers. Alternatively, an axonal excitability measurement, *e.g.*, using threshold tracking, might have been able to detect analgesic effect of PF-05089771, since dysfunction of the $Na_V1.7$ may lead to changes in the conduction velocity.[26,27]

It is also possible that the sample size may have been too small to detect smaller effect sizes than were previously reported, Although the clinical significance of such effects may be questionable. The sample size for this study was based on conservative estimates from two previous methodology studies with the same test battery and is more commonly used in single-dose human evoked pain model testing.

Another possible explanation for the negative findings in our study may be related to the dose of PF-05089771 administered in the current study which could have been too low to exert analgesic effects large enough to measure using this analgesic test battery in healthy volunteers. Analgesic effects were demonstrated in a phase II clinical trial for the treatment of patients with IEM, but these patients were administered with a single dose of 1600 mg.[14] The dose used in the current study was based on the selected dose for the DPN study (150 mg BID, oral dose) which was conducted in parallel to the current study. The dose selected for the DPN study was based on results from a prior dental pain study (NCT01529346). Although both the dental pain study and the DPN study[13] demonstrated trends towards pain relief, the magnitude of efficacy observed in either study did not meet pre-specified decision criteria. The current study confirmed these results suggesting that PF-05089771 does not have a strong analgesic effect. The current study highlights the predictive value of the battery of pain models as a tool that may contribute to go/no go decisions.

It is to be considered whether peripheral $Na_V1.7$ blockage is sufficient or if CNS access is required to produce sufficient analgesia. Peripheral blockage was believed to be adequate to achieve analgesic efficacy since $Na_V1.7$ plays an important role in activation of the threshold current for action potential initiation in the peripheral terminals of primary afferent nociceptors.[5,13] A more complex role for

$Na_V1.7$ in primary afferent nociceptors, with evidence for a contribution to the upstroke of the action potential and consequential role in axonal conduction as well as both central and peripheral neurotransmitter release has been recently highlighted.[28] An inability to penetrate through the blood-brain barrier may be a key factor as to why PF-05089771 is unable to satisfactory modulate C-fibre nociceptive transmission, both along the sheathed peripheral nerve as well as neurotransmitter release in the dorsal horn of the spinal cord.[13] Despite the limited success to date with $Na_V1.7$ blockers, it is likely that they will remain a key analgesic target. It remains to be seen whether it is sufficient to selectively target $Na_V1.7$ blockade or whether co-targeting other Na_V channels involved in nociceptive transmission, such as $Na_V1.8$, $Na_V1.9$ and even $Na_V1.3$ is required, whilst avoiding subtypes $Na_V1.1$ and $Na_V1.2$ for their CNS side effects, and $Na_V1.4$ and $Na_V1.5$ for their cardiovascular effects. Alternatively, combining a $Na_V1.7$ blocker with an opioid may be promising.[29] Research with $Na_V1.7$ null mutant mice showed that mechanical and cold allodynia can still be induced under certain conditions.[30] $Na_V1.7$ related analgesia may be enhanced by opioid signaling.[31,32] Minnet et al. were able to reverse a pain free state in mice with a deletion of SCN9A encoding for $Na_V1.7$ with naloxone, which was replicated in one human subject with an identical deletion.[32] The exact mechanism by which $Na_V1.7$ influences the opioid system is unclear and needs further investigation, but it may hold promise for development of more effective $Na_V1.7$ channel blockers.

Administration of PF-05089771 concomitantly with pregabalin was included in this study because it was to be considered as a treatment for neuropathic pain, especially in patients with painful DPN. Pregabalin, and other alpha2delta anticonvulsants such as gabapentin, are useful as an adjuvant therapy together with opioids for neuropathic (cancer) pain.[33-36] With the current dose levels in this study, no additive PD effect was observed compared to pregabalin alone.

CONCLUSION

This study did not demonstrate analgesic properties of PF-05089771, alone or when administered concomitantly with pregabalin, compared to placebo or pregabalin respectively. The use of the battery of human evoked pain models did confirm the analgesic profile of pregabalin and ibuprofen as established in previous studies with the PainCart test battery.

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Table 1 – SUMMARY OF RESULTS FOR THE primary analysis.

Endpoint	PF-05089771		PF-05089771 + Pregabalin		Pregabalin	Ibuprofen
	LSMean difference (90% CI)*	Probabilities associated with decision	LSMean difference (90% CI)*	Probabilities associated with decision	LSMean difference (90% CI)*	LSMean difference (90% CI)*
Normal heat PDT	0.08 (-0.25, 0.42)	0.66	-0.24 (-0.57, 0.08)	0.11	**0.63 (0.32, 0.93)**	0.18 (-0.13,0.50)
UVB heat PDT	-0.01 (-0.44, 0.43)	0.49	0.30 (-0.14, 0.74)	0.87	**0.53 (0.11, 0.96)**	**1.26 (0.82, 1.70)**
Electrical stimulation PTT	0.97 (0.91, 1.03)	0.22	0.97 (0.91, 1.04)	0.23	1.03 (0.98, 1.09)	0.98 (0.93, 1.04)
Pressure stimulation PTT	1.01 (0.95, 1.07)	0.58	1.03 (0.97, 1.10)	0.81	**1.10 (1.04, 1.18)**	**1.08 (1.01, 1.15)**
Cold pressor PTT	1.03 (0.95, 1.11)	0.70	1.01 (0.93, 1.09)	0.54	**1.22 (1.14, 1.32)**	1.08 (1.00, 1.17)

*) LSMean differences for PF-05089771 alone, pregabalin and ibuprofen are relative to placebo, whereas for PF-05089771 + pregabalin the differences are relative to pregabalin. PTT endpoints were analysed on the log scale, so results are presented as back-transformed LS mean ratios and 90% CIs for treatment differences. / Criteria 1: At least 95% confident that PF-05089771 effect was greater than placebo. / Criteria 2: At least 95% confident that PF-05089771 + pregabalin effect was greater than pregabalin. / Statistically significant result in bold. / CI = confidence interval; PDT - Pain Detection Threshold; PTT = Pain Tolerance Threshold.

Table S1 – Summary of demographic characteristics.

		All subjects
Number of subjects		25
Sex		Male
AGE (YEARS)		
	<18	0
	18-44	24
	45-55	1
	Mean (SD)	26.8 (8.0)
	Range	18-46
RACE		
	White	20
	Other	5
WEIGHT (KG)		
	Mean (SD)	79.1 (10.9)
	Range	57.9-105.0
HEIGHT (CM)		
	Mean (SD)	185.3 (7.7)
	Range	169.3-203.9
BODY MASS INDEX (KG/M^2)		
	Mean (SD)	23.0 (2.2)
	Range	18.5-27.0

Body mass index was defined as weight/(height x 0.01)^2 / SD = Standard Deviation

Table S2 – Descriptive summary of plasma PF-05089771 pharmacokinetic parameter values following single oral dose of capsule 2 × 150 mg PF-05089771 alone and concomitantly with pregabalin 300 mg.

Parameter (units)	Parameter summary statisticsa by PF-05089771 treatment	
	PF-05089771 300mg	PF-05089771 + Pregabalin 300 mg
N	24	23
AUC_{last} (ng•h mL^{-1})	68,600 (37)	69,700 (31)
C_{max} (ng mL^{-1})	14,730 (26)	14,250 (25)
T_{max} (h)	2.00 (2.00-5.00)	3.00 (2.00-5.07)

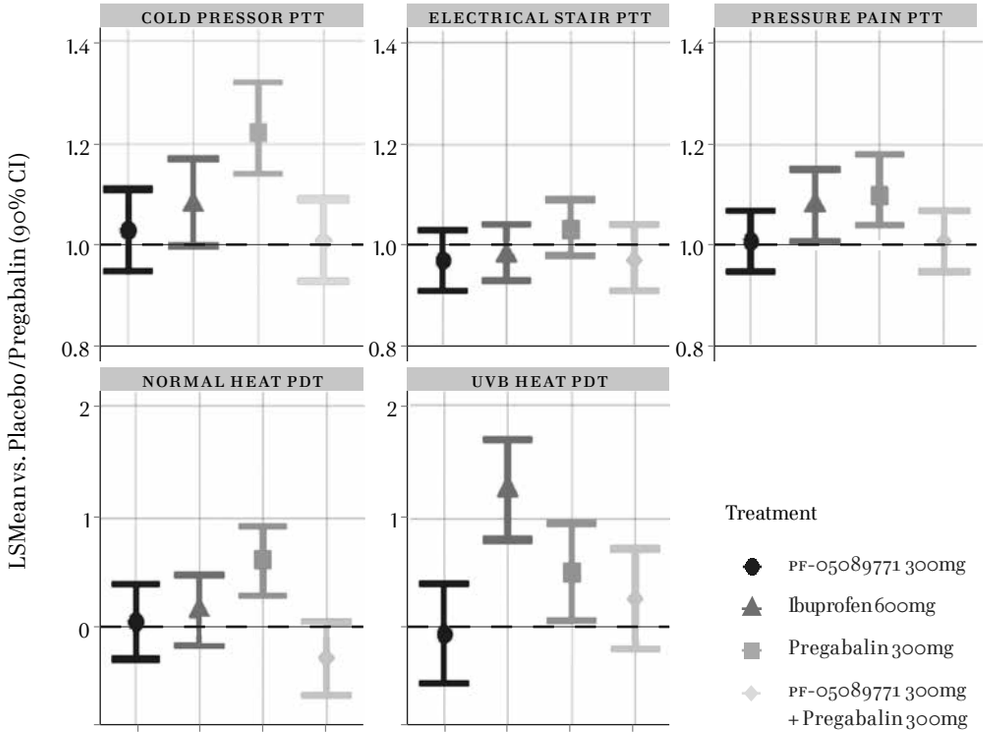
AUC_{last} = area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last}); C_{max} = maximum observed plasma concentration; N = number of subjects in the treatment group and contributing to the geometric mean estimation; T_{max} = time for C_{max}. / a Geometric mean (geometric %CV) for all except: median (range) for T_{max}

Table S3 – Incidence of adverse events. All causality (Treatment related).

Number of subjects with an adverse event	PF-05089771 300mg	PF-05089771 300mg + Pregabalin 300mg	Pregabalin 300mg	Ibuprofen 600mg	Placebo
Subjects evaluable for AEs	24	23	24	23	23
Number of AEs	27 (18)	49 (45)	53 (47)	14 (8)	17 (9)
Number of subjects with AEs	13 (12)	21 (21)	23 (23)	8 (6)	8 (6)
Somnolence	3 (3)	10 (10)	13 (13)	1 (1)	4 (4)
Dizziness	1 (1)	13 (13)	12 (12)	1 (1)	1 (1)
Headache	8 (5)	4 (2)	4 (2)	1 (1)	2 (0)
Fatigue	3 (2)	4 (4)	6 (6)	3 (3)	2 (1)
Euphoric mood	0	2 (2)	4 (4)	0	0

Subjects were counted only once per treatment in each row. Only reporting adverse events that were reported in 4 or more subjects for a treatment group. / Body mass index was defined as weight/(height x 0.01)^2 / SD = Standard Deviation.

Figure 1 – Primary analysis results. The comparisons of PF-05089771 vs. placebo, and PF-05089771 + pregabalin vs. pregabalin alone (*).was made with LSMeans averaged over 1 to 10 hours. The comparison of pregabalin vs. placebo was made with LSMeans averaged over 6 hours. The comparison of ibuprofen vs. placebo was made with LSMeans averaged over 4 hours. The purple horizontal dashed line represents no effect relative to placebo/pregabalin. PTT endpoints are presented on the fold-change to placebo scale, whereas PDT endpoints are presented on the absolute difference to placebo scale.



CI: confidence interval; LSMeans: least squares mean; PDT: pain detection threshold; PTT: pain tolerance threshold; UVB: ultraviolet B

Figure S1 – Disposition of subjects

Randomized (n = 25)

Allocation

Allocation to treatment sequence (n = 25)

Received allocated intervention:

Treatment	Period					
	1	2	3	4	5	Total
PF-05089771	n = 5	n = 5	n = 4	n = 5	n = 5	n = 24
PF-05089771 + pregabalin	n = 5	n = 5	n = 5	n = 3	n = 5	n = 23
Pregabalin	n = 5	n = 5	n = 4	n = 5	n = 5	n = 24
Ibuprofen	n = 5	n = 4	n = 5	n = 5	n = 4	n = 23
Placebo	n = 5	n = 4	n = 5	n = 5	n = 4	n = 23

Follow up

Completed the study (n = 23)

- No longer willing to participate in the study (n = 1)
- Does not meet entrance criteria (n = 1)

Analysis

Analysed:

Treatment	Pharmacodynamics	Pharmacokinetics	Safety
PF-05089771	n = 24	n = 24	n = 24
PF-05089771 + pregabalin	n = 23	n = 23	n = 23
Pregabalin	n = 24	n = 24	n = 24
Ibuprofen	n = 23	n = 23	n = 23

SECTION II

Chapter 5

REPRODUCIBILITY OF A BATTERY OF HUMAN EVOKED PAIN MODELS TO DETECT PHARMACOLOGICAL EFFECTS OF ANALGESIC DRUGS

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ABSTRACT

Although reproducibility is considered essential for any method used in scientific research, it is investigated only rarely; thus, strikingly little has been published regarding the reproducibility of evoked pain models involving human subjects. Here, we studied the reproducibility of a battery of evoked pain models for demonstrating the analgesic effects of two analgesic compounds.

A total of 81 healthy subjects participated in four studies involving a battery of evoked pain tests in which mechanical, thermal, and electrical stimuli were used to measure pain detection and tolerance thresholds. Pharmacodynamic outcome variables were analysed using a mixed model analysis of variance, and a coefficient of variation was calculated by dividing the standard deviation by the least squares means.

A total of 76 subjects completed the studies. After receiving pregabalin, the subjects' pain tolerance thresholds in the cold pressor and pressure stimulation tests were significantly increased compared to the placebo group. Moreover, the heat pain detection threshold in UVB-irradiated skin was significantly increased in subjects who received ibuprofen compared to the placebo group. Variation among all evoked pain tests ranged from 2.2% to 30.6%.

Four studies using a similar design showed reproducibility with respect to the included evoked pain models. The relatively high consistency and reproducibility of two analgesics at doses known to be effective in treating clinically relevant pain supports the validity of using this pain test battery to investigate the analgesic activity and determine the active dosage of putative analgesic compounds in early clinical development.

INTRODUCTION

Demonstrating the analgesic potential of a new analgesic compound in pain patients can be inherently difficult, particularly in the concurrent presence of other symptoms such as fever and/or malaise. Moreover, pain can occur in patients with clinical conditions and diseases that are usually treated with therapeutic interventions that can Alter the perception of pain, for example by inducing side effects such as sedation.[15] An alternative to studying new putative analgesic compounds in patients with pain is to assess their analgesic potential in healthy subjects using an evoked pain model. In recent decades, a variety of pain models have been developed for measuring pain thresholds.[3,9,10,13,14,17,25,28,40,41,46-48,53,56] Historically, these models have been used as a single test; however, based on studies measuring the effect of analgesic compounds on evoked pain it has become clear that some drugs can yield significant results in one pain model but can fail to have an analgesic effect when using a different pain model.[4,11,61] This inconsistency is due – at least in part – to the wide variety of pain signalling mechanisms and pathways at the peripheral and spinal levels, which are sensitive to different analgesics. Therefore, when using only one evoked pain model, selecting the correct model is essential. For example, using a single test can increase the likelihood of obtaining a false negative result, and decisions based on that result are not only costly, but could also lead to the incorrect decision to terminate the development of a potentially active analgesic compound. To minimise this possibility, the pharmacological mechanism of action of a compound should be assessed using a battery of pain models, thereby increasing the likelihood of accurately measuring nociceptive activity and helping predict the optimal dosing range for putative analgesic compounds. A battery of evoked pain models involving human subjects provides the opportunity to use several pain models in the same study and allows the researcher to profile the analgesic in an early phase of clinical development. For example, such a battery can help determine whether the compound acts centrally or peripherally, the modality of pain for which the compound is best suited (*e.g.*, nociceptive, neuropathic, or inflammatory pain), and whether additional effects such as sedation and/or tolerance contribute to the compound's mode of action.[31,37,39,40,58,63] Moreover, results obtained using a batter of pain models can provide important information regarding dose finding and can provide proof-of-analgesia for new analgesic compounds.[5,39]

The ability to consistently obtain reproducible results is an essential attribute of pain models, particularly in the early stages of clinical drug development. Unfortunately, however, reproducibility can be overshadowed by publication bias, in which innovation and/or the strength of the study's findings serve as the primary basis for the submission and ultimate acceptance of a research article. Reproducibility is an important feature in science, as it can be used to verify prior results, to clarify whether results can be generalised to a wider population or other populations, and/or to test the hypotheses proposed in the initial study.[20] Before proper conclusions can be drawn from a study, unknown factors and dependent variables should be minimised, and comparability between studies should be optimised, as inconsistencies and/or contrasting results between studies can be confusing and can lead to the wrong – or even a potentially dangerous – conclusion.[49] In a recent study entitled 'The Reproducibility Project', investigators attempted to reproduce the methods in 100 studies published in top psychology journals, but concluded that only 39% of the trials could be reproduced unambiguously.[42] Although this project was based on psychology studies, a similar discussion in the field of biomedical research has recently emerged, with similar outcomes regarding a lack of reproducibility of the results in the original publications.[6,7,18,20,35] Despite its underlying message, no clear conclusions can be drawn from The Reproducibility Project, as no single study can provide a definitive outcome; rather, it is the cumulative process that increases the reliability of a method. Staahl and colleagues concluded that their models are generally reproducible, but noted that overall variation can be high in certain cases due to a variety of factors, including stimulation duration, the site of stimulation, and the age and gender of the subjects.[57] High variability is not unusual when using evoked pain models with human subjects,[51,60] as differences in the subjects' pain perception can cause high inter-subject variability. Moreover, using a study design that is appropriate to each study compound is particularly important.

Here, we compared the results of four recent studies that used an identical battery of evoked pain models in healthy subjects; specifically, these studies used a nearly identical study design as well as identical drugs and doses. The aim of our study was to examine the reproducibility of using a battery of pain models, including thermal, electrical, and mechanical stimuli, to profile the analgesic effects of two commonly used analgesics.

METHODS

Design

All four studies had a randomised, double-blind, placebo-controlled, crossover single-dose design and were registered with EudraCT (2013-003443-28, 2014-003015-12, 2014-003553-34, and 2014-004468-39, referred to hereafter as

studies I through IV, respectively); in addition, studies II-IV were also registered at Clinicaltrials.gov (NCT02238717, NCT02260947, and NCT02349607). The studies were designed to investigate the ability of new and established analgesics to induce analgesia in healthy subjects and used a panel of pain models. Subjects participated in up to three phases: (1) a screening/training phase; (2) four daily in-house periods (for studies I and II) or five daily in-house periods (for studies III and IV) at 7-day intervals; and (3) a post-treatment (follow-up) call scheduled 6-8 days after the last study drug administration (for study I) or 7-10 days after the last study drug administration (studies II-IV). At screening, each subject underwent a complete medical screen as well as the battery of pain tests. In addition, during the screening phase, a trained physician determined each subject's minimal erythema dose (MED) 24±2 hours after applying six ascending intensities of ultraviolet B (UVB) irradiation to the subject's skin.

Each in-house period started on day 1 with exposure to UVB at three times the subject's MED (3MED). After an overnight stay in the research unit, the subject's baseline pharmacodynamics (PD) profile was measured twice 24±2 hours after UVB irradiation. A baseline blood sample for pharmacokinetics (PK) analysis was drawn before dosing, and PD and PK were assessed 0.5, 1, 2, 3, 4, 6, 8, and 10 hours after dosing.

All trials were conducted at the clinical research unit of the Centre for Human Drug Research in Leiden, the Netherlands. Each subject received the study treatment regimen based on his/her randomised assignment. Both the subjects and the investigators were blinded with respect to the treatment regimen. The studies were conducted in accordance with the Declaration of Helsinki and its amendments and in accordance with established guidelines for Good Clinical Practice. All protocols were approved by a Medical Ethics Committee (MREC); study I was approved by the MREC of Leiden University Medical Center (Leiden, the Netherlands), and studies II-IV were approved by the Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, the Netherlands).

Subjects

A total of 81 healthy subjects enrolled in the four studies; three subjects participated in both study II and study IV. Studies II, III, and IV had identical inclusion and exclusion criteria, whereas study I used slightly different criteria.

Females were allowed to participate in study I but were not included in studies II-IV. The age range for participating in study I was 18-45 years; the age range for participating in studies II-IV was 18-55 years. For participation in study I, the allowed range for BMI was 18-30 kg/m^2; for studies II-IV, the allowed range for BMI was 17.5-30.5 kg/m^2. Finally, for study I the allowed values for systolic and diastolic blood pressure were 100-160 and 50-95 mmHg, respectively; for studies II-IV, the allowed values for systolic and diastolic blood pressure were ≥50 and ≤95 mmHg, respectively.

For all studies, subjects were excluded if they had evidence of clinically relevant findings while taking the medical history and/or during the physical examination, or evidence of clinically significant abnormalities with respect to the subject's ECG, vital signs, blood chemistry, and/or haematology results. In all four studies, only healthy subjects with a Fitzpatrick skin type of IV or lower were included. Additional exclusion criteria included wide-spread acne, tattoos and/or scarring on the back, any clinically significant medical condition (particularly any existing condition that would affect the subject's sensitivity to cold), and subjects who indicated an intolerability for nociceptive tests at screening or who achieved tolerance at >80% of maximum input intensity for any nociceptive test involving cold, pressure, heat, or electrical stimuli. Other exclusion criteria included a positive drug test or urine-based pregnancy test (for female subjects, relevant only to study I), hypersensitivity to the study treatments, and the use of prescription and/or non-prescription drugs or dietary supplements within 7 days or 5x the half-life (whichever was longer) prior to the first study dose. Finally, subjects were instructed to avoid excessive exercise, dietary restrictions, and alcohol, nicotine, and caffeine for 24 hours prior to dosing and while at the clinical research unit.

All participating subjects provided written informed consent.

Treatment

Pregabalin (300 mg) was administered to the subjects in all four studies, and ibuprofen (600 mg) was administered to the subjects in studies I, III, and IV. For study I, pregabalin was supplied by Pfizer and ibuprofen was provided by Reckitt Benckiser Healthcare (Hoofddorp, the Netherlands); for studies II-IV, pregabalin and ibuprofen (study II excluded) was supplied by Pfizer. The Pharmacy Department at Leiden University Medical Center prepared the study compounds together with identical placebos. To ensure blinding of the participants and researchers, a double-dummy design was used in studies II, III, and IV; in study I, all study drugs were over-encapsulated in order to render them indistinguishable from each other. All compounds and placebos were taken orally with 240 mL of water.

Pharmacodynamics

Pharmacodynamics was measured using an integrated battery of pain models designed to measure various modalities of pain. These models have been described previously,[25] and all assessments were performed by trained personnel. In all four studies, tasks were performed in order to measure the pain detection threshold (PDT) and the pain tolerance threshold (PTT). For each pain model (with the exception of the heat pain model), pain intensity was measured continuously, and the subject rated their pain intensity using a 100 mm electronic visual analogue scale (eVAS), with 1 mm and 100 mm defined as PDT and PTT, respectively. The testing equipment automatically terminated the pain assessment when PTT was reached or when the safest maximum stimulation was applied, whichever occurred first. Electrical PTT, pressure PTT, cold pressor PTT, and heat pain PDT were the primary endpoints of interest.

Electrical stimulation

This method was adapted from the protocol reported by Olofsen and colleagues.[13,41] For inducing cutaneous electrical pain, two Ag/AgCl electrodes were placed on a clean patch of skin overlying the left tibia; specifically, the centre of the first electrode was placed 100 mm distal from the caudal end of the patella, and the centre of the second electrode was placed 135 mm below the first electrode. Electrical resistance between the electrodes was verified as <2 kW.

Each stimulus (a 10 Hz tetanic pulse lasting 0.2 ms) was administered by a computer-controlled constant current stimulator. Current intensity began at 0 mA and increased in 0.5 mA increments every second; the maximum current intensity was 50 mA. Pain intensity after each stimulation was measured using the eVAS, and the stimuli continued until the subject's pain tolerance level was reached or until a maximum of 50 mA was applied, whichever occurred first.[13,41]

Pressure stimulation

This method was adapted from the protocol reported by Polianskis and colleagues.[47,48] An 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz am Neckar, Germany) was placed over the gastrocnemius muscle, and the pressure applied was increased linearly by 0.5 kPa/s under the control of a model ITV1030-31F2N3-Q electro-pneumatic regulator (SMC Corporation, Tokyo, Japan) driven by a Power1401 analogue-to-digital converter and Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK). During the test, the subject was seated comfortably with the feet flat on the floor; pain intensity was rated using the eVAS, and the pneumatic pressure was increased until the subject indicated his/her pain tolerance threshold or until a maximum pressure of 100 KPA was applied (whichever occurred first), at which point the device released the pressure on the tourniquet.

Cold pressor

The method for applying cold pressor pain was based on the methods reported by Eckhardt and colleagues[17] and Jones and colleagues.[28] During the test, the subject placed his/her non-dominant hand in a water bath at 35±0.5°C (minimal depth: 200 mm) for 2 minutes. After 1 minute and 45 seconds, a blood pressure cuff on the upper arm was inflated to 20 mmHg below the subject's resting diastolic pressure. At 2 minutes, the subject was instructed to remove the hand from the warm water bath and place the same hand immediately into a similar sized water bath at 1.0±0.5°C. The subject was then instructed to indicate when the pain detection threshold was reached (based on the initial change in sensation from cold and non-painful to painful) and to record the increase in pain intensity by moving the eVAS slider. When the subject's pain tolerance was reached (*i.e.*, the sensation was no longer tolerable, defined as 100 mm on the eVAS) or after 120 seconds (whichever occurred first), the subject was instructed to remove his/her hand from the cold water bath, at which point the blood pressure cuff deflated.

Heat pain and inflammatory heat pain

Heat pain was assessed using a method adapted from Bishop and colleagues.[9] At the screening visit, UVB irradiation was applied using a TL01 narrow-band UV lamp (Phillips) in ascending doses (corresponding to different durations of irradiation) at six separate 1 cm × 1 cm patches of skin on the subject's upper back in order to determine that subject's minimal erythema dose (MED), defined as the minimum UVB dose that produced the first clearly discernible erythema.

For each subject, a 3 cm × 3 cm patch of skin on the back was exposed to 3 times the individual MED of UVB 24±2 hours prior to the first battery of tasks; this irradiation was applied to the subject's back in order to produce a homogeneous patch of skin with erythema and hyperalgesia.

A 3 cm × 3 cm thermode (Medoc, Ramat Yishai, Israel) was used to measure the thermal pain detection threshold (PDT) using an initial temperature of 34°C, which was increased at a rate of 0.5°C/s. First, the average PDT from three stimuli

was measured on a non-irradiated patch of skin (contralateral to the site of UVB irradiation), followed by the same measurements on the UVB-irradiated skin.

Sampling for pharmacokinetics

Throughout each study, blood samples (8 mL) were collected from each subject in order to provide the minimum volume of 4 mL plasma required for PK analysis. Blood samples were collected in tubes containing K_2-EDTA. Plasma concentration was measured over time, and for each subject the maximum concentration (C_{max}), the area under the concentration-time curve (AUC) from time 0 to the last sample above the limit of quantification (AUC_{last}), the AUC from time 0 extrapolated to time infinity (AUC_{inf}), the time of C_{max} (T_{max}), and the half-life ($T_{½}$) were calculated for both pregabalin and ibuprofen, where applicable.

Statistical analyses

The primary comparison was the mean effect of pregabalin compared to placebo up to 5 hours in study I and up to 6 hours in studies II-IV. The mean effect of ibuprofen compared to placebo was analysed up to 4 hours in all four studies. In each study the least squares means (LSMeans) and standard deviation (SD) were analysed using a mixed model analysis of variance, with treatment, time, and treatment by time as fixed factors and subject, subject by treatment, and subject by time as random factors; the average baseline measurement was used as a covariate. Different times for the duration of effect were selected based on the half-life of the respective drug.

For each pain model, reproducibility across subjects was evaluated for both pregabalin and ibuprofen using the coefficient of variation (CV). The CV expressing the inter-subject variability was calculated by dividing the SD by the LSMeans and is expressed as a percentage. The confidence interval (CI) reported for study I is the 95% CI; the CI in studies II-IV is the 90% CI. In studies II-IV, the confidence intervals of the heat pain assessments (in UVB-irradiated and non-irradiated skin) were back-transformed.

RESULTS

A total of 81 healthy subjects (8 women and 73 men) satisfied the inclusion and exclusion criteria and were randomised to receive analgesic or placebo. All 81 subjects received pregabalin, and 61 subjects (8 women and 53 men) received ibuprofen. The demographics of the subjects were similar between all four studies and are summarised in Table 1. Of the 81 subjects who enrolled in the study, 76 completed their respective studies and were included in the final analyses. In both studies I and III, one subject dropped out; in study IV, two subjects dropped out; finally, one subject withdrew from study III due to abnormalities on ECG. No study drug-related reasons were cited by the subjects who dropped out, and the ECG abnormality that caused the subject in study III to withdraw was not deemed to be related to the study drug.

A summary of the CV values, p-values, and LSMeans effect versus placebo (primary outcomes) are presented in Table 2. In all four studies, subjects who received pregabalin had a significantly higher PTT in the cold pressor test compared to subjects who received placebo. Specifically, the LSMeans (estimate of the difference) in studies I, II, III, and IV was 25.1 seconds (46.4%), 23.3 seconds (29.7%), 32.4 seconds (21.4%), and 21.0 seconds (22.4%), respectively. Moreover, compared to placebo pregabalin also had a significant effect on PTT in the pressure stimulation test and on PDT in the heat pain assessment of the non-irradiated area. For the pressure stimulation test, the LSMeans (estimate of the difference) in studies I, II, III, and IV was 47.6 KPA (14.1%), 43.7 KPA (12.1%), 57.1 KPA (7.0%), and 37.8 KPA (10.5%), respectively (with significance in studies I, II, and IV). With respect to the heat pain assessment of the non-irradiated area, the LSMeans (estimate of the difference) in studies I, II, III, and IV was 45.1°C (4.1%), 47.3°C (0.6%), 47.1°C (0.7%), and 47.0°C (1.4%), respectively (with significance in studies I and IV). Compared to placebo, ibuprofen had a significant on the PDT in the heat pain assessment in all three studies in which ibuprofen was administered, with an LSMeans (estimate of the difference) in studies I, III, and IV of 40.2°C (4.0%), 42.1°C (3.4%), and 41.8°C (3.1%), respectively. Similar results were obtained with respect to the other pain models.

The coefficient of variance (CV), which reflects inter-subject variability, was also analysed for both pregabalin and ibuprofen for each pain model separately in each study. With respect to pregabalin, the heat pain assessment of both the UVB-irradiated skin and the non-irradiated skin had the lowest variability, with all CV values <5%. The CV values for the electrical and pressure stimulation assessments were approximately 20%, and the cold pressor assessment had the highest CV values, ranging from 19.8% to 30.6%. Similar results were obtained for ibuprofen; specifically, the CV values for the heat pain assessment were <5%, both electrical and pressure stimulation had CV values ranging from 14.5% to 25.4%, and highest variation was measured for the cold pressor assessment, with CV values ranging from 19.4% to 28.9%.

Fig. 1 shows the profile of pregabalin based on the battery of pain models up to 6 hours after dosing (or up to 5 hours after dosing in the case of study I). Overall, the cold pressor and pressure pain models were most sensitive to the effects of pregabalin, whereas the heat and electrical models were the least sensitive to pregabalin. The electrical, cold pressor, and heat pain in non-irradiated skin models were all more sensitive to pregabalin in study I compared to studies II, III, and IV. The effects of ibuprofen analysed up to 4 hours after dosing were similar to the effects of pregabalin; the profile of ibuprofen based on the battery of pain models is shown in Fig. 2. All three studies with ibuprofen yielded a similar profile.

DISCUSSION

Here, we report that using a battery of five pain models yielded highly consistent results with respect to the qualitative and quantitative analgesic effects of both pregabalin and ibuprofen. This high reproducibility means that obtaining a profile for a given drug using this battery of pain tests can reveal the 'trait' of that drug. Our results demonstrate that inter-subject variability is relatively low with respect to assessing heat-induced pain in both UVB-irradiated and non-irradiated skin; thus, we found an overall consistent level of efficacy for two commonly used analgesics compared to placebo. Importantly, ibuprofen had a significant analgesic effect in all three studies that used this drug, suggesting that the UVB-induced heat pain model is highly sensitive to this class of compounds. This finding is consistent with expected pharmacodynamic outcomes using this pain model, as the UVB model is considered a suitable model for inflammatory pain and is therefore highly sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs).[9,61] The lack of an effect of ibuprofen in the other pain models was also highly consistent, supporting the high reproducibility of these models. The lack of an effect with ibuprofen with the other pain models can be explained by the fact that these models are not based on inflammation-related hyperalgesia, but rather are mediated by acute nociceptive pain. The fact that the PD profile for ibuprofen differs from the PD profile for pregabalin indicates that specific compounds can produce a highly specific profile in a battery of pain models; moreover, we recommend than several modalities be included in the design of future studies in order to obtain a more complete profile of the test compound. Such an approach will likely yield important information that can be used to make a more informed decision regarding the next phases in the drug development process.

With respect to pregabalin, a wide range of pain models have been used to demonstrate significant analgesic effects, and pregabalin has anxiolytic, anticonvulsant, and analgesic properties.[8,19,21,27,34,43,50,52] Pregabalin is a structural analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and binds to the α2δ auxiliary subunit of voltage-gated calcium channels in the central nervous system. In clinical trials, pregabalin has been shown to have analgesic effects in diabetic neuropathy, post-herpetic neuralgia, and spinal cord injury.[38,44,59] In controlled clinical trials involving peripheral neuropathic pain, 35% of pregabalin-treated patients had an improvement in their pain scores.[52] It is important to note, however, that pregabalin has also been shown to provide analgesic effects in nociceptive pain, including dental patients following extraction of the third molar[27] and in patients suffering from lower back pain.[34] This broad analgesic efficacy is reflected in the profile shown in Fig. 1, in which the majority of pain models were sensitive to pregabalin. This finding suggests that combining these well-established pain models – which together represent distinct, complementary mechanisms – can have high predictive value with respect to the efficacy of an analgesic in clinical practice.

Using our battery of evoked pain models, we were able to detect differences in both pharmacological and analgesic properties. Both ibuprofen and pregabalin produced a unique profile of analgesic effects in pain evoked using the models included in these studies. Pregabalin is known to induce somnolence, which can affect its analgesic outcome. We recently reported that this battery of pain models is not affected by sedation, as the classic H1 antihistamine promethazine served as a negative control.[62] Most of the analgesic effects that we observed for ibuprofen and pregabalin are consistent with previous reports and with their expected pharmacodynamics and pharmacokinetics profiles, and both compounds have been shown to provide analgesic effects in clinical practice in nociceptive and/or neuropathic pain at the doses used in our studies. The analgesic profile that drugs exhibit in this multimodal pain test setting in healthy subjects may possibly be linked in the future to the subgroups of patients with neuropathic pain –divided based on phenotypical differences tested with *e.g.*, QST- who appear to respond better to certain treatments than other phenotypical subgroups, but this needs further exploration.[65,66]

Inconsistencies that emerge from a reproducibility study do not necessarily indicate that the method is unreliable. For example, the profile for pregabalin in study I differed from the profiles obtained in the other three studies (see Fig. 1); however, this difference was likely due to the use of a different formulation of pregabalin in the first study. The dissolution and absorption of pregabalin could have

been altered by this different formulation, which could have affected the plasma concentration over time. Additional analyses of the PK profiles among the various studies revealed that the systemic exposure of pregabalin was higher in study I than in the other three studies. Importantly, additional PK/PD model-based analyses of each endpoint and the concentration-response curves increased reproducibility by accounting for differences in exposure between subjects and studies (data not shown). Although this additional analysis confounded the reproducibility by dose, it provided direct insight into the test battery's sensitivity to differences in the plasma concentration of an analgesic compound, and it demonstrated reliability by concentration. The electrical stimulation assessment, the cold pressor assessment in particular, and – to a lesser extent – the heat pain assessment of non-irradiated skin were affected more strongly by pregabalin in the first study than in the other three studies. This suggests that the maximum effective concentration of pregabalin may not have been reached in studies II-IV and that this battery of pain models can be used to distinguish between dose-dependent and concentration-dependent effects of pregabalin. Thus, using ascending doses may improve the profile of a drug's analgesic effects, and testing more than one dose of a compound in the battery of pain models will likely provide a more complete overview of the compound's analgesic potential. Another factor that may have contributed to the difference in outcome between study I and the other three studies is that study I included both male and female subjects, whereas the other three studies were restricted to male subjects only.[39]

When using pain models to assess the putative analgesic properties of a drug, it is important to determine the precision of the measurements obtained with these pain models. Unfortunately, however, the reproducibility of major findings published in high-profile journals is strikingly low, ranging from 10% to 25%.[45,49] Thus, increasing the reproducibility of a method is an important step in the scientific method, allowing science to progress by building on previous research. Achieving this goal requires the submission of both the data and the computational and analytical tools that were used to generate the results; without this information, the results cannot be verified and built upon. Adherence to established guidelines regarding the conduct of experimental research is also important, as is proving access to the protocol and the data collected.[7] On the other hand, a single well-defined scientific method that results in a constructive scientific process is – at best – debatable. Additional submission of the data may lead to mistrust among researchers and possible over-regulation with respect to the acceptance of manuscripts based on seemingly narrow technical criteria. Misconduct has always been a part of science, with surprisingly few consequences. Distrust by the public is likely higher thanks to the apparent variability among scientific conclusions.[16] Consensus regarding how this variability can be addressed is unlikely. A combination of approaches has been used, including an assessment of test-retest reliability, intraclass correlation coefficient, and the level of agreement.[12,22,24,36,54,55] Reliability analysis has also been used widely, generally yielding high reliability among results.[1,2,22,23,26,29,30, 32,33,36,64] The aim of our study was to measure consistency of the profile of two analgesic drugs using our pain test battery. When the drug profile is consistent among populations, the profile can be regarded a 'trait' – in other words, a pharmacological biomarker. Our approach revealed consistently reproducible results with respect to the analgesic profiles of both pregabalin and ibuprofen in a heterogeneous study population, suggesting that variability regarding the perception of pain among subjects likely plays only a small role. As discussed above, using the appropriate study design for each compound under investigation is particularly important.

Our finding that the results obtained using our battery of pain models are reproducible – thus yielding a reliable profile of analgesic effects when testing different compounds – supports the notion that this test battery can be used reliably in the early stages of clinical drug development. For example, this battery of tests can be used to screen drugs for their analgesic potential and/or to determine the analgesic dose/concentration range of new analgesic compounds in the early stages of clinical development. Importantly, creating an extensive database containing the profiles of established analgesic compounds can provide a series of benchmarks for comparing new compounds to existing analgesic drugs and can help researchers predict the efficacy of new compounds in specific patient populations.

CONCLUSIONS

Here, we report that four separate studies with a similar design and using a battery of evoked pain models involving healthy human subjects yielded highly reproducible results with a low coefficient of variation. The consistency and the reproducibility of the analgesic profile at clinically effective doses validates the use of this pain test battery as a tool for demonstrating analgesic activity and for helping establish the optimal active dose in early clinical drug development.

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Table 1 – Participant characteristics. Age and BMI are presented as the mean (range).

Study	I (n = 16)	II (n = 20)	III (n =20	IV (n = 25)
Age, years	21.9 (19 – 25)	31.8 (18 – 50)	26.0 (18 – 43)	26.9 (18 – 46)
BMI, kg/m²	21.9 (19.4 – 25.1)	23.3 (18.5 – 27.2)	23.7 (18.2 – 29.8)	23.0 (18.5 – 27.0)
Male/Female	8/8	20/0	20/0	25/0
FITZPATRICK SKIN TYPE				
Type I	2 (12.50%)	0	0	0
Type II	2 (12.50%)	4 (20.00%)	1 (5.00%)	6 (24.00%)
Type III	9 (56.25%)	11 (55.00%)	12 (60.00%)	13 (52.00%)
Type IV	3 (18.75%)	5 (25.00%)	7 (35.00%)	6 (24.00%)

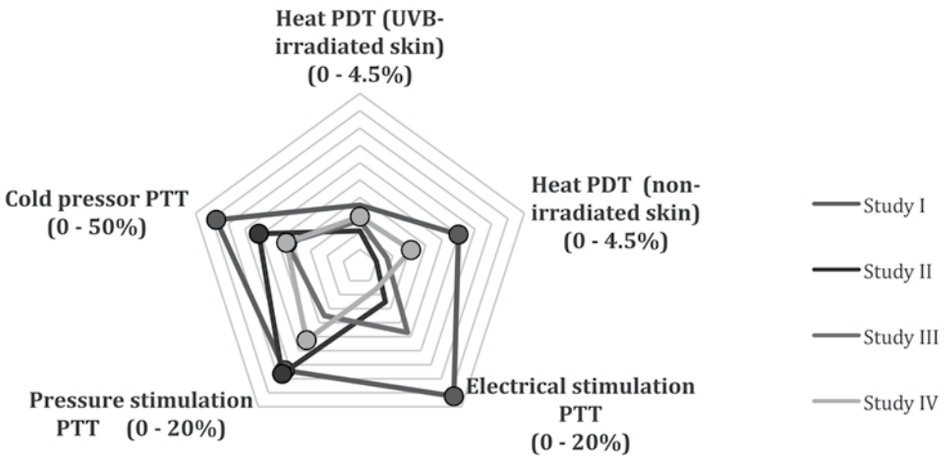
BMI, body mass index.

Table 2 – Significant clusters of the pharmacodynamic measurements. Average effects compared with placebo: study I: up to 5 hours post-dose for pregabaline and ibuprofen; study II-IVL pregabalin up to 6 hours post-dose, ibuprofen up to 4 hours post-dose. Contrasts were calculated within the repeated measures mixed model. Significant values shown in bold. Confidence interval LOG transformed, heat pain (UVB and normal skin) in study II-IV are back transformed.

	Study I			Study II			Study III			Study IV		
	CV (%)	p-value	LSMean effect vs. placebo (95% C.I.)	CV (%)	p-value	LSMean effect vs. placebo (90% C.I.)	CV (%)	p-value	LSMean effect vs. placebo (90% C.I.)	CV (%)	p-value	LSMean effect vs. placebo (90% C.I.)
PREGABALIN												
Electrical stimulation PTT	18.8	**0.0121**	1.46 [1.02; 1.20]	22.8	0.3697	1.31 [0.96; 1.15]	22.1	0.0701	1.22 [1.01; 1.19]	14.8	0.3489	1.22 [0.98; 1.09]
Pressure stimulation PTT	25.0	**0.0052**	1.13 [1.04; 1.25]	21.1	**0.0085**	1.05 [1.06; 1.26]	22.1	0.1420	1.09 [0.99; 1.15]	19.8	**0.0124**	1.03 [1.04; 1.18]
Cold pressor PTT	28.9	**<0.0001**	1.14 [1.27; 1.69]	30.6	**0.0005**	1.15 [1.16; 1.48]	26.6	**0.0008**	1.09 [1.11; 1.34]	19.8	**<0.0001**	1.10 [1.14; 1.32]
Heat pain PDT (UVB treated skin)	4.2	0.2671	4.10% [0.99; 1.03]	4.2	0.4483	0.21 [-0.45; 1.22]	3.3	0.1138	0.35 [-0.02; 0.95]	3.4	**0.0380**	0.63 [0.11; 0.96]
Heat pain PDT (normal skin)	4.6	**0.0049**	1.20% [1.01; 1.07]	3.5	0.5705	0.38 [-0.41; 0.84]	2.8	0.2121	0.45 [-0.11; 0.81]	2.4	**0.0009**	0.53 [0.32; 0.93]
IBUPROFEN												
Electrical stimulation PTT	19.0	0.7525	1.03 [0.94; 1.09]	-	-	-	18.0	0.5835	1.05 [0.95; 1.11]	14.5	0.5974	1.08 [0.93; 1.04]
Pressure stimulation PTT	25.4	0.2576	1.01 [0.96; 1.15]	-	-	-	18.0	0.1589	1.03 [0.99; 1.14]	19.4	0.0603	0.98 [1.01; 1.15]
Cold pressor PTT	28.9	0.6850	1.05 [0.89; 1.19]	-	-	-	22.6	0.4250	1.06 [0.95; 1.15]	19.4	0.1007	1.08 [1.00; 1.17]
Heat pain PDT (UVB treated skin)	4.5	**0.0006**	1.70% [1.02; 1.06]	-	-	-	2.8	**<0.0001**	-0.13 [0.91; 1.87]	3.4	**<0.0001**	0.18 [0.82; 1.70]
Heat pain PDT (normal skin)	4.7	0.2080	4.00% [0.99; 1.05]	-	-	-	2.7	0.6191	1.39 [-0.57; 0.31]	2.2	0.3383	1.26 [-0.13; 0.50]

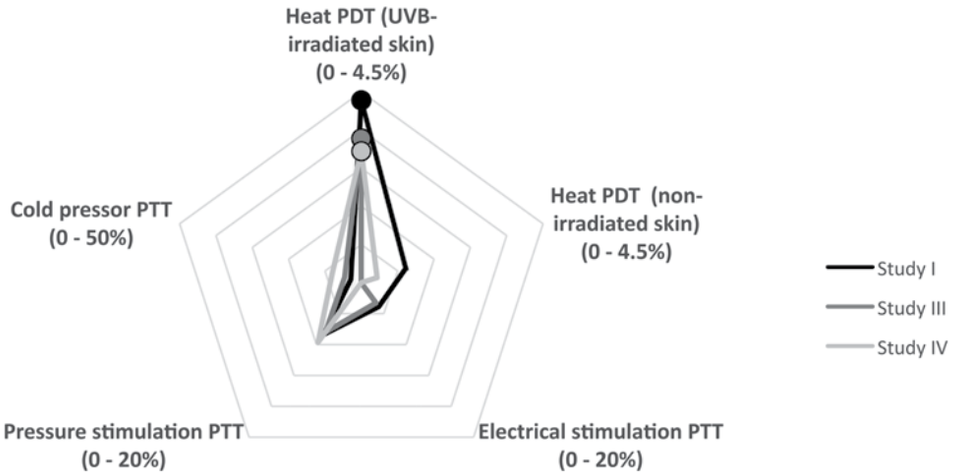
CI: confidence interval; CV: inter-individual coefficient variability; LSMeans: least squares means; PDT: pain detection threshold; PTT: pain tolerance threshold; UVB: ultra violet B radiation.

Figure 1 – Star plot summarising the effects of pregabalin (300 mg) on the indicated pain assessments in studies I through IV. The percentages shown for each assessment reflect the difference between pregabalin and placebo. Values marked with a circle are significantly different (p<0.05) compared with placebo.



PDT, pain detection threshold; PTT, pain tolerance threshold.

Figure 2 – Star plot summarising the effects of ibuprofen (600 mg) on the indicated pain assessments in studies I, III, and IV. The percentages shown for each assessment reflect the difference between ibuprofen and placebo. Values marked with a circle are significantly different (p<0.05) compared with placebo.



PDT, pain detection threshold; PTT, pain tolerance threshold.

Chapter 6

EFFECT PROFILE OF PARACETAMOL, Δ9-THC AND PROMETHAZINE USING AN EVOKED PAIN TEST BATTERY IN HEALTHY SUBJECTS

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ABSTRACT

A battery of evoked pain tasks (PainCart) was developed to investigate the pharmacodynamic properties of novel analgesics in early phase clinical research. As part of its clinical validation, compounds with different pharmacological mechanisms of actions are investigated. The aim was to investigate the analgesic effects of classic and non-classic analgesics compared to a sedating negative control in a randomized placebo-controlled crossover study in 24 healthy volunteers using the PainCart.

The PainCart consisted of pain tasks eliciting electrical, pressure, heat, cold and inflammatory pain. Subjective scales for cognitive functioning and psychotomimetic effects were included. Subjects were administered each of the following oral treatments: paracetamol (1000 mg), Δ9-THC (10 mg), promethazine (50 mg) or matching placebo. Pharmacodynamic measurements were performed at baseline and repeated up to 10 hours post-dose.

Paracetamol did not show a significant reduction in pain sensation or subjective cognitive functioning compared to placebo. Promethazine induced a statistically significant reduction in PTT for cold pressor and pressure stimulation. Furthermore, reduced subjective alertness was observed. Δ9-THC showed a statistically significant decrease in PTT for electrical- and pressure stimulation. Δ9-THC also demonstrated subjective effects, including changes in alertness and calmness, as well as feeling high and psychotomimetic effects.

This study found a decreased pain tolerance due to Δ9-THC and promethazine, or lack thereof, using an evoked pain task battery. Pain thresholds following paracetamol administration remained unchanged, which may be due to insufficient statistical power. We showed that pain thresholds determined using this pain test battery are not driven by sedation.

INTRODUCTION

The complex clinical reality of pain medicine demands novel therapeutics. A multi-modal battery of evoked pain tasks could be a useful tool to investigate the analgesic properties of novel compounds, but needs to be pharmacologically validated for specific classes of compounds. In the present study the effects of three oral drugs were investigated and compared to placebo: Δ9-tetrahydrocannabinol (Δ9-THC), paracetamol and promethazine.

Different cannabinoids have previously been shown to be effective in various pain conditions, including neuropathic pain related to oncological disease.[55] Δ9-THC has been shown to be an effective analgesic in preclinical studies and clinical trials. However, previous formulations of cannabinoid Δ9-THC are also known for variable pharmacokinetic profiles and pharmacodynamic responses.[22] To overcome barriers in clinical application, novel formulations and cannabinoids are under development.[23]

Even though paracetamol is one of the most widely used medications in the world, there is still debate regarding its exact mechanism of action. Paracetamol is thought to be a weak inhibitor of prostaglandins (PG) synthesis. The subsequent main driving mechanism of paracetamol analgesia is not completely understood. It has been proposed that it exerts most of its effects through COX-2 inhibition, but also inhibition of endocannabinoids has been proposed. In addition, various neurotransmitter systems (*e.g.*, serotonergic, opioid and noradrenaline) are thought to be involved.[8,11,19,24]

To investigate the role of sedation rather than analgesic effects of psychoactive compounds a negative control was included in the current study in the form of the H1 antihistaminergic promethazine (50 mg). Even though it has been observed in preclinical research that H1 antihistaminergic drugs may have analgesic potential, this has not been replicated in clinical practice for oral formulations administered alone.[44,48] Therefore we considered this sedative compound suitable as a comparator drug without analgesic effects

The primary aim of this study is to investigate the analgesic effects of classic and non-classic analgesics compared to a sedating negative control in a randomized placebo-controlled crossover study in 24 healthy volunteers using the PainCart. As a secondary objective, by comparing the effects of the 3 compounds within each subject in a crossover design, and comparing the analgesic profile to the profiles of other analgesic compounds that we recently investigated using the battery of evoked pain tasks, we aimed to further elucidate the still unknown pharmacological mechanism of action of Δ9-THC and paracetamol analgesia.

The battery of evoked pain tasks has been pharmacologically validated by investigating a broad range of analgesics from various classes, with diverse but well-known mechanisms of action.[40] This first pharmacological validation study demonstrated the necessity of utilising a range of pain tasks in early-phase drug research. Namely, each compound provided a unique fingerprint of effects on the test battery. These findings emphasized the importance of utilising a range of pain tasks, rather than a single pain task, when determining the profile of analgesic effects of a compound in early phase drug development. Building on this knowledge, the present study investigated the effects of two (classes of) analgesics, paracetamol and Δ9-THC, and additionally the effects of sedation using promethazine as a negative control.

METHODS

Subjects and study design

The study was a double blind, double-dummy, single dose, randomized, placebo-controlled, crossover study in which the effects of paracetamol, Δ9-THC and the negative control promethazine were compared to placebo. The study was conducted at the Centre for Human Drug Research in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. This study was registered in the public registry of the Centrale Commissie Medisch Onderzoek (CCMO) in the Netherlands, under registration number: NL54643.056.15

Each subject provided written informed consent before any screening procedures were performed. A total of 24 healthy subjects (12 males and 12 females) between 18 and 45 years of age with a body mass index of 18 to 30 kg/m² were enrolled. The subjects underwent a full medical screening, including medical history anamnesis, a physical examination, blood chemistry and haematology, urinalysis, electrocardiogram (ECG) and assessment of the minimal erythema dose (MED) for ultraviolet B (UVB) light to assess eligibility. Subjects with a clinically significant known medical condition, in particular any existing condition that

would affect sensitivity to cold or pain were excluded. Subjects with Fitzpatrick skin type V or VI, widespread acne, tattoos or scarring on the back were excluded due to the inability to accurately assess MED. Also any subject, who was a regular user of any illicit drugs, had a history of drug abuse or a positive drug screen at screening was excluded. Smoking and the use of xanthine-containing products were not allowed during dosing days. Alcohol was not allowed at least 24 hours before each scheduled visit and during the stay in the research unit.

Study drugs

Paracetamol (1000 mg), Δ9-THC (10 mg), promethazine (50 mg) or placebo were given as a single dose. Paracetamol 1000 mg is within the labelled dose range in the European Union (EU) and has been shown to be effective in reducing various types of pain. The currently used formulation of Δ9-THC (Namisol®, Echo Pharmaceuticals) has been administered in multiple studies including healthy volunteers[23] and different patient populations.[3,54,57] Δ9-THC has potential side effects, but is generally considered well-tolerated, even in high dosages. Promethazine is a classic H1-antihistamine with some anticholinergic effects. Daily doses up to 150 mg are prescribed for the treatment of allergic rhinitis and motion sickness. Single doses up to 50 mg are prescribed to induce mild sedation.

Due to unequal formulations (Δ9-THC was formulated as an oral tablet, whereas paracetamol and promethazine were formulated as capsules), matched placebo tablets for each treatment were administered in a double-dummy fashion to maintain blinding of treatment for participants and researchers.

Pharmacodynamic assessments

Pain detection and tolerance thresholds were measured using a battery of evoked pain tasks, as described previously.[21,37-40] The test battery consists of an integrated range of pain tasks for measuring different modalities of pain. Assessments were conducted twice pre-dose (double baseline) and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose by trained personnel. Each measurement round was performed in a fixed order and took approximately 30 minutes to complete. To eliminate the risk of tissue damage, all pain tasks had a maximum safety cut-off. The aim of the test battery is to assess as objectively as possible the levels of pain induced by different noxious mechanisms in human subjects. A training session was included as part of the screening examination to reduce learning effects during the study and exclude non-responders (*i.e.*, subjects who reach PDT at >80% of the maximum at any of the nociceptive tasks, excluding the heat pain task) or extreme responders (subjects indicating to be intolerable to any of the nociceptive tasks). All measurements were performed in a quiet room with ambient illumination. Per session, there was only one subject present in the same room. To reduce variability from affects associated with fear of pain, the subjects themselves were responsible for starting and ending each pain task.

The battery of evoked pain tasks consists of the following tasks for nociception: the electrical stimulation task, pressure stimulation task, thermal (heat) pain and the cold pressor tasks. Furthermore, the test battery includes a model for inflammatory pain, the UVB model and a paradigm to quantify Conditioned Pain Modulation (CPM), formerly known as Diffuse Noxious Inhibitory Control (DNIC).

For the electrical stimulation task, the pressure stimulation task and the cold pressor task, pain intensity was measured continuously (beginning from when the first stimulus was applied until the end of the test) using an electronic visual analogue scale (eVAS) scale ranging from 0 (no pain) to 100 (most intense pain tolerable). Equipment was programmed to cease giving stimuli if the recorded pain intensity reaches the maximum pain score (100) or when the maximum safety level was reached. For the abovementioned pain tasks, the pain detection threshold (PDT) (defined as eVAS score > 0), pain tolerance threshold (PTT) (defined as eVAS score of 100) and Area Under the Curve (AUC) or Area Above the Curve (AAC) (Cold Pressor only) were determined. Additionally, a post-test Visual Analogue Scale (VAS) score (anchored with no pain (0) and worst pain imaginable (100)) was performed to retrospectively assess the worst pain experienced during the pain task. For the thermal pain task (normal skin and UVB exposed skin) only the (average of triplicate) PDT was determined, since assessment of heat PTT is prone to inducing tissue damage. For all nociceptive tasks were a PTT is determined (all except thermal pain) the primary endpoint is the PTT. For the thermal pain tasks (normal skin and UVB exposed skin), the PDT is the primary endpoint of the measurement. However, since each parameter (PDT, PTT, AUC/AAC) provides information on different aspects of the nociceptive system and pain perception, all variables are taken into account.

In addition to the evoked pain tasks, subjective assessment of sedation and psychotomimetic effects were included as PD outcome measures. Visual analogue scales (VAS) as originally described by Norris[36] have often been used previously to quantify subjective effects of a variety of sedative agents.[14,36] A set of VAS scales assessing alertness, mood, and calmness (Bond and Lader)[9] were used for subjective assessment of sedation. The VAS allows the subjects to evaluate their current subjective states. Each VAS scale consists of 2 words representing

opposite feelings placed to the left and right of a horizontal line. The subject is asked to mark his/her current feelings. Subjective psychotomimetic (psychedelic) effects were evaluated using VAS Bowdle. This scale has been used extensively to quantify subjective psychotomimetic effects of psychoactive compounds, including ketamine.[10] Bowdle Psychotomimetic Effects Scores consist of thirteen visual analogue lines ranging from 0 ('not at all) to 100 ('extremely'),[58] addressing various (abnormal) states of mind.

Sample size and randomisation

Based on literature, PDT for the cold pressor assessment was used for the sample size calculation as this assessment has been shown sensitive to the effects of Δ9-THC in previous research.[12] For the cold PDT, a sample size of 24 subjects has 80% power to detect a difference in means of 35%, assuming a standard deviation of differences of 0.5, using a paired t-test with a 0.05 two-sided significance level. For the sample size calculation, placebo data from a previous study with the battery of pain tasks were used to determine variability.[40] The balanced Williams design randomization code was generated using SAS version 9.1.3 by a study-independent statistician.

Statistical analysis

To establish whether significant treatment effects could be detected on the PD outcome variables, variables were analysed with a mixed model analysis of variance with treatment, time, sex, treatment by time and treatment by sex as fixed factors and subject, subject by treatment and subject by time as random factors and the average baseline measurement as covariate. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters were estimated using the restricted maximum likelihood method. The general treatment effect and specific contrasts were reported with the estimated difference and the 95% confidence interval, the least square mean estimates and the p-value. Graphs of the Least Squares Means estimates over time by treatment were presented with 95% confidence intervals as error bars. All calculations of the pharmacodynamic parameters were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The main SAS procedure that was used in the analysis was "PROC MIXED". No adjustments for multiple comparisons were employed. The contrasts for the relevant time periods based on the expected PK profiles of the compounds of 0-4 hours are presented.

RESULTS

A total of 25 subjects were randomized, of which 23 subjects completed study participation. Two (2) subjects withdrew consent to participate for personal reasons, one of which was replaced. A summary of the baseline demographics is provided in Table 1.

Pharmacodynamics

Time profiles of the pharmacodynamic responses on PTT for each pain task, except heat pain (Normal skin and UVB skin) for which PDT is displayed, are presented in Figure 1. This figure also includes a graphical presentation of CPM (Delta PTT for electrical pain). PTT and PDT measurements were log (ln) transformed before analysis, due to the log normal distribution of the data. The results are presented as % change from baseline over a 10 hour period. A detailed description of the results of the Least Square Means (LSMeans) analyses for each treatment as well as contrasts compared to placebo (0-4 hours) can be found in Table 2. The results of the LSMeans analyses for the primary endpoints (PTT) are summarized in Figure 2. Each spoke represents one of the pain tasks, resulting in an effect profile compared to placebo per treatment. Here, the dashed placebo line represents the value to which other treatment effects are normalized. A contrast distal from placebo indicates that the LSMeans PTT for that treatment is greater than placebo, proximal indicates a LSMeans PTT lower than placebo.

Furthermore, the results for the subjective scales for cognitive functioning and psychotomimetic symptoms are presented in Table 3. Paracetamol did not show a significant reduction in pain sensation compared to placebo. A small increase in AUC (p=0.0314) was observed for the pressure pain task, indicating a slight increase in perceived pain sensation. Treatment with paracetamol did not lead to any observable changes in subjective cognitive functioning or mood. Promethazine demonstrated a statistically significant reduction in PTT for the cold pressor pain task (p=0.0189) and for the pressure stimulation task (p=0.0149), as well as an increase in AUC (p=0.0032), indicating an increase in pain sensation. In addition to the pharmacodynamic effects of promethazine on the pain task battery, a reduction in subjective alertness (p=0.0002) was observed. Δ9-THC did not show a statistically significant analgesic effect on any of the pain tasks. For the electrical stimulation task, the PTT was significantly decreased by -12.7%, (p=0.0134), also indicating an increase in pain sensation.

Furthermore, a significant reduction was observed for the pressure stimulation task PTT (p=0.0126) and AUC (p=0.0001). In addition to the effects observed on the pain task battery, Δ9-THC also demonstrated other pharmacodynamic effects, including a reduction on the composite scale for alertness (p=<.0001) and an increase on the composite scale for calmness (p=<.0001) compared to placebo. Moreover, significant psychotomimetic effects were observed expressed in changes in internal perception (p=<.0001) and external perception (p=<.0001), measured using the VAS Bowdle, as well as VAS Feeling high (p=<.0001). Of note, psychotomimetic effects were virtually absent after placebo treatment, thereby leading to high significance levels even at small effect sizes.

Safety

During the execution of this study, a total of 79 Treatment Emergent Adverse Events (TEAE) were registered. The majority (N=43, 54%) of these were recorded after treatment with Δ9-THC, after which 20 out of 25 subjects reported any event. Out of all TEAE, seven (8.8%) were considered moderate, all others were deemed mild. For Δ9-THC treatment, 60% of subjects reported an adverse event in the System Organ Class (SOC) Nervous system disorders, most of which were dizziness (40%) and headache (20%). Furthermore, 3 subjects (12%) reported euphoric mood and 3 subjects (12%) mild auditory hallucinations. A total of four subjects experienced TEAE of moderate intensity after treatment with Δ9-THC, leading to one or more missing measurement. For treatment with promethazine, most prominently somnolence (N=7, 30.4%) and fatigue (N=6, 26.1%) were observed. For paracetamol treatment, a total of six events were recorded, which is comparable to placebo treatment.

To investigate whether adverse events may have impacted the outcome of the pain tasks, a subgroup analysis was performed in which the 4 subjects that experienced at least one adverse event of moderate intensity were omitted from the analyses, as a moderate adverse event may have impacted pain tasks adjacent to its occurrence. This analysis had no significant impact on the interpretation of the results, therefore it was decided to report the results on the intention-to-treat (ITT) population.

DISCUSSION

The main objective of the present study was to investigate the effects of a classical (paracetamol) and a non-classical (Δ9-THC) analgesic on a battery of pain tasks (PainCart®), compared to placebo and a negative control (promethazine). The effects of the different treatment effects on each pain task are summarized in Figure 2, demonstrating the differential effect profile of each compound for the different pain tasks. Contrary to our expectation we found that paracetamol was not effective at reducing any of the pain modalities measured using the battery of evoked pain tasks. Furthermore, Δ9-THC did not show any acute analgesic effect, and even showed a hyperalgesic effect on two of five pain tasks, namely electrical and pressure pain. Finally, the negative control promethazine showed an increase in pain sensation for cold, pressure and inflammatory pain. In addition to the pain tasks, cognitive tests were performed to assess subjective alertness, mood, and psychotomimetic symptoms, which were moderately affected by treatment with Δ9-THC (alertness, calmness, internal and external perception) or promethazine (alertness).

This study did not demonstrate and acute analgesic effect of Δ9-THC, even though the subjective psycho-active effects were clearly present. As such we can conclude that the subjective psycho-active effects are not responsible for producing nociceptive analgesia. Moreover, the present study helped to further elucidate the mechanism of action of paracetamol as our results enable comparison to other analgesics with known mechanisms of action. Finally, when combining the findings of the current study with the existing body of evidence from this battery of evoked pain tasks, we have shown this battery to be a robust tool to determine analgesic effects that are specific, and thus not merely expressing sedation, otherwise the observed subjective sedation would have resulted in analgesia. This is an important finding for future studies in order to benchmark the effects of novel analgesics that may demonstrate a degree of sedation, including subtype selective $GABA_A$ agonists or novel mixed MOP/NOP receptor agonists.

At first glance it may have been surprising that the battery of evoked pain tasks was not sensitive to detect analgesic effects of paracetamol over a period of 4 hours post-dose, as it is among the most widely used analgesics worldwide. It has been shown to be effective in the treatment of different types of clinical pain, Although not all. While it is effective at reducing postoperative pain,[30,60] episodic tension headache[51] and acute migraine,[15] there is no evidence for its effectiveness in treating lower back pain[50,61] or pain related to osteoarthritis.[29] However, when looking at available literature on human evoked pain tasks in healthy volunteers, the image becomes more diffuse. For each of the pain tasks that were investigated in more than one clinical trial, positive as well as negative results have been reported: mixed results were obtained using the Cold pressor,[31,32,52,64] there was a single negative study for contact heat,[52] and again mixed results for

electrical pain,[5,16,41,52] mixed results for pressure pain[41,43,47] and only a single study showing analgesic effects on inflammatory pain using the UVB model.[42] Interestingly, the published studies measuring pain experience (post-test NRS or post-test VAS) tend to be more likely to show analgesia by paracetamol than studies measuring the more objective pain thresholds. This may indicate that paracetamol exerts its analgesic effect on the aspect of subjective pain experience by means of pain modulation rather than exerting changes in nociceptive pain perception thresholds. This differential effect was not observed in the present study. Additionally, the analgesic effects of paracetamol in human evoked pain models tend to be more subtle than the effect sizes that were used for the power calculation, therefore the study may have been underpowered. This applies specifically to for the Cold pressor task, where a non-significant increase in pain thresholds was observed. Summarising, based on the findings in literature and the aforementioned hypothesis, the outcome might have been different if a two-way crossover compared to placebo design was used in which different endpoints, *i.e.*, Laser Evoked Potentials,[4,34,35] were investigated.

Medicinal use of cannabis dates back tens of thousands of years.[1] In the last decade the role for (plant-derived or synthetic) cannabinoids has shifted from complementary medicine to regular care for pain related to oncology[2] and neuropathic pain resulting to spinal cord injury[62] or Multiple Sclerosis (MS).[49,53] The oral formulation of Δ9-THC (Namisol®) that was used in the current study has been shown to be effective in reducing neuropathic pain in a recently performed study in 24 patients suffering from progressive MS after 4 weeks of chronic treatment.[57] However, given its interaction with the endocannabinoid system it cannot be considered an "antinociceptive" analgesic, even if it may have analgesic effects in some conditions. This is reflected in the results of clinical studies using human evoked pain models to investigate pharmacology and mechanism of action. Only two studies investigating the effects of either inhaled cannabis or oral Δ9-THC showed a statistically significant reduction in pain sensation on the cold pressor task[12] or the heat pain task.[20] Two other studies investigating the effects on heat pain alone, did not demonstrate this improvement.[45,46] The results of the present study are in line with the results of Naef et al.[33] and Kraft et al.[25] who showed lack of analgesia on a set of pain tasks and even a significant or non-significant increase in pain sensation for electrical pain and cold pressor. The finding of Δ9-THC induced hyperalgesia has also been observed in the clinic.[6] A possible explanation is that this effect is dose-related, due to a bell-shaped effect curve. As proposed by Walter et al.,[59] this narrow therapeutic window may be the result of co-activation of TRPA1 and TRPV1 channels along with CB1 receptors by Δ9-THC at higher concentrations. The dose of 10 mg of the oral formulation of Δ9-THC was the highest single dose that is administered to healthy volunteers of this formulation to date.[23] Due to inter-subject variability this dose may have been too high for some, as in four subjects pharmacodynamic assessments were delayed or omitted as a result of adverse events associated with subjective effects and nausea. However, on a group level only a reasonable reduction in subjective alertness was reported. Furthermore, a post-hoc analysis excluding the measurements that may have been affected by AEs of moderate intensity did not lead to a different interpretation of the results compared to the ITT analysis. Therefore the ITT analysis was maintained and reported here. On the other hand, it is known that chronic and even acute exposure to Δ9-THC can induce a "transient amotivational state",[26] which may be misinterpreted as an apparent hyperalgesic state. This hyperalgesic state is in fact the result of the psychotropic effect profile of Δ9-THC, as subjects become less motivated to complete the pain tasks. Despite our efforts, human evoked pain tasks remain also sensitive to the affective components of pain sensation, and thus susceptible to detect changes in motivation as well as pure analgesia.

Over the recent years some evidence has gathered for the effectiveness of antihistaminergic drugs as adjuvant in the treatment of various pain states.[7,17,18] However, there is no evidence for any acute analgesic effect in humans. As such, promethazine (50 mg) was selected as a negative control for Δ9-THC and to investigate the effects of sedation on the battery of evoked pain tasks. In addition to an increased sensitivity for electrical and pressure pain, a decreased pain detection threshold for inflammatory pain was observed. Even though histamine is involved in the initial phase of erythema development, this role is not prominent in the delayed erythemic response[63] and as such administration 24 hours after UVB exposure is not likely to have influenced the pathophysiology of the UVB induced erythema. Thus, the results of promethazine treatment may indicate a reduction of pain endurance, which could result from reduced motivation associated with sedative effects (expressed as a reduction in subjective alertness), rather than suppositious analgesia resulting from delayed or impaired responsiveness.

The present study adds to a body of research studies in which this exact battery of evoked pain tasks was used to investigate various analgesic compounds alone[38-40] or combined.[37] As such, the battery of evoked pain tasks is pharmacologically validated for the effects of cannabinoids and sedatives. The battery of evoked pain tasks was not sensitive to detect analgesic effects of paracetamol, but that finding by itself provides information on the much debated and yet unrevealed pharmacological mechanism of action, as we are able to compare the

results to other compounds with known mechanism of action. As recognized before,[27,28,57] translatability of findings from human evoked pain models to clinical pain remains elusive. Nonetheless, if used prudently, this battery of pain tasks can provide invaluable information on pharmacodynamic and pharmacokinetic relationships in the early phases of drug development, especially when combined with other neurocognitive assessments.

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Table 1 – Summary demographic and baseline characteristics for all subjects (N=25)

AGE (YEARS)		
	Mean (SD)	24.0 (5.6)
	Median	23
	Min, Max	18, 45
BMI (KG/M^2)		
	Mean (SD)	23.5 (2.9)
	Median	23.7
	Min, Max	18.2, 29
SEX (N)		
	Female (%)	12 (48%)
	Male (%)	13 (52%)
RACE		
	Other	1 (4%)
	White	24 (96%)
FITZPATRICK SKIN TYPE		
	II: Always burns & tans min	6 (24%)
	III: Burns moderate & tan grad	11 (44%)
	IV: Burns minimal & tans well	8 (32%)
MED (MJ/CM)		
	Mean (SD)	777 (249)
	Median	702
	Min, Max	351, 1321

BMI = Body Mass Index; MED = Minimal Erythema Dose.

Table 2 – Summary of LSMeans analyses for battery of evoked pain tasks.

Endpoint	Placebo	Paracetamol		Promethazine		Δ9-THC	
	LSMean (95%CI)	LSMean (95%CI)	Contrast vs. placebo* (95%CI)	LSMean (95%CI)	Contrast vs. placebo (95%CI)	LSMean (95%CI)	Contrast vs. placebo (95%CI)
COLD PRESSOR (S)							
PTT	13.4 (12.5-14.3)	13.9 (13-14.8)	5.2% (-4.6%, 16.0%) p=0.3090	12.1 (11.3-13)	**-11.1% (-19.3%, -1.9%) p=0.0189**	13 (12.2-13.9)	2.5% (-7.0%, 12.9%) p=0.6183
PDT	3.2 (2.5-4)	3.3 (2.6-4.1)	3.5% (-15.0%, 26.0%) p=0.7292	2.7 (2.2-3.5)	-17.0% (-31.8%, 1.2%) p=0.0648	3.1 (2.5-4)	-0.8% (-18.5%, 20.7%) p=0.9335
AAC	860 (803-922)	902 (841-968)	7.8% (-3.8%, 20.9%) p=0.1938	780 (727-837)	-10.6% (-20.2%, 0.2%) p=0.0548	845 (789- 905)	4.1% (-7.0%, 16.6%) p=0.4806
VAS	56.9 (52.8-61)	58.3 (54.2-62.4)	2.59 (-0.45, 5.63) p=0.0940	58.4 (54.3-62.5)	1.48 (-1.56, 4.52) p=0.3366	57.6 (53.5-61.8)	0.11 (-2.93, 3.15) p=0.9444
ELECTRICAL STIMULATION (MA)							
PTT	22.1 (20.3-24.1)	19.7 (18.1-21.6)	-8.7% (-18.2%, 1.9%) p=0.1023	20.5 (18.8-22.4)	-7.7% (-17.2%, 3.0%) p=0.1495	20.1 (18.4-21.9)	**-12.7% (-21.5%, -2.8%) p=0.0134**
PDT	9.43 (8.44-10.53)	8.13 (7.27-9.1)	-9.9% (-22.8%, 5.3%) p=0.1896	8.95 (8-10.01)	0.8% (-13.7%, 17.8%) p=0.9211	9.19 (8.22-10.26)	-2.7% (-16.6%, 13.4%) p=0.7222
AUC	3244 (3112-3376)	3438 (3302-3573)	171.37 (-21.12, 363.87) p=0.0805	3324 (3189-3460)	76.86 (-115.81, 269.53) p=0.4311	3379 (3246-3512)	196.58 (6.98, 386.19) p=0.0423
VAS	54.6 (51.8-57.4)	56 (53.1-58.8)	1.71 (-1.18, 4.61) p=0.2414	52.5 (49.6-55.4)	-1.38 (-4.28, 1.52) p=0.3467	54.3 (51.4-57.1)	-0.68 (-3.53, 2.17) p=0.6372
CPM: ELECTRICAL STIMULATION (DIFFERENCE PRE-POST COLD PRESSOR) (MA)							
PTT	1.07 (0.64-1.51)	1.00 (0.56-1.45)	0.101 (-0.772, 0.973) p=0.8206	0.85 (0.41-1.29)	-0.186 (-1.050, 0.678) p=0.6716	0.77 (0.32-1.23)	0.099 (-0.785, 0.983) p=0.8254

Table 2 – continued

PDT	1.07 (0.64-1.51)	1.00 (0.56-1.45)	0.678 (-0.566, 1.921) p=0.2841	0.85 (0.41-1.29)	0.052 (-1.186, 1.290) p=0.9339	0.77 (0.32-1.23)	-0.144 (-1.398, 1.109) p=0.8206
AUC	-147 (-192--102)	-113 (-159--67)	-24.74 (-107.64, 58.17) p=0.5574	-108 (-154--61)	13.44 (-69.10, 95.98) p=0.7488	-79 (-125--33)	9.63 (-73.60, 92.87) p=0.8200
VAS							
PRESSURE STIMULATION (KPA)							
PTT	39.9 (36.3-43.9)	38.9 (35.3-42.8)	-5.1% (-11.9%, 2.2%) p=0.1653	37.1 (33.7-40.8)	**-8.9% (-15.4%, -1.8%) p=0.0149**	36.5 (33.1-40.1)	**-9.0% (-15.6%, -2.0%) p=0.0126**
PDT	16.7 (14.2-19.6)	15.5 (13.2-18.2)	-8.5% (-19.1%, 3.5%) p=0.1552	14.8 (12.6-17.4)	-7.1% (-17.8%, 5.1%) p=0.2392	14.3 (12.2-16.8)	-9.9% (-20.3%, 1.9%) p=0.0972
AUC	6761 (6457-7064)	6906 (6601-7211)	**248.01 (22.33, 473.69) p=0.0314**	7072 (6767-7377)	**341.93 (115.96, 567.89) p=0.0032**	7180 (6876-7484)	**446.38 (221.09, 671.67) p=0.0001**
VAS	50.2 (44.2-56.2)	51.4 (45.4-57.4)	1.12 (-1.60, 3.85) p=0.4176	50.3 (44.2-56.3)	-0.20 (-2.92, 2.52) p=0.8841	50.4 (44.4-56.5)	0.35 (-2.35, 3.06) p=0.7964
NORMAL HEAT (°C)							
PDT	45.1 (44.7-45.6)	45.5 (45-46)	0.5% (-0.9%, 2.0%) p=0.4434	45 (44.6-45.5)	-0.6% (-2.0%, 0.8%) p=0.3830	45.3 (44.8-45.8)	0.3% (-1.1%, 1.7%) p=0.7229
UVB HEAT (°C)							
PDT	39.7 (39.1-40.2)	39.8 (39.3-40.3)	0.2% (-1.1%, 1.4%) p=0.8033	38.8 (38.3-39.3)	**-2.8% (-4.1%, -1.6%) p=<.0001**	39.4 (38.9-40)	-1.0% (-2.3%, 0.3%) p=0.1220

PTT = Pain Tolerance Threshold; PDT = Pain Detection Threshold; AAC = Area Above the Curve; AUC = Area Under the Curve; VAS = Visual Analogue Scale; CPM = Conditioned Pain Modulation/ * Contrasts over 0-4 hours post dose.

Table 3 – Summary of LSMeans analyses for subjective cognitive functioning and psychotomimetic symptoms.

	Placebo	Paracetamol		Promethazine		Δ9-THC	
	LSMean (95%CI)	**LSMean (95%CI)**	**Contrast vs. placebo* (95%CI)**	**LSMean (95%CI)**	**Contrast vs. placebo (95%CI)**	**LSMean (95%CI)**	**Contrast vs. placebo (95%CI)**
VAS BOND & LADER							
Alertness (mm)	49.7 (48.7-50.7)	49.8 (48.8-50.8)	0.07 (-1.55, 1.68) p=0.9339	47.2 (46.2-48.2)	**-3.11 (-4.75, -1.48) p=0.0002**	45.7 (44.7-46.7)	**-5.83 (-7.45, -4.21) p=<.0001**
Calmness (mm)	51.6 (50.6-52.6)	51.1 (50-52.1)	-0.73 (-2.57, 1.12) p=0.4369	51.9 (50.9-53)	0.46 (-1.38, 2.30) p=0.6236	53.6 (52.6-54.7)	**3.97 (2.11, 5.82) p=<.0001**
Mood (mm)	50.4 (49.7-51.1)	50.2 (49.5-50.9)	-0.42 (-1.39, 0.55) p=0.3914	50.7 (50-51.4)	0.10 (-0.88, 1.07) p=0.8408	51 (50.3-51.6)	0.76 (-0.21, 1.74) p=0.1244
VAS BOWDLE							
Feeling High (LOGmm)	0.33 (0.28-0.39)	0.31 (0.26-0.37)	-0.0295 (-0.1347, 0.0757) p=0.5804	0.35 (0.29-0.4)	0.0189 (-0.0859, 0.1237) p=0.7225	0.71 (0.65-0.77)	**0.7232 (0.6164, 0.8300) p=<.0001**
Internal perception (LOGmm)	0.32 (0.29-0.34)	0.31 (0.29-0.34)	-0.0061 (-0.0470, 0.0348) p=0.7677	0.33 (0.31-0.35)	0.0269 (-0.0139, 0.0678) p=0.1951	0.4 (0.38-0.42)	**0.1705 (0.1292, 0.2117) p=<.0001**
External perception (LOGmm)	0.33 (0.28-0.37)	0.32 (0.27-0.36)	-0.0143 (-0.0957, 0.0670) p=0.7279	0.34 (0.29-0.39)	0.0148 (-0.0664, 0.0960) p=0.7189	0.55 (0.5-0.59)	**0.4289 (0.3466, 0.5112) p=<.0001**

VAS = Visual Analogue Scale; * Contrasts over 0-4 hours post dose / Bold values signify that the p-value <0.05

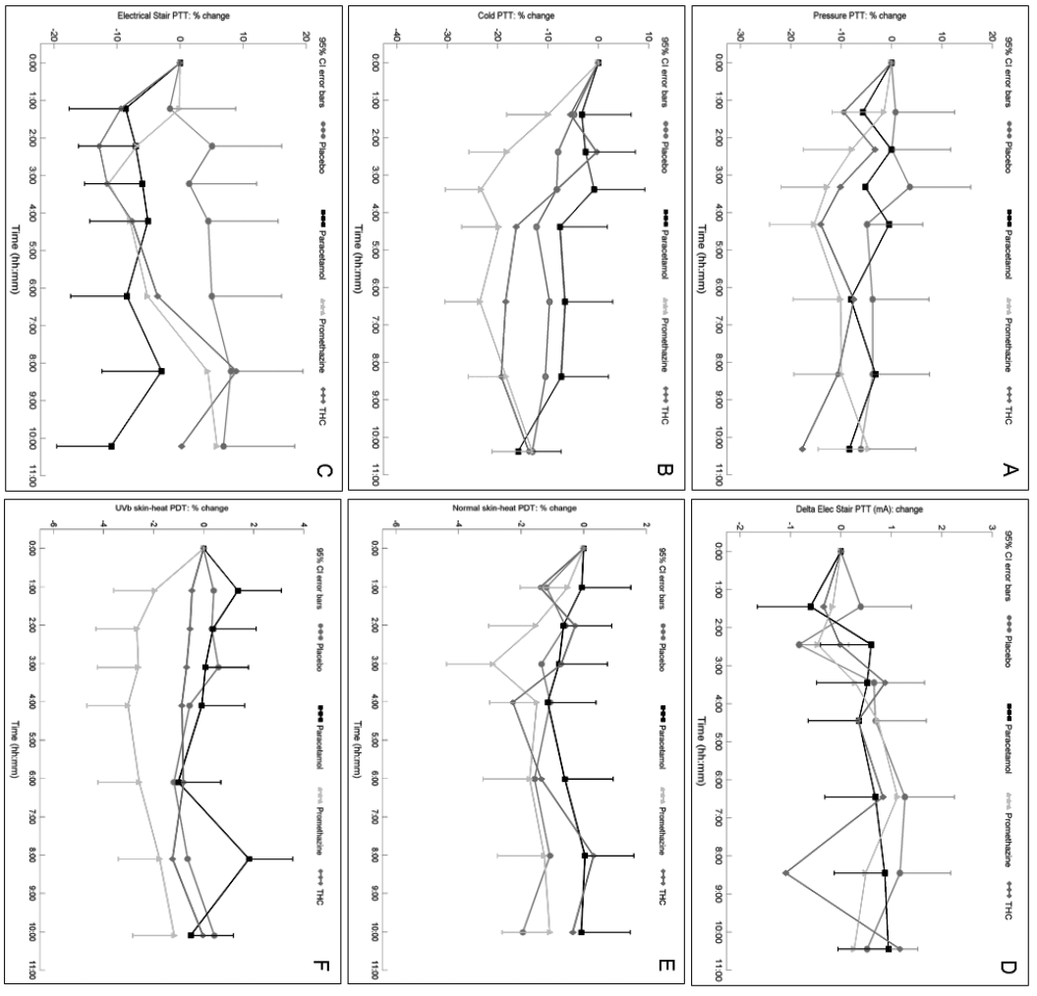
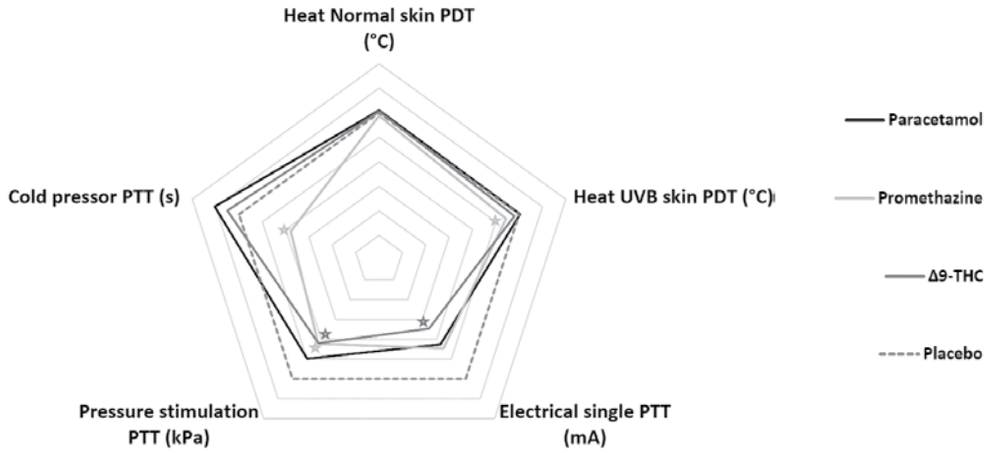


Figure 1 – Overview of Change from Baseline time profiles for battery of evoked pain tasks.

Panel A = Pressure pain task in KPA (PTT); Panel B = Cold pressor in S (PTT); Panel C = Electrical pain task in MA (PTT); Panel D = Conditioned Pain Modulation (CPM) in delta MA (PTT) Panel E = Thermal pain normal skin in °C (PDT); Panel F = Thermal pain UVB skin in °C (PDT) / Lines with Circles (●) = placebo; lines with squares (■) = paracetamol; lines with triangles (▲) = promethazine; lines with diamonds (♦) = Δ9-THC / PTT = Pain Tolerance Threshold; PDT = Pain Detection Threshold.

Figure 2 – Spider plot overview of Pharmacodynamic response profile for battery of evoked pain tasks normalized to placebo (0-4 hours. Dashed placebo line represents the value to which other treatment effects are normalized. Distal from placebo indicates Least Square Mean PTT greater than placebo, proximal indicates Least Square mean PTT lower than placebo. Actual values are described in Table 2. A star (*) indicates a statistically significant (P<0.05) difference compared to placebo for treatment on pain task.



Chapter 7

THE ULTRAVIOLET B INFLAMMATION MODEL: POSTINFLAMMATORY HYPERPIGMENTATION AND VALIDATION OF A REDUCED UVB EXPOSURE PARADIGM FOR INDUCING HYPERALGESIA IN HEALTHY SUBJECTS

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ABSTRACT

Pain models are commonly used in drug development to demonstrate analgesic activity in healthy subjects and should therefore not cause long-term adverse effects. The ultraviolet B (UVB) model is a model for inflammatory pain in which three times the minimal erythema dose (3MED) is typically applied to induce sensitisation. Based on reports of long-lasting postinflammatory hyperpigmentation (PIH) associated with 3MED, it was decided to investigate the prevalence of PIH among subjects who were previously exposed to 3MED at our research centre. In addition, re-evaluation of the UVB inflammation model using a reduced exposure paradigm (2MED) was performed in healthy subjects.

In the first study, all 142 subjects previously exposed to 3MED UVB were invited for a clinical evaluation of PIH. In the second study, 18 healthy subjects were exposed to 2MED UVB, and heat pain detection threshold (PDT) and PIH were evaluated.

In total, 78 of the 142 subjects responded. The prevalence of PIH among responders was 53.8%. In the second study, we found a significant and stable difference in PDT between UVB-exposed and control skin 3 hours after irradiation; 13 hours post-irradiation, the least squares mean estimate of the difference in PDT ranged from -2.6°C to -4.5°C (p<0.0001). Finally, the prevalence of PIH was lower in the 2MED group compared to the 3MED group.

The 3MED model is associated with a relatively high prevalence of long-lasting PIH. In contrast, 2MED exposure produces stable hyperalgesia and has a lower risk of PIH and is therefore recommended for modelling inflammatory pain.

INTRODUCTION

Evoked pain models in human subjects are commonly used in the early stages of clinical drug development for demonstrating analgesic activity and determining the compound's active dose. Ideally, a pain model should be easy to perform and should provide reproducible, reliable results under standardised conditions, but it must not cause tissue damage or have long-term adverse side effects.

The ultraviolet B (UVB) pain model is a commonly used model for studying inflammatory pain, as its effects are sensitive to anti-inflammatory analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs).[43,48] This model consists of exposing a patch of skin to UVB irradiation, which leads to a localised reduction in the heat pain threshold due to inflammation; this phenomenon is known as heat allodynia. Typically, three times the minimal erythema dose (3MED) is used to induce hyperalgesia.[3,16-18,20,28,29,34,44] Although some groups reported the use of 1x (1MED) and 2x (2MED) the minimal erythema dose.[6,24,25]

From 2012 to 2015, our research centre applied the 3MED UVB model in six studies involving a total of 142 subjects. Beginning in 2015 onwards, some subjects started to report hyperpigmentation of the area of skin that was exposed to UVB, which lasted longer than expected (Fig. 1). This postinflammatory hyperpigmentation (PIH) is an acquired form of hypermelanosis that can occur after skin inflammation and/or injury. Although PIH can occur in all skin types, it is generally more common among individuals with skin of colour, including African Americans, Hispanics/Latinos, Asians, Native Americans, Pacific Islanders, and persons of Middle Eastern descent.[14] A wide range of aetiologies have been described, including skin diseases (*e.g.*, acne vulgaris, atopic dermatitis, impetigo, plaque psoriasis, and lichen planus), bacterial, fungal, and viral infections, allergic reactions, medication-induced PIH, and cutaneous injuries as a result of topical irritants, sunburns and other types of burns, and cosmetic procedures.[45]

Hyperpigmentation results from either melanin overproduction or the irregular dispersion of melanin (*i.e.*, pigmentary incontinence) following cutaneous inflammation.[14] Pigmentary incontinence results from the destruction of the basal cell layer,[30] which allows macrophages to accumulate in the upper dermis, where they phagocytise degenerating basal keratinocytes and melanocytes. The release of melanin from these melanocytes is believed to result in hyperpigmentation.[45] Although occasional exposure to sunlight has been associated with a reduced risk of skin cancer,[26] cumulative exposure to solar UV radiation – particularly UVB radiation – is a major risk factor for developing basal cell carcinoma, squamous cell carcinoma, melanoma, and cataract, and should therefore not be taken lightly.[4,5,13,23]

Based on our finding that the 3MED UVB model appeared to be associated with long-term PIH in some patients, it was decided to perform two studies. The aim of the first study was to measure the prevalence of PIH among all subjects who were previously exposed to the 3MED UVB inflammation model at our centre. The second study was designed to evaluate the short-term tolerability and efficacy of inflammation using two times the minimal erythema dose (2MED) in order to test whether this lower amount of UVB exposure is associated with a lower frequency and/or severity of PIH in healthy subjects.

METHODS

Both studies were conducted at the Clinical Research Unit of the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands. Both studies were conducted in accordance with the Declaration of Helsinki and its amendments and in accordance with the Guidelines for Good Clinical Practice. Both protocols were approved by the Medical Ethics Committee approval prior to their initiation; Study I was approved by the Medical Ethics Research Committee of Leiden University Medical Centre (Leiden, the Netherlands), and Study II was approved by the Foundation BEBO (Stichting Beoordeling Ethiek Biomedisch Onderzoek) in Assen, the Netherlands. The studies were registered under ToetsingOnline number NL60563.058.17 (Study I) and NL63598.056.17 (Study II).

Study I

Study design

In this observational study, all 142 subjects who previously participated in the studies CHDR0729, CHDR1311, CHDR1422, CHDR1425, CHDR1431, and CHDR1440[21,29,34,49] and were exposed to 3MED UVB irradiation were contacted and invited to visit our clinical research unit in order to evaluation the area(s) that were exposed to UVB irradiation. In order to maximise the number of respondents, the subjects were given the option to complete the questionnaire (see below) at home and provide a self-made photograph of the skin.

All participants provided written informed consent. The evaluation included a medical interview, a physical examination of the exposed area(s) where

applicable (see Fig. 3), completion of the Dermatology Life Quality Index (DLQI) questionnaire, and a photo assessment of the exposed area(s). A standardised set of photographs of the exposed area(s) was taken using the same lighting conditions using Bodystudio ATBM (FotoFinder Systems GmbH, Birnbach, Germany). All photographs were taken with a QPcard 201 attached to the subject's skin for colour correction using QPcolorsoft software, followed by analysis using ImageJ software (NIH, Bethesda, MD).

After this initial assessment, all subjects who presented with PIH were invited to return to our facility for follow-up assessment of PIH every 6 months. However, insufficient numbers of subjects returned for these follow-up visits; therefore, these data are not presented.

Statistical analysis

The role of potential risk factors on the occurrence of hyperpigmentation was assessed using a set of patient characteristics and study-specific variables, which were identified based on clinical considerations and included skin type measured using the Fitzpatrick scale, gender, ethnicity, study enrolment, and baseline MED. We then calculated the frequency of subjects with hyperpigmentation and the frequency of subjects without hyperpigmentation in the various risk factor categories.

Study II

Study design

This study was designed to determine the efficacy of using the 2MED UVB irradiation paradigm to induce hyperalgesia, and to assess the prevalence of PIH in subjects exposed to the 2MED UVB paradigm. Each subject visited the clinical research unit for general medical screening and to assess the minimal erythema dose applied to a region of the skin on the subject's back; this visit was performed 3-28 days prior to application of the 2MED UVB paradigm. During the clinical study 2MED was administered, and hyperalgesia was monitored over the following 36 hours. The subjects then returned to the clinical research unit for two follow-up visits (6 weeks and 6 months after 2MED exposure), during which the irradiated area was inspected visually and PIH was assessed.

All 18 healthy subjects provided written informed consent. The subjects were divided into three groups containing six subjects each; each group followed a specific measurement protocol in which no measurements were performed for several hours in order to ensure a period of undisturbed sleep (Fig. 2). The aim was to minimise the burden placed on the subjects while obtaining the most complete overview of the effects of 2MED UVB with respect to heat pain threshold over time. This design ensured that for each hour after UVB exposure, at least 12 subjects were scored with respect to their heat pain detection threshold (PDT).

Subject selection

After screening and application of the inclusion and exclusion criteria, 18 subjects (9 males and 9 females) 18-45 years of age were determined to be eligible and were included in the study. The following exclusion criteria were applied: any current clinically significant medical condition that would have affected sensitivity to pain; history or presence of PIH; the use of concomitant medication (except contraception); dark skin colour (Fitzpatrick skin type IV-VI); widespread acne, freckles, tattoos, and/or scarring on the back; and an MED >355 mJ/cm^2 at screening.

UVB model

During the screening visit, UVB irradiation was applied using a narrow-band UVB (TL01) lamp (Philips). To determine the MED, six ascending doses (corresponding to increasing duration of irradiation) were applied to separate 1 cm × 1 cm areas of skin on the subject's upper back. This dosing schedule is based on the average MED of various skin phototypes as reported by Sayre et al.,[40] and ranged from 64 mJ/cm^2 to 1321 mJ/cm^2. Twenty-two to twenty-six hours after exposure to the 6 UVB doses, the skin's erythemic response was assessed, and MED was determined visually based on the lowest UVB dose that produced clearly discernible erythema.

During the clinical study, 2 times the individual subject's UVB MED (*i.e.*, 2MED) was applied to the skin over the right scapula prior to the first pain task: the subjects in group 1 received UVB irradiation 8 hours prior to the first pain task, and groups 2 and 3 received UVB irradiation 1 hour prior to the first pain task (see Fig. 2). The area of irradiated skin was 3 cm × 3 cm, which matched the dimensions of the thermode used to determine the heat pain threshold (see Fig. 3).
Inflammatory pain was assessed first on a patch of control skin contralateral to the site of UVB irradiation, followed by the UVB-irradiated site. Heat PDT was measured using a 3 cm × 3 cm TSA-II thermode (Medoc Ltd., Ramat Yishai, Israel). During the test, the thermode temperature started 32°C and increased linearly by

0.5°C/s until the subject reported that the stimulus was first perceived as painful by clicking a mouse button. During each assessment, the average PDT measured using three stimuli was calculated. The schedule for assessing PDT in groups 1, 2, and 3 is shown in Fig. 2.

Statistical analysis

Heat PDT was analysed using a mixed-model analysis of variance with the following fixed factors: treatment (non-irradiated vs. UVB-irradiated), time and treatment by time, random factor subject, repeated factor time within subject by treatment with a first order autoregressive variance/covariance structure and the pre-value as covariate. The difference in PDT between non-irradiated skin and UVB-irradiated skin was calculated within each time point in the model.

The 2MED and 3MED (extracted from four previous studies at CHDR) heat PDT data were analysed using a mixed-model analysis of variance using the following fixed factors: treatment (2MED vs. 3MED), time and treatment by time, random factor subject, and a repeated factor time within subject with a first order autoregressive variance/covariance structure. The difference in PDT between the 2MED and 3MED groups was calculated within the model using the 8 post-UVB time points that were common to both the 2MED and 3MED groups (23, 25, 26, 27, 28, 30, 32, and 34 hours post-UVB irradiation).

RESULTS

Study I

Six studies conducted in 2012 to 2015 were included in the analysis, comprising a total of 142 subjects (37 women and 105 men). Five of the male subjects participated in two studies. The characteristics of these 142 subjects are summarised in Table 1.

Of the 142 subjects that were contacted, a total of 78 subjects (54.9%) responded; 6 of these respondents opted to participate from home, and 72 respondents visited our clinic. The mean (SD) age of the respondents was 27.8 (±7.2) years (range: 19-50 years). Forty-two of the participating subjects (53.8% of respondents) had PIH; the mean age of the participants with PIH was 27.2 (±6.8) years (range: 19 48 years). Table 1 summarises the prevalence of PIH by ethnicity, gender, MED, Fitzpatrick skin type, and time since UVB irradiation. Our analysis revealed that gender, ethnicity, and Fitzpatrick skin type were not associated with the prevalence of PIH. However, the remaining study variables were associated with the prevalence of PIH. The prevalence of PIH was the lowest among the subjects in first study group (CHDR0729) and increased with each subsequent study (data not shown). In addition, the MED dose (determined at the initial screening) was generally correlated with the prevalence of PIH.

Overall, the mean total DLQI score among all responding subjects was 2.1±2.8 (range: 0-15). The mean DLQI score for the subjects with PIH was 2.7 ± 3.3 (range: 0-15) and mean score for the subjects without PIH was 1.4±2.0 (range: 0-9). The distribution of DLQI scores among the participants is summarised in Table 2.

Study II

A total of 18 subjects (9 males and 9 females) completed the study and were included in the final analysis. The mean age of the subjects was 27.1±7.0 years (range: 20-41 years). The characteristics of the subjects in this study are summarised in Table 3.

Before UVB exposure, the baseline mean PDT on the skin for control (non-irradiated) and test (irradiated) skin was 44.0±3.6°C and 43.7±4.1°C, respectively. Analysis of the primary endpoint (heat PDT at the irradiated area versus the contralateral non-irradiated area) revealed a significant difference beginning at 3 hours post-irradiation (estimate of the difference: 1.58°C, 95% CI: 0.26-2.90, p=0.0188) onwards; this difference remained significant through the final measurement at 36 hours post-irradiation. Beginning 13 hours after irradiation, the LSMEANS estimate of the difference in heat PDT relative to baseline in the irradiated and non-irradiated skin patches ranged from -2.6°C to -4.45°C (p<0.0001). Fig. 4 shows a time course of the LSMEANS estimates in the irradiated and non-irradiated patches.

The time course for the change in PDT relative to baseline following 2MED and 3MED is presented in Fig. 5, which shows that UVB irradiation with 2MED caused in qualitatively similar hyperalgesia compared to 3MED exposure at the same time points; 24-36 hours after irradiation, the average change in PDT following 2MED irradiation was approximately 3-4°C, compared to an average change of approximately 6°C in the 3MED group.

Lastly, we performed a physical examination and examined the photographic data in order to evaluate the incidence of PIH in all 18 subjects both 6 weeks and 6 months after 2MED UVB irradiation. At the 6-week time point, 11 out of 18 subjects (61.1%) had either minimal (5 subjects), mild (4 subjects), or moderate (2 subjects) hyperpigmentation at the irradiated area. After 6 months, 5 of the 18 subjects (27.8%) had either minimal (1 subject) or mild (4 subjects) hyperpigmentation.

DISCUSSION

This study demonstrates that long-term effects of the UVB model using the 3MED paradigm poses a major problem for the subjects. Retrospective analysis and assessment of 142 healthy subjects in 6 previous studies revealed that the prevalence of long-term PIH in subjects exposed to 3MED UVB for inducing transient hyperalgesia model can be as high as 53.8%. This relatively prevalence of PIH may be an overestimation, as patients with PIH may have been more likely to respond to request to participate in a survey of PIH compared to patients without PIH. On the other hand, even our most conservative estimate of nearly 30% is meaningful, and should be considered unacceptable for a model that should not have unwanted, long-lasting, and possibly even permanent side effects in healthy subjects.

To the best of our knowledge, such a high prevalence of long-term UVB-induced PIH has not been reported previously. One study that evaluated long-lasting molecular changes in the skin of subjects after repetitive UV irradiation reported one subject who developed hyperpigmentation 520 days after repetitive, cumulative UV exposure.[9] The high prevalence in our cohort, combined with the paucity of reports regarding UVB-induced PIH, suggests the possible under-reporting of long-lasting side effects in healthy subjects. The significant and widespread under-reporting of adverse drug reactions is a well-known phenomenon;[22] in contrast, virtually no data is available with respect to adverse reactions associated with the UVB inflammation model. Several factors may contribute to this phenomenon. First, an assessment of harm by clinicians may not necessarily represent the subjects' experience. Second, even if harm is detected, it may not be reported appropriately by the investigators, or its reporting may be influenced by the study sponsors, particularly in the case of commercial sponsors. Finally, short-term follow-up might not be adequate to detect potential long-side effects.[41] This last fact may be particularly relevant here. The under-reporting of long-term side effects can prevent researchers from learning from these incidents in order to improve both the safety of study subjects and the design of future studies.

UVB-induced inflammation and PIH are complex processes involving both molecular and cellular changes that lead to the overproduction of melanin and/or the irregular dispersion of pigment following inflammation. Mechanistically, the release of prostanoids, cytokines, chemokines, and other inflammatory factors is stimulated in both UVB-induced inflammation and PIH.[14] Moreover, several studies found the leukotrienes C4 and D4, prostaglandin E2, prostaglandin D2, thromboxane-2, interleukin-1 (IL-1), IL-6, tumour necrosis factor alpha (TNF-α), epidermal growth factor, and reactive oxygen species such as nitric oxide[10,35,45,46] have melanocyte-stimulating properties. Pro-inflammatory cytokines such as IL-1 also increase the expression of bradykinin, a potent algogenic compound that is produced following tissue injury and may mediate UVB-induced hyperalgesia and pain.[15,32] Bradykinin-induced pain and erythema are mediated in UVB-inflamed skin, possibly via an up-regulation or de novo expression of receptor proteins.[15,36] For example, the bradykinin receptor is sensitised by inflammatory mediators, particularly prostaglandins,[38,47] which are produced in UVB-inflamed skin,[12,42] ultimately causing the sensitisation of cutaneous nociceptors.[27,37] Furthermore, Andersen et al. systematically examined the correlation between an UVB dose and inflammation,[2] however, some details regarding the underlying mechanism are still unknown, and the notion that a higher UVB dose increase the molecular and/or cellular changes in the dermis that lead to PIH warrants further investigation.

Although most of the subjects with PIH in the observational study reported that their daily life was not severely affected by the hyperpigmented area(s), many reported that several aspects were affected, including self-consciousness, social well-being, and interpersonal relationships. Nevertheless, based on the DLQI scores, the PIH had less of an effect compared to other hyperpigmentation-related disorders.[31] Although analysis of possible risk factors for hyperpigmentation, including ethnicity, skin colour and heat hyperalgesia, did not reveal a clear correlation between these factors and the prevalence of PIH, it was found that generally speaking subjects with a high MED had a higher risk of PIH; in addition, the prevalence of PIH generally decreased with increasing time following UVB exposure. Several epidemiological studies found that PIH tends to occur more frequently among dark-skinned individuals compared to individuals with lighter skin tones.[1,11,19] This study could not confirm these results, as ethnicity and Fitzpatrick skin type were not distributed evenly among the subjects.

In study II subjects were excluded with a MED score > 355 mJ/cm² and a Fitzpatrick skin type IV. The MED was multiplied by two instead of a threefold in order to try to keep the occurrence of PIH to a minimum. No cases of PIH have been described in literature when using the 2MED UVB model. Validation of the 2MED UVB model confers hyperalgesia is consistent with previous studies using the same paradigm.[8,24,25,39] We also found that 2MED UVB model induced primary hyperalgesia as early as 3 hours after irradiation, and this response was relatively stable for up to 36 hours after irradiation.

Compared to 3MED UVB, 2MED UVB caused slightly less pronounced hyperalgesia, which was reflected by a difference in average PDT of approximately 2°C

between 2MED and 3MED; this finding is consistent with previous reports of dose-dependent sensitisation of cutaneous nociceptors.[7,8,18,20] However, the relatively high prevalence of long-term PIH in the 3MED cohort indicates that the 3MED model should be used with caution, as its use can lead to a negative risk-benefit balance. Nevertheless, the UVB inflammation model is an established model for inducing cutaneous hyperalgesia, making it valuable for use in studies designed to investigate the investigating effects of analgesics in the setting of hyperalgesia.[29,34,44,49] Importantly, this models provides a consistent level of efficacy with low inter-subject variability (unpublished data) and high test-retest reliability.[33] Given these advantages, the UVB model is also considered suitable for modelling inflammatory pain and is therefore used to measure the effects of non-steroidal anti-inflammatory drugs (NSAIDs).[8,48]

Because of the study design, the effect of analgesics on hyperalgesia induced by 2MED UVB exposure was not examined. Thus, an important question that remains to be addressed is whether or not the slightly lower hyperalgesia induced by 2MED is still sufficient to test the efficacy of analgesic compounds. To date, one study used the 2MED paradigm and was able to demonstrate the efficacy of a combination of paracetamol and ketorolac in reducing hyperalgesia 25 Finally, one of the main reasons to execute this study was to develop a valid pain model with minimal risk of hyperpigmentation. The prevalence of PIH 6 months after exposure to 2MED is similar to the prevalence in the 3MED group after a follow-up between 901-1128 days. As the prevalence of PIH among the subjects exposed to 3MED declines over time as demonstrated by the lower prevalence of PIH among subjects with a longer time since exposure than among patients with a shorter time since exposure (see Table 1), it is to be expected that PIH will fall well below 20%. To test this hypothesis, follow up will continue for subjects in whom hyperpigmentation was still present 6 months after exposure.

In conclusion, our observational study revealed that long-lasting PIH is relatively common among healthy subjects previously exposed to 3MED UVB irradiation, providing the first report of this adverse side effect in association with this model. Given that 2MED UVB is associated with a reduced prevalence of PIH yet still produces stable hyperalgesia, this model should be tested for use in evaluating the efficacy of analgesics in the early stages of development.

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Table 1 – Summary of the subjects included in the observational study involving subjects following 3MED UVB exposure.

	Total cohort, N (%)	Responders, N (%)	Responders with PIH, N (%)
Subjects	142 (100)	78 (100)	42 (53.8)
GENDER			
Female	37 (26.1)	21 (26.9)	11 (52.4)
Male	105 (73.9)	57 (73.1)	21 (54.4)
ETHNICITY			
Caucasian	122 (85.9)	67 (85.9)	35 (52.2)
Non-Caucasian	20 (14.1)	11 (14.1)	0 (0)
FITZPATRICK SKIN TYPE			
I	2 (1.4)	1 (1.3)	0 (0)
II	25 (17.6)	10 (12.8)	5 (20.0)
III	75 (52.8)	48 (61.5)	28 (58.3)
IV	40 (28.2)	19 (24.4)	9 (47.4)
TIME SINCE IRRADIATION (DAYS)			
500 -750	25 (17.6)	18 (23.1)	12 (66.7)
751-1000	69 (48.6)	37 (47.4)	27 (73)
1001-1250	39 (27.5)	19 (24.4)	7 (36.8)
>1751	9 (6.3)	4 (5.1)	0 (0)
MED (MJ/cm^2)			
251	1 (0.7)	0 (0)	0 (0)
256	1 (0.7)	1 (1.3)	0 (0)
351	4 (2.8)	3 (3.8)	1 (33.3)
355	7 (4.9)	4 (5.1)	1 (25.0)
362	1 (0.7)	0 (0)	0 (0)
467	5 (3.5)	2 (2.6)	1 (50.0)
496	23 (16.2)	13 (16.7)	8 (61.5)
502	9 (63)	2 (2.6)	1 (50.0)
660	17 (12.0)	10 (12.8)	5 (50.0)
702	27 (19.0)	18 (23.1)	11 (61.1)
710	4 (2.8)	2 (2.6)	2 (100)
934	14 (9.9)	8 (10.3)	4 (50.0)
993	22 (15.5)	13 (16.7)	10 (76.9)
1321	7 (4.9)	2 (2.6)	2 (100)

MED: minimal erythema dose; PIH: postinflammatory hyperpigmentation.

Table 2 – Measurement of dermatology quality of life index. Calculations made by summing the score of each question resulting in a maximum of 30 and a minimum of 0. The higher the score, the more the quality of life is impaired.

Sum of the DLQI scores	Total responding group		PIH +		PIH -	
	N	%	N	%	N	%
0-1 'no effect at all on patient's life'	49	62.8	23	56.1	23	74.2
2-5 'small effect on patient's life'	20	25.6	11	26.8	6	19.4
6-10 'moderate effect on patient's life'	8	10.3	6	14.6	2	6.8
11-20 'very large effect on patient's life'	1	1.3	1	2.4	0	0
21-30 'extremely large effect on patient's life'	0	0	0	0	0	0

DLQI: Dermatology Quality of Life Index; N: number; PIH+: subject with postinflammatory hyperpigmentation; PIH–: subjects without postinflammatory hyperpigmentation.

Table 3 – Summary of subject characteristics in Study II. Body mass index was defined as weight/(height × 0.01)².

Number of subjects	18
GENDER	
Female	9
Male	9
AGE	
Mean (SD)	27.1 (6.8)
Range	20 – 41
ETHNICITY	
White	17
Mixed	1
FITZPATRICK SKIN TYPE	
II	14
III	4
MED (MJ/CM²)	
251	1
351	5
355	12
WEIGHT (KG)	
Mean (SD)	74.5 (14.4)
Range	49.4 – 95.4
HEIGHT (CM)	
Mean (SD)	176.1 (11.8)
Range	157.9 – 193.8
BMI	
Mean (SD)	23.8 (2.4)
Range	19.6 – 27.9

BMI: Body mass index; mj/cm²: millijoule/square centimetre; SD: standard deviation.

Figure 1 – Example images of two subjects in the observational study with post-inflammatory hyperpigmentation at the irradiated areas (arrows). The images in the top row were taken 18, 24, and 30 months after UvB irradiation in one subject. The images in the bottom row were taken 6, 12, and 18 months after UvB irradiation in a different subject.

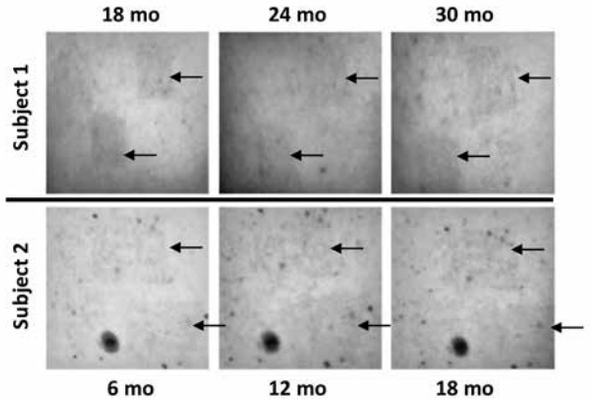


Figure 2 – Schematic showing the timing of the PDT measurements obtained in the second study. Eighteen subjects were randomly assigned to three groups. The light shaded boxes indicate PDT measurements.

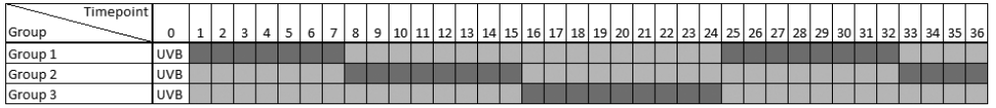


Figure 3 – Schematic diagram showing the approximate location of the regions used to determine the MED and the irradiated and non-irradiated regions used to induce hyperalgesia. To determine the MED, six 1 cm² patches of skin were irradiated at increasing doses. After MED was determined, a separate 3 cm × 3 cm patch of skin was irradiated; a non-irradiated patch of skin on the contralateral side was used as a control.

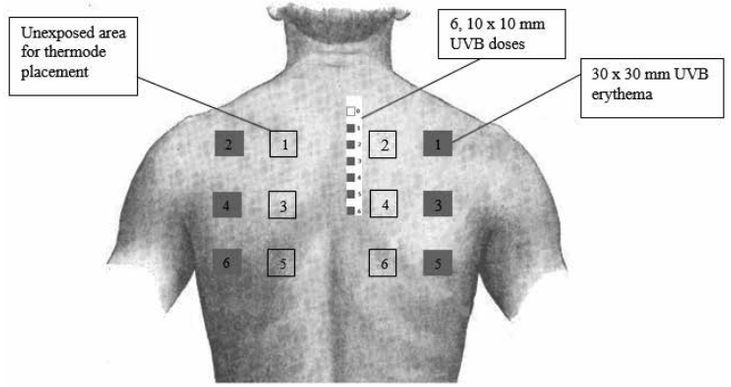


Figure 4 – Time course of change in pain detection threshold (PDT) following 2MED UVB exposure. The change in PDT was measured in both the irradiated and non-irradiated areas and is expressed relative to baseline. The data are expressed as the least square means with 95% CI.

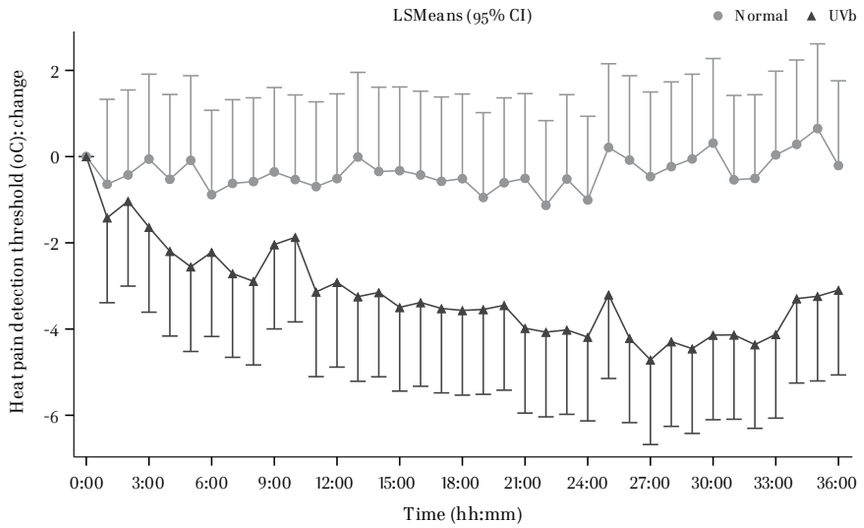
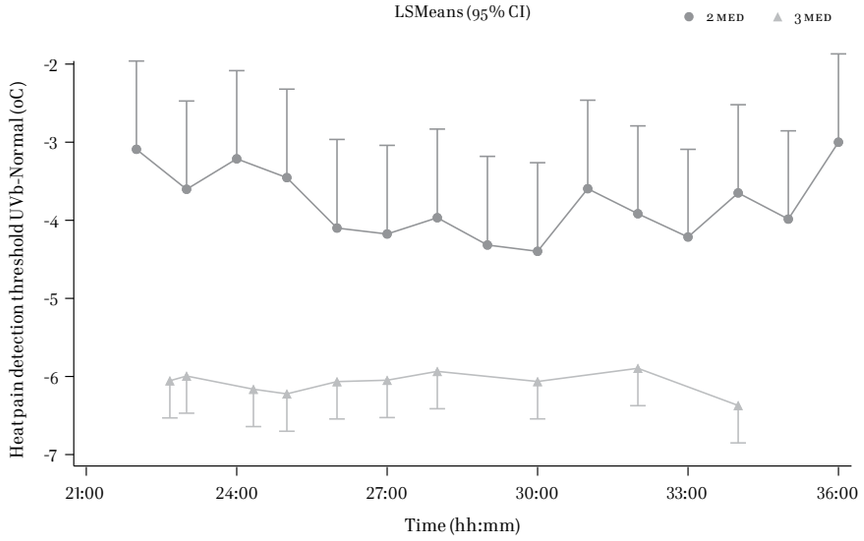


Figure 5 – Time course of the difference in PDT between the irradiated area and the control non-irradiated area following either 2MED or 3MED UVB exposure. The data are expressed as the least square means with 95% CI.



Chapter 8

THE CAPSAICIN-INDUCED HYPERALGESIA MODEL: VALIDATION AND INCORPORATION IN A MULTI-MODAL NOCICEPTIVE AND HYPERALGESIA PAIN TEST BATTERY TO DETECT ANALGESIC EFFECTS OF DRUGS IN HEALTHY SUBJECTS

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INTRODUCTION

Continuing efforts are made to improve pain models, for example by simulation of clinical pain symptoms, such as hyperalgesia.[1-21] A frequently used hyperalgesia model is the capsaicin-induced hyperalgesia/allodynia model, in which application of the red chili pepper extract capsaicin induces a temporary hyperalgesia, resulting in a lower heat pain threshold, and a temporary mechanical allodynia.[5-7,19,21-23,26,28,37,51] Capsaicin is a highly selective agonist for transient receptor potential cation channels subfamily V member 1 (TRPV1). TRPV1 channels are transducers of physically and chemically evoked sensations.[29] The vanilloid 1 subtype is activated by noxious heat (≥ 43°C) and is expressed on C-fibers and on a subset of Aδ-fibers.[30,31] The transient effects of applying topical capsaicin are burning sensations, hyperalgesia, allodynia, and erythema. By periodically heating the zone of primary hyperalgesia to a non-painful temperature (prekindling and rekindling), a longer-lasting hyperalgesia can be maintained. Previous studies have shown that the topical application of capsaicin induces peripheral sensitization by primary mechanical allodynia/thermal hyperalgesia.[7,37] Primary hyperalgesia is caused by modulation of peripheral afferents and is therefore restricted to the site of injury, *i.e.*, peripheral sensitization. Capsaicin may also induce central sensitization by secondary mechanical allodynia, an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input (secondary hyperalgesia/allodynia). The secondary hyperalgesia is thought to be a transient state of central sensitization where an increase in excitability of the dorsal horn neurons is induced, which can also be caused by nerve injury.[7,24] In addition, it triggers the release of pro-inflammatory agents at peripheral terminals, such as substance P and calcitonin gene-related peptide (CGRP).[32,33]

Thermal primary and secondary hyperalgesia, after application of capsaicin, can be evaluated via laser evoked potentials (LEP) using an EEG recording system. Laser stimulation (LS) uses energy to heat up the epidermis and parts of the dermis with brief and powerful stimuli.[34] This type of stimulation causes a characteristic double pain sensation, consisting of an initial sharp pinpricking-like pain (Aδ-fibers) and a second longer burning pain (C-fibers).[35-36] LS can stimulate the skin in a well-reproducible manner making it useful as a tool to elicit evoked potentials. Evoked potentials are monophasic deflections of spontaneous EEG and are time and phase locked on the onset of the stimuli.[27] These waveforms are typically characterized by their polarity, latency, amplitude, and measurement position on the scalp. Evoked potentials via EEG can be registered due to the brief nature of the stimulus of which the timing can be controlled and may provide important information on (central) pain processing.[25-27] Combined LS and EEG registration may quantify the pain stimulus in a more objective manner.

The use of multimodal, multi-tissue pain testing is a strategy to improve the predictive value of evoked pain models in clinical drug development. Effect outcomes, however, are only valuable when the pain models don't interfere with each other. The aim of the present study was to evaluate the validity and reliability of the capsaicin-induced hyperalgesia model. The model was evaluated in a multimodal pain test setting to determine whether the capsaicin-induced hyperalgesia model can contribute to the analgesic profile of the drug tested without influencing the other pain models.

METHODS

Study design and study drugs

This was a two part study which is registered in the European Clinical Trial database under No. 2017-000480-32. Part A was a method validation study of the capsaicin-induced hyperalgesia model. Part B was a double-blind, single dose, randomized, placebo-controlled, 3-way cross-over study. The latter study was setup to establish the usefulness of the capsaicin model, when used in the context of the pain test battery, to detect the effects of drugs used in the treatment of neuropathic pain. For both Part A and B screening occurred between 28 and 8 days before first study drug administration.

In Part A of the study, subjects were to attend the clinic on 2 occasions, with a wash-out period of at least 7 days. Subjects were divided in to 2 groups: those who were to receive pre-/rekindling (rekindle) or those who were not to receive pre-/rekindling (no-rekindle). Both groups followed the same schedule of assessments. Prekindling was performed immediately before application of the capsaicin patch (duration: 5 min; temperature: 45°C), rekindling was done immediately before the contact heat stimulation on the capsaicin treated skin at 0.5, 3, 5 and 8 hours post-capsaicin application (duration: 5 min; temperature: 40°C).

In Part B subjects were to attend the clinic on three separate occasions, with a wash-out period of at least 7 days. Each subject received on one occasion a single dose of duloxetine 60 mg, tramadol 100 mg, or placebo. Both tramadol and duloxetine are known to be effective in the treatment of neuropathic pain and are being used in the clinic for this indication.[38-41] The Pharmacy Department of

the Leiden University Medical Center prepared the study treatments and over-encapsulated all treatments to ensure blinding. A battery of human evoked pain models was used to demonstrate analgesic properties.

The study was approved by the Medical Ethics Committee Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki.

Study participants

A total of 20 healthy male subjects (1:1 ratio rekindle: no-rekindle) between 18 and 45 years of age, were to be included in the study in Part A and 18 healthy male subjects in Part B. All subject provided written consent prior to any study activities. Subjects with a Fitzpatrick skin type I-IV, without widespread acne, tattoos or scarring on the volar forearms and who were willing and able to comply with all scheduled visits were included. Subjects indicating a NRS (0-10) > 8 after removal of the capsaicin or < 3 after LS were excluded. In part B, subjects were also excluded when they were not able to tolerate nociceptive assessment at screening or those who did not achieve tolerance at >80% of maximum input intensity for the mechanical, electrical or cold thermal nociceptive assessments, or who had less than 1°C decrease of the average pain detection threshold (PDT) between control and capsaicin treated skin during the contact heat pain model. Any condition that would affect sensitivity to pain or cold, or a confirmed significant allergic reaction (urticaria or anaphylaxis) to duloxetine or tramadol, or multiple drug allergies, would lead to exclusion.

Evoked pain tests

Primary hyperalgesia to heat (contact heat and LEPs) and secondary hyperalgesia and mechanical allodynia (LEPs and pin-prick) were evaluated in the capsaicin-induced hyperalgesia/allodynia model in Part A. Only contact heat and LEPs (primary hyperalgesia) were assessed in Part B. For these assessments, a 3x3 cm surface on the right volar forearm was used for the application of occlusive topical 1% capsaicin cream FNA (Formulary of Dutch pharmacists) for 30 minutes, whereas the left volar forearm served as a non-sensitized control.

Pain detection thresholds to contact heat were measured using a 3x3 cm contact thermode (TSA-II, Medoc Ltd., St. Ramat Yishai, Israel) on the capsaicin treated skin and control area (initial temperature 32°C, ramp 0.5°C/s, cut-off 50°C, average of 3 stimuli). Measurements were performed twice pre-dose (baseline measurements were performed once before capsaicin application and once after capsaicin removal. Both baseline measurements were performed before study drug administration), and 0.5, 1, 3, 5, 8 and 10 hours post-capsaicin in Part A, and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-study drug administration in part B.

LEPs were recorded in a in a quiet room with minimal illumination. Both subject and investigator wore protective goggles. Laser stimuli (5 ms stimulus duration, 5 mm diameter, 2 Joules, random stimulus intervals of 6-8 seconds) were generated by a ND:YAP laser (neodymium:yttrium-aluminium perovskite, STIMUL 1340, Electronic Engineering). To avoid skin damage and nociceptor sensitization/habituation, the site of stimulation was moved after each laser stimulus.[25,45-46] LS was always performed on the secondary area first, then control area and last on the primary area in Part A, and on the control area first and then on the capsaicin treated area in part B. 20 stimulations were administered per location per stimulus block and were performed twice pre-dose (baseline measurements were performed once before capsaicin application and once after capsaicin removal. Both baseline measurement were before study drug administration), and 1, 3, 6 (part B only) and 8 hours post-dose. After each block of stimulations the subject was asked to indicate the most painful moment during this series on a numeric rating scale (0-10 with 0= 'no pain' and 10= 'worst pain imaginable'). LS was recorded using an EEG system (TMSi Polybench, Twente Medical Systems international (TMSi), Oldenzaal, the Netherlands). EEG data was collected with a cap using the 10-20 system. In order to minimize artefacts on the EEG recording, subject were instructed to focus, stay relaxed and keep the eyes open as much as possible. A reaction button had to be pushed as fast as possible after a stimulus was felt. Measurement parameters included the amplitude (μV) and latency (ms) of N2, P2 and N2P2 peaks.

Secondary allodynia was measured via mechanical pin-prick assessment with Von Frey filaments (Part A only). At each visit pre-capsaicin application, individualized pain perception was evaluated using Von Frey filaments ranging from 128-512 mN. The filament where the subject reported 'nearly painful' was then used to determine the area of secondary mechanical allodynia. Eight spokes dividing a circle in equal parts were used to assess secondary allodynia. Stimulation started 3.5-4 cm from the center of the primary area moving to the middle with steps of 5 mm. Once a change in sensation was felt, from nearly painful to painful, that point was determined to be the border of the secondary allodynia area. To minimize the risk of measuring false positive central sensitization, the primary area

was surrounded by a border of 5 mm in which no secondary allodynia measurements was performed. Due to a high variation and a relative high non-responder group in Part A of the study, analysis of this paradigm was not possible.

Part B nociceptive tasks were integrated in a range of pain assessments measuring different modalities of pain on different tissues, now included with the capsaicin-induced hyperalgesia model (no-rekindle), see Figure 1. The electrical stimulation, pressure stimulation and cold pressor assessment have been described previously.[42-44] The battery of evoked pain tasks also consisted of a paradigm quantifying conditioned pain modulation (CPM). These assessments were conducted twice pre-dose (double baseline) and 1, 2, 3, 4, 6 and 10 hours post-dose by trained personnel. A training session was included as part of the screening examination to familiarize subjects with the procedure. To eliminate the risk of tissue damage, all pain tasks have a maximum safety cut-off.

Statistical analysis

Part A and B were analyzed separately.
Repeatedly measured pharmacodynamic data of Part A were analyzed with a mixed model analysis of covariance with fixed factors group, treatment, visit, time, group by treatment, group by visit, group by time, treatment by visit, treatment by time, visit by time, group by treatment by visit, group by treatment by time, group by visit by time, treatment by visit by time and group by treatment by visit by time and random factors subject, subject by treatment, subject by visit and subject by time and the average pre-value as covariate.

Repeatedly measured pharmacodynamic data of part B were analyzed with a mixed model analysis of variance with the fixed factors treatment, period, time and treatment by time, random factors subject, subject by treatment and subject by time and the average pre-value as covariate. Within each pain model the contrast of tramadol and duloxetine compared with placebo was calculated over the full testing period (0 -10 hours). Per variable results were generated with estimates of the difference of the different contrasts and a back transformed estimate of the difference in percentage for log transformed parameters, 95% confidence intervals (in percentage for log-transformed parameters) and Least Square Means (LSMeans) (geometric means for log transformed parameters), and the p-value of the contrasts.

All calculations of the pharmacodynamic parameters were performed using SAS for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA). The main SAS procedure that was used in the analysis was "PROC MIXED". No adjustments for multiple comparisons were employed.

The test-retest reliability of the PDTS for the contact heat pain model was calculated using intra-class correlation (ICC). Calculations were based on all measurements performed on the control area of all subjects in Part A, and on all baseline measurements on the capsaicin and control area of the subjects in Part A. Assessment of variability included the calculation of the intra-subject coefficient variation (CV) of the PTT for the pressure stimulation, cold pressor and electrical stair of the current study and 4 previous studies conducted with these models: Study I-IV (Eudract no. 2014-003015-12, 2014-003553-34, 2014-004468-39 and 2015-003496-30).[63-66] Least squares means PDTS and PTTS per time point for all 5 studies were calculated.

RESULTS

Demographics and safety

21 male subjects were included in Part A and 18 male subjects were randomized to receive treatment in part B. One subject in Part A was replaced as he developed flu-like symptoms during occasion 1 and was unable to complete the study. This adverse event was not assessed as related to the application of the capsaicin or the study procedures. A total of 38 subjects completed the study and were included in the final analysis. Table 1 shows an overview of the subject characteristics. Table 2 shows an overview of the treatment related adverse events (AE) observed in part B. All AEs were transient and needed no medical intervention. One AE (dizziness) was moderate in intensity, while all others were mild.

Part A

Capsaicin-induced hyperalgesia contact heat model

LSMeans PDTS were measured in the rekindle and no-rekindle groups on each day the subjects attended the clinic. The time course of the heat PDTS is presented in Figure 2. Analysis of the contrasts showed a significant difference between the PDTS on the capsaicin treated skin versus the control area (LSMeans 40.5°C; estimate of the difference -3.85°C; 95%CI-4.45 – -3.26; p<.0001). Between the rekindle and no-rekindle groups no significant differences were observed, neither on the capsaicin treated skin (LSMeans 40.8°C; estimate of the difference 0.69°C; 95% CI -0.74-2.12; p=0.3205) nor on the control area (LSMeans 44.4°C; estimate of the difference 0.08°C; 95% CI -1.35-1.50; p=0.9116). The heat PDT's measured

on occasion 1 and occasion 2 were comparable (LSMeans 42.6°C; estimate of the difference 0.29°C; 95% CI -0.26-0.84; p=0.2886). The ICC on the control area was 0.784, for all baseline measurements 0.813, and for all data points (control and capsaicine) 0.700.

Part B

Capsaicin-induced hyperalgesia contact heat model

The heat PDT values were measured on the capsaicin treated skin and on the control skin (Figure 3A). Tramadol led to a significant increase in PDT for heat pain on the control area (LSMeans 44.9; estimate of the difference 1.08; 95% CI 0.42-1.75; p=0.0023) over the complete period. No significant difference between duloxetine and placebo was detected on the control area (p=0.1375). Neither tramadol nor duloxetine could be shown to lead to significant effects on primary heat hyperalgesia compared to placebo (tramadol: p=0.0753; duloxetine p=0.1915). A summary of the endpoints is presented in Table 3A and a time course profile of the heat PDT on the three treatments occasions is presented in Figure 4.

Laser evoked potentials

On the control area, the placebo parameters were unchanged throughout the study period. Latency was not significantly affected by tramadol or duloxetine compared with placebo. Tramadol had a significant effect compared with placebo on the N2 amplitude (LSMeans -6.98 µV; estimate of difference 1.885 µV; 95% CI 0.61-3.16; p=0.0051), the N2P2 amplitude (LSMeans 15.07 µV; estimate of difference -3.106 µV; 95% CI -5.744 – -0.468; p=0.0226), and the reaction time (LSMeans 427.43 ms; estimate of difference 38.761 ms; 95% CI 13.426-64.097, p=0.0041), on the normal skin. A time course for the N2P2 amplitude has been depicted in Figure 4. Duloxetine had no significant effect compared with placebo on any of the endpoints in the normal skin. Also, there were no statistically significant differences from placebo on any of the endpoints on the capsaicin treated skin for tramadol or duloxetine. Perceptive thresholds after LEP were unaffected for both treatments compared with placebo (p>0.2). A summary of effects (LSMeans, estimate of the difference, 95% CI and p-values) are presented in Table 3B.

Mechanical, thermal (cold) and electrical stimulation models

Time courses and the analgesic profile for each treatment on the primary endpoints are shown in Figure 4 and 5. The results of the analyses for the PTT for the he pressure stimulation, cold pressor and electrical models are presented. Tramadol increased the PTT significantly compared with placebo for the pressure stimulation (LSMeans 50.5; estimate of the difference 18.7%; 95% CI 12.3%-25.5%; p<.0001), cold pressor (LSMeans 31.5; estimate of the difference 37.7%; 95% CI 24.3%-52.5%; p<.0001), and electrical stair model (LSMeans 25.8; estimate of the difference 17.6%; 95% CI 6.6%-29.8%; p=0.0023) over the complete 10 hours of testing. CPM (The delta PTT for electrical pain) effect was not demonstrated by either tramadol (LSMeans 1.71; estimate of the difference -0.241; 95% CI -1.119-0.636; p=0.5791) or duloxetine (LSMeans 1.70; estimate of the difference -0.243; 95% CI -1.127-0.640; p=0.5784). Duloxetine had no significant effect on any of the pain models. The effects of tramadol and duloxetine on evoked pain are depicted in Figure 3B and Table 3A.

Reliability

The average PDT and PTT per time point per study were comparable (Table 4). The pressure stimulation, cold pressor and the electrical stimulation all had PTTs e in the range of the previous studies. The study-to-study variability (current study and studies I-IV)[34-36,45] of the mean PTT in the pressure stimulation, cold pressor and electrical stair were small. The pressure stimulation had a CV of 8.2% in the current study and 13.1%, 12.0%, 12.2% and 13.8% in the previous studies. The cold pressor CV ranged around 15% with an exception in Study I where the variation was higher (14.9%, 22.7%, 15.9%, 12.8% and 15.4%). The variability of the electrical stair was 14.3%, 16.5%, 14.4%, 11.2% and 16.7%.

DISCUSSION

The capsaicin-induced hyperalgesia/allodynia model has been used in numerous experimental and clinical pain studies.[5-7,19,21-23,26,28,37,51] In this study we aimed to incorporate the capsaicin-model in a battery of multimodal pain testing. The validity and reliability of the capsaicin model was evaluated, as well as the analgesic profile of two known analgesics by testing the effects in comparison

with placebo on a range of experimental pain tests. Incorporation of this pain model should lead to a more complete analgesic profile of the compounds under investigation increasing the confidence in dose selection at an early stage of drug development and the probability of success in patient studies.[43,47-50]

Hyperalgesia to heat was robust and lasted for the full 10 hours of testing. This could be contributed to the capsaicin cream which was applied topically and not intra-dermally where skin trauma could influence heat hyperalgesia. It has been suggested that the model becomes more stable over a longer period of time due to a synergistic effect of heat (pre-/rekindling) and capsaicin.[19,21] This synergistic effect was not seen in the present study. Heat PDTs in Part A of the study in the rekindle versus no-rekindle group did not differ from each other on any time point nor between each visit. The rekindle interval in the present study might have been too long for a synergistic effect. Additionally, the two groups were evaluated with contact heat stimulation on the primary area which might have neutralized the synergistic effect. In future studies, it would be advantageous to compare the rekindle effect with a secondary allodynia model, for example with mechanical stimulation on the secondary area. In the present study this measurement paradigm proved to be too variable to analyze. The area of mechanical hyperalgesia did not increase in about half of the subjects, as it would have been expected.[19,26,51] Intra-subject variation within and between days of mechanical stimulation on the secondary area was too high in both groups giving reason to omit the assessment in Part B of the study.

A key learning from the current study is a better understanding of the influence of topical formulations on drug penetration. Capsaicin in cream and ethanol solutions are used to induce hyperalgesia, and intra-dermal injection are used to administer the capsaicin directly in to the target site.[5-7,19,21-23,26,28,37,51] Topical drug delivery depends on the ability to overcome biological barriers. Drug penetration and permeation through the skin are greatly influenced by the structural properties of the skin – mainly provided by the stratum corneum where the corneocytes, in a matrix of intercellular lipids, serve as the primary barrier of the skin, a regulator of water loss and prevent permeation of potentially harmful substances and microorganisms – and the physicochemical properties of the drug, like partition coefficient, molecular size, solubility and hydration.[67] Penetration of the active ingredient through the skin can be challenging, which might also have been the case in the present study. A low drug absorption could have resulted in a lower efficacy, reflected by the absence of secondary hyperalgesia in our study.

Chemical penetration enhancers (CPE) interact with the skin to stimulate drug flux and can be used to increase permeation of the topical drug.[76] CPEs are pharmacologically inactive compounds, or vehicles, that may partition and/or diffuse into the membrane and interact with the stratum corneum.[77] Ethanol is an often used CPE as it can permeate rapidly through the skin and interact with the skin in various ways. Ethanol can increase permeant solubility and delay depletion, resulting in a raise of the flux.[78] It Alters the tissue solubility, improving the drug partitioning into the membrane,[78] and ethanol increases the thermodynamic activity of the drug by a rapid evaporative loss from the application site. As ethanol disappears, the drug concentration may increase beyond the saturated solubility, yielding a supersaturated state with a greater driving force for permeation.[78] With this knowledge it can be expected that a topical formulation of capsaicin in an ethanol solution would have resulted in a better partition and solubility, increasing the efficacy and produce a stable secondary hyperalgesia. This has been confirmed is in studies using an ethanol solution.[5,6,23,25,28]

Demonstrating reliability is a distinctive feature in science as it verifies scientific evidence. In Part A the capsaicin model demonstrated a high between-day reliability assessed via primary hyperalgesia to heat. This is in line with the known literature where reliability of the capsaicin pain model mainly proved to be high.[21,26] Although these studies used intra-dermal injection of capsaicin and reliability was not assessed via primary heat hyperalgesia, but via secondary allodynia with mechanical stimulation[21] or ongoing pain ratings.[26] In opposition to Dirks et al., Geber et al. was not able to confirm the test-retest correlation of the areas of pin-prick hyperalgesia, since it was too poor to reach significance.[21,26] In the present study secondary mechanical allodynia also proved to be too variable. In Part B we incorporated the capsaicin model in the context of a test battery which has been used to determine the analgesic profile of new drugs in previous studies.[63-66] The capsaicin model did not interfere with the other pain models included in the test battery. The PDTs and PTTs of the pain models in the pain test battery were comparable to the ones observed in the previous studies (Study I-IV) conducted without the capsaicin model. This suggests that the newly incorporated model does not affect the results of the existing models which is an essential factor in multimodal pain testing. Several factor should be considered. First, the study design should have a cross-over design including a placebo arm. Sequence of treatment should be randomized (*e.g.*, Williams design). Repeated measures should be performed and analysis should be done with each subject's baseline recording of that study visit to deal with inter-subject variation.[50,52] Additionally, different test locations of each pain model can decrease the chance of interference. The volar forearms were used for the capsaicin-induced hyperalgesia

model, whereas the contralateral hand (compared with arm of capsaicin application) was being used to submerge in water for the cold pressor test, the anterior side of the lower legs were used for the electrical and pressure stimulation was performed on the contralateral lower leg. The latter does not automatically have to be the case since conditioned pain modulation is not limited by stimulus location.

Tramadol and duloxetine each showed a different analgesic profile where tramadol demonstrated an analgesic response on the thermal, electrical and the mechanical models, while duloxetine did not show any significant analgesic effects (Figure 5). Tramadol, the more broadly effective analgesic of the two, produces antinociception mainly by activation of the μ-opioid receptor and inhibition of monoamine reuptake, such as serotonin, but it also has an effect on various G protein coupled receptors, ion channels, and transporters. All mechanisms contribute to the analgesic effect by inhibiting pain transmission in the central nervous system. In literature, tramadol shows analgesic effects on cold[53-56] and heat,[57] electrical[58] mechanical models,[56] and even in chemical models.[59] In contrast to previous statements, where it is believed that opioids mainly attenuate pain intensities above the PDT,[47,54] our study did also demonstrate significant effects on heat PDT.

Duloxetine is a serotonin noradrenalin reuptake inhibitor (SNRI). The antidepressant activity and pain inhibitory property of duloxetine is believed to be related to its potentiation of serotonergic and noradrenergic activity in the central nervous system where it increases descending pain inhibition by inhibiting reuptake of spinal noradrenalin and serotonin.[60,72,73] In humans, descending pain modulation can be evaluated with CPM. The pain stimulus, measured before and after another modulating pain stimulus, usually has an inhibiting effect, at least in healthy subjects.[71,74] In patients with painful diabetic neuropathy, duloxetine can improve CPM efficiency, Although this correlation was only seen in patients with a higher drug efficacy.[75] Neuropathic pain is efficaciously treated by duloxetine, but only in patients with inefficient CPM, and vice versa. Thus, patients that did not, or to a lesser extent, experience pain alleviation from duloxetine, did not show a significant change in CPM.[75] These patients already had an efficient CPM pretreatment. It is expected that the healthy subjects in our study have the same efficient CPM, explaining why there is a lack in significant CPM effects in the current study. An Alternative consideration is whether this specific CPM paradigm produces such a strong effect that it becomes difficult to increase the CPM after administration of a drug. It has been suggested that CPT temperatures between 4°C and 12°C can be sufficient to induce inhibition.[79-83] If so, duloxetine would not have been able to increase the CPM because the ceiling effect has already been reached. Finally, a lack of CPM effect could have to do with the timing of the dose administration which needs careful consideration since the duration of the cutaneous sensitization is fairly short. Recommended is to use compounds with the T_{max} of 2 to 3 hours.[21] However, after ingestion of the duloxetine, there is a median two hour lag until absorption begins because of the enteric coating and the maximal plasma concentrations (C_{max}) of the drug occurs at approximately six hours.[68-69] Due to the pharmacokinetics of duloxetine it is possible that the hyperalgesic effect of the capsaicin might have already been worn off before duloxetine could reach its potential analgesic threshold. In addition, multiple doses of duloxetine administered over a longer period could potentially yield positive results since the analgesic onset only is observed within the first week of administration. In contrast, Schaffler et al.[28] used a similar design with a single-dose of duloxetine and was able to demonstrate a reduction in the peak-to-peak amplitude. Possibly this has to do with the study designs where the present study used a pre-defined fixed stimulation intensity in contrast to individualized intensities or with the type of laser (ND:YAP vs. CO2). Duloxetine has not been studied as extensively as tramadol in human evoked pain models. Three studies reported outcomes where duloxetine was tested in a heat model[28,61] and mechanical models.[62] The latter was tested in patients with central nerve pain and should therefore be interpreted differently than in healthy humans.

CONCLUSIONS

The capsaicin-induced hyperalgesia/allodynia model leads to a stable primary hyperalgesia to heat after a 30 minute application of a 1% capsaicin cream, but not to a stable secondary hyperalgesia/allodynia to heat or mechanical stimulation. The test-retest analysis proved that the between-day variation was low. Moreover, the capsaicin model does not seem to interfere with other pain model after it was incorporated in the PainCart®, giving it validity in multimodal testing. Tramadol significantly affected multiple pain models in the PainCart, but duloxetine could not be demonstrated to affect the pain thresholds. For the detection of analgesic effects of duloxetine, it may be necessary to generate a stable secondary mechanical allodynia. Additional studies will now focus on improvement of the capsaicin model (*e.g.*, topical formulation) in such a way that primary and secondary hyperalgesia are both stable.

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Table 1 – Summary of Demographic Characteristics.

	Part A	Part B
Number of subjects	21	18
Sex	Male	Male
AGE		
Mean (SD)	24.2 (4.9)	26.1 (5.1)
Range	19 – 42	20 – 38
RACE		
White	17	13
Asian	1	0
Hispanic	1	1
Mixed	1	0
Other	1	2
WEIGHT (KG)		
Mean (SD)	78.2 (11.6)	78.0 (10.3)
Range	52.4 – 107.2	64.0 – 102.6
Height (cm)		
Mean (SD)	184.7 (7.3)	185.2 (6.1)
Range	166.7 – 200.4	175.8 – 197.9
BMI		
Mean (SD)	23.2 (2.7)	22.8 (2.8)
Range	18.8 – 29.8	19.4 – 28.6

Body mass index was defined as weight/(height × 0.01)² / SD = standard deviation.

Table 2 – Treatment-related Adverse Events. Subjects were counted only once per treatment in each row. Included all data collected since the first dose of study drug.

MedDra (v19.1) Preferred Term	Placebo	Tramadol	Duloxetine
Nausea		4	5
Somnolence	1	4	2
Dizziness		3	4
Fatigue	1		3
Diarrhoea			2
Vomiting		1	1
Dry mouth		1	
Muscular weakness		1	
Abdominal pain		1	
Headache		1	
Stress			1
Retching			1

MEDDRA = Medical Dictionary for Regulatory Activities; V = version.

TABLE 3A – Summary of the primary endpoints in Part B.

	Tramadol - Placebo 0-10hr					Duloxetine - Placebo 0-10hr				
			95% CI					95% CI		
	LSMean	Estimate of the difference	Lower	Upper	p-value	LSMean	Estimate of the difference	Lower	Upper	p-value
Heat thermode - normal skin PDT	44.9	1.08	0.42	1.75	**0.0023**	44.3	0.5	-0.17	1.16	0.1375
Heat thermode – capsaicin skin PDT	41.9	0.76	-0.08	1.59	0.0753	41.7	0.57	-0.3	1.44	0.1915
Electrical stair PTT	25.8	17.6%	6.6%	29.8%	**0.0023**	23.0	4.5%	-5.3%	15.2%	0.3646
Cold pressor PTT	31.5	37.7%	24.3%	52.5%	**<.0001**	23.5	2.7%	-7.2%	13.7%	0.5944
Pressure stimulation PTT	50.5	18.7%	12.3%	25.5%	**<.0001**	44.0	3.4%	-2.2%	9.4%	0.2242

TABLE 3B – Summary of laser evoke potential endpoints in Part B. Clusters of analgesic effects for tramadol and duloxetine compared with placebo. Significant effect are in bold.

	Tramadol - Placebo 0-10hr					Duloxetine - Placebo 0-10hr				
			95% CI					95% CI		
	LSMean	Estimate of the difference	Lower	Upper	p-value	LSMean	Estimate of the difference	Lower	Upper	p-value
NORMAL SKIN										
Amplitude N2	-6.98	1.885	0.61	3.16	**0.0051**	-8.32	0.541	-0.73	1.812	0.3919
Amplitude P2	8.14	-1.207	-2.752	0.337	0.1190	9.21	-0.131	-1.687	1.424	0.8626
Amplitude N2P2	15.07	-3.106	-5.744	-0.468	**0.0226**	17.66	-0.516	-3.153	2.122	0.6923
Latency N2	203.36	5.947	-2.383	14.276	0.1525	203.14	5.727	-2.590	14.044	0.1668
Latency P2	319.32	5.813	-2.494	14.120	0.1628	314.16	0.653	-7.608	8.915	0.8724
Reaction time	427.43	38.761	13.426	64.097	**0.0041**	393.24	4.574	-20.739	29.887	0.7136
CAPSAICINE SKIN										
Amplitude N2	-7.74	0.352	-1.101	1.805	0.6097	-8.47	-0.376	-1.837	1.084	0.5883
Amplitude P2	7.74	-0.409	-1.812	0.993	0.5519	9.22	1.067	-0.385	2.519	0.1426
Amplitude N2P2	15.41	-0.605	-2.875	1.665	0.5864	18.04	2.032	-0.285	4.348	0.0829
Latency N2	208.54	2.691	-3.484	8.867	0.3807	205.51	-0.341	-6.534	5.853	0.9113
Latency P2	323.86	-5.074	-15.125	4.978	0.3092	321.38	-7.557	-17.596	2.481	0.1339
Reaction time	401.11	17.514	-5.632	40.659	0.1327	380.960	-2.630	-25.701	20.440	0.8174

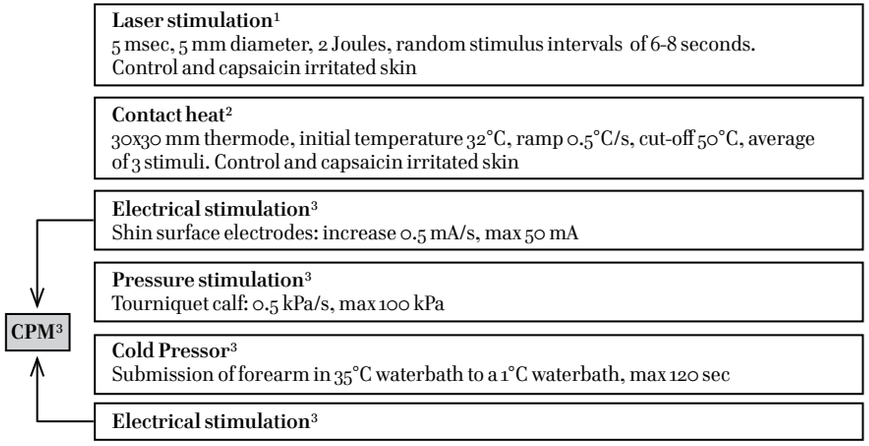
CI= Confidence Interval; LSMeans = Least squares Means; PDT = Pain Detection Threshold; PTT= Pain Tolerance Threshold

Table 4 – Pain thresholds per time point per study. Overview of the average pain detection and tolerance thresholds per study per measurement time point. PDT is given first, then PTT.

	Time (hr)	Present study	Study I	Study II	Study III	Study IV
Pressure stimulation PDT/PTT (kPa)	1	11.4/41.8	12.6/40.5	21.0/48.6	12.5/34.5	17.0/40.5
	2	11.0/42.3	11.9/37.8	16.5/51.1	12.3/33.1	16.6/40.3
	3	12.2/44.5	13.0/40.5	18.3/51.4	12.5/35.2	16.7/41.7
	4	12.6/42.4	11.3/37.1	19.7/51.3	12.7/33.2	15.7/38.3
	6	11.5/43.0	13.1/38.6	23.0/50.4	12.0/33.5	16.2/38.7
	10	12.6/41.4	11.6/39.1	21.8/47.7	12.3/33.0	17.7/37.8
Cold pressor PDT/PTT (sec)	1	4.0/23.0	4.7/18.4	8.8/25.3	4.2/16.6	3.4/14.1
	2	3.9/23.5	4.2/17.9	7.2/26.6	4.4/17.5	3.5/13.6
	3	3.7/22.9	4.9/19.2	8.2/27.3	4.0/17.5	3.2/13.6
	4	3.8/22.2	5.3/18.4	7.0/28.5	4.1/17.4	2.7/13.0
	6	3.1/23.7	4.4/17.7	7.6/24.8	3.7/16.1	3.1/13.4
	10	3.4/21.9	3.6/17.4	6.3/24.2	3.7/15.6	3.2/12.9
Electrical stair PDT/PTT (mA)	1	6.0/22.3	6.6/19.3	10.8/22.2	7.9/19.7	9.0/20.9
	2	5.1/21.8	6.0/19.2	7.6/21.5	9.6/20.3	8.0/22.3
	3	6.5/21.7	7.2/18.8	8.3/22.4	8.5/20.0	9.5/21.5
	4	6.3/21.8	7.0/19.5	10.4/23.0	8.3/20.8	8.9/22.2
	6	5.6/22.2	5.0/17.8	10.1/23.4	8.8/20.6	10.2/22.3
	10	5.3/22	5.6/18.3	10.6/22.8	9.9/20.7	10.8/22.7

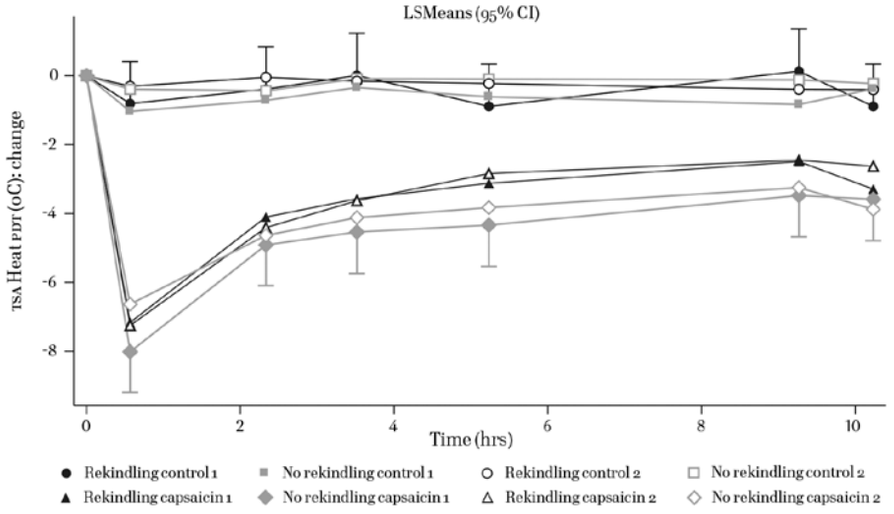
kPa= kilopascal; mA= milliampere; PDT= Pain Detection Threshold; PTT= Pain Tolerance Threshold; SEC= seconds.

Figure 1 – Order of pain assessments in Part B.



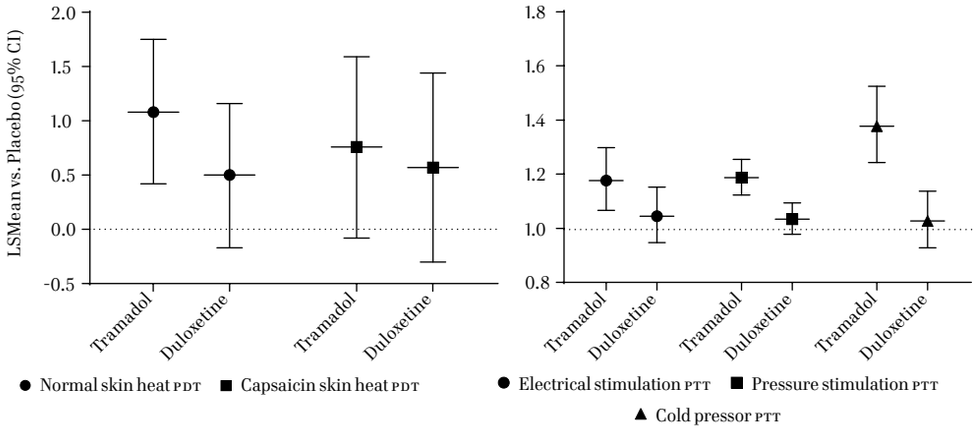
1 Laser stimulation was performed 1, 3, 6 and 8 hours post-dose. / 2 Contact heat was performed 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose. /3 Electrical stimulation, pressure stimulation, cold pressor and CPM were performed 1, 2, 3, 4, 6 and 10 hours post-dose. / CPM = Conditioned Pain Modulation

Figure 2 – Time course of contact heat pain assessment in Part A. Change from baseline graph of the least squares mean PDTs over time on the capsaicin treat area and control area in the rekindle and no-rekindle groups for visit 1 and 2.



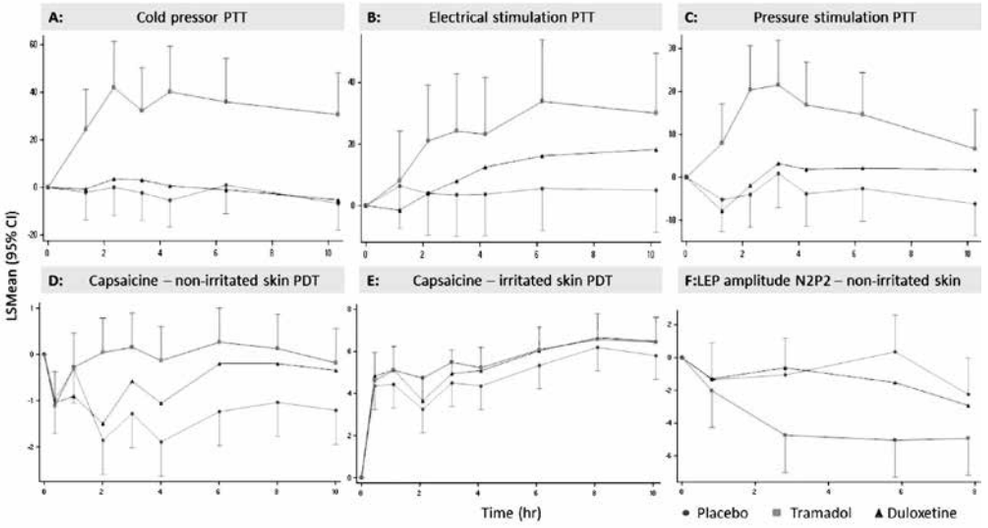
C = Celsius; CI = Confidence Interval; LSMeans = Least squares Means; PDT = Pain Detection Threshold;

Figure 3 – Primary analysis results Part B. Comparison of tramadol and duloxetine versus placebo with LSMeans and 95% confidence interval over the 0 – 10 hour period. Figure 3A is presented as the absolute differences to placebo, Figure 3B as changes to placebo in percentages. The dotted line represents no significant effect.



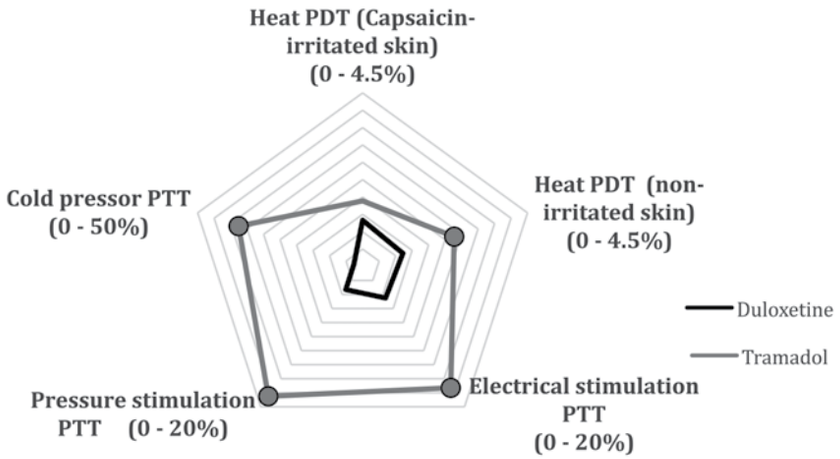
CI = Confidence Interval; LSMeans = Least Squares Mean; PDT = Pain Detection Threshold; PTT = Pain Tolerance Threshold.

Figure 4 – Time course of treatment effect on the pain models in Part B. Overview of change from baseline time profiles for battery of evoked pain tasks. (A) cold pressor in seconds (PTT); (B) electrical pain task in mA (PTT); (C) pressure pain task in kPa (PTT); (D) capsaicin-induced hyperalgesia model on the capsaicin irritated skin assessed with the contact heat paradigm in°C (PDT); (E) capsaicin-induced hyperalgesia model on the non-irritated (control) skin assessed with the contact heat paradigm in°C (PDT); (F) laser evoked potential on the non-irritated (control) skin N2P2 amplitude in µV.



Lines with Circles (●) = placebo; lines with squares (■) = tramadol; lines with triangles (▲) = duloxetine. PTT = pain tolerance threshold; PDT = pain detection threshold.

Figure 5 – Star plot of effects of tramadol 100mg and duloxetine 60mg on the different pain assessments. Star plot of effects of tramadol 100 mg and duloxetine 60 mg on the pre-defined primary endpoints. Values shown are differences compared with placebo. Values marked with a dot are significantly different ($p<0.05$) compared with placebo.



PDT = Pain Detection Threshold, PTT = Pain Tolerance Threshold.

Chapter 9

GENERAL DISCUSSION

Pain is a complex, multifactorial symptom that remains poorly understood and an unmet clinical need. Pain is influenced by many factors, like psychological factors, functional activities, genetics, gender, race, emotional functioning, social context, and education level and socioeconomical concerns. Pain is an individualized unpleasant sensory and emotional experience which is associated with actual or potential tissue damage, or described in terms of such damage.[8] The ideal species for the study of pain is the human being, in particular chronic pain patients. However, pain in these patients is almost always influenced by fear, emotion, anxiety, cognitive and autonomic responses, general malaise, etcetera.[3] Various approaches in search of the "magic drug" for pain palliation have arisen, leading to an exponential increase in health care costs.[1,2] This process is complicated by a number of factors: our access to the human organism is limited as ethical restrictions apply to all manipulations involving healthy subjects or patients, a lack of understanding about the underlying pathophysiological mechanisms, and the poor predictive validity of the current models of evoked pain used for the screening of novel compounds.

Healthy subject studies with human evoked pain models are an Alternative to investigate (novel) analgesics. With these pain models researchers can explore different pain mechanisms in a controlled setting. Different modalities of pain (*e.g.*, mechanical, thermal, electrical, or chemical) can be applied to different tissues (*i.e.*, skin, muscles, or viscera) for the assessment of various pain pathways. When intensity, duration, frequency, and localization of the stimulus can be controlled and a stable and reproducible outcome can be measured, a valid pain model is established. This can subsequently be used for the evaluation of analgesic activity and to demonstrate active dose ranges in early clinical phase of drug development. Pain models are able to induce a single or a composition of multiple positive somatosensory symptoms, making them suitable to investigate phenomena like nociceptive and inflammatory pain, and, to a lesser degree, hyperalgesia and allodynia, demonstrating some similarities to mechanisms present in neuropathic pain. These paradigms aim to activate different nociceptors and evoke pain through specific pathways and mechanisms, but difficulties remain in the exact determination of the activated pathways and pain mechanisms.[9] Studies measuring the effect of analgesic compounds on evoked pain make it clear that some drugs can yield significant results in one pain model but can fail to have an analgesic effect when using a different pain model.[10-12] Multimodal testing gives the opportunity to activate multiple receptor types and mechanisms. This multimodal test approach has shown its value in this thesis. For example, the high dose of PF-06372865, a $GABA_A$ positive allosteric modulator, evaluated in **Chapter 2**, increased the PTT to pressure stimulation and the cold pressor test, but not of the CPM or heat stimuli, and in **Chapter 3** a high dose of PF-06273340 (pan-Trk inhibitor) significantly affected the heat pain threshold, but not the cold pressor pain thresholds, or the electrical or pressure pain thresholds. Hence, these combined models may increase the knowledge regarding effects of analgesic compounds on peripheral and central pain mechanisms, and are therefore better suited for pharmacological testing. Combining the testing of various pain mechanisms provides the opportunity to obtain a more complete impression of the analgesic profile of a drug and increase the predictive value of nociceptive testing in healthy subjects for analgesic efficacy in patients with pain. The analgesic profiles of drugs appear to be unique and related to the pharmacology of the drug, in which case they may turn out to be specific for drug class, evidence of which may be seen in **Chapter 2 -4 & 6**.

Comparable to the uniqueness of the profile of analgesics with respect to their effects on a battery of human evoked pain models, patients with chronic pain show selectivity in their response to different analgesics. Particularly in patients with neuropathic pain, a high degree of variability in pain relief is observed, even among patients with identical diagnostic aetiologies, such as diabetic neuropathy or post-herpetic neuropathy. This high interpatient variability has frustrated responses to analgesics, both in clinical practice as in clinical trials.

Historically, neuropathic pain is classified based on aetiology (*e.g.*, nerve lesion, infection or diabetes), Although similar symptoms and signs are frequent across different aetiologies. It is this heterogeneity in pain patients that may have obscured positive results in certain subgroups due to the presence of multiple pain mechanisms within a diagnostic patient population. It has become apparent that this approach to classify pain patient might not be adequate, supported by the obtained results in late stage trials where promising candidate analgesic have failed to produces satisfying pain reductions.[13-15] Better patient stratification might improve clinical trial outcome, for example by classification based on somatosensory phenotypes. This more mechanism of action based approach is justified because variability between different pain syndromes was found to be smaller than between patients.[4,5,16]

Somatosensory phenotypes are patterns of somatosensory abnormalities and their likely underlying mechanisms.[14,16-21] These different phenotypical presentations possibly reflect different dysfunctions in somatosensory processing and defining them might give a better understanding to the underlying mechanisms of pain generation. Quantitative sensory testing (QST) is a comprehensive way of assessing the somatosensory phenotype in patient with pain.[22,23] QST evaluates

somatosensory modalities, such as temperature, touch, vibration, and pain. It provides information on the condition of peripheral sensory nerves, as well as central sensitization and pain perception. QST allows for the evaluation of the functional status of the small (Aδ, C) and large (Aβ) fiber sensor systems.[6,23] It enables one to create a sensory profile of the patient, which in some ways is comparable to the way human evoked pain models aid in the definition of an analgesic profile of a drug.

Before implementation of this new mechanism based approach, the challenge is to characterize the somatosensory phenotypes and the response each phenotype demonstrates on different analgesics. Identification of phenotypic profiles with the most predictive value in analgesic efficacy ideally are then linked to the analgesic profiles created by the PainCart. Subsequently, this process can be reversed: a mechanism of action based approach can be deployed where the predictive value of the PainCart leads to the selection of a cluster(s) of somatosensory phenotypes with a similar mechanistic aetiology, increasing the chance to yield positive outcomes.

This thesis focused on profiling novel and currently existing analgesic compounds using the PainCart. The analgesic profiles are part of the mechanism of action based approach and may serve as a predictive tool to select the correct somatosensory phenotype for further evaluation of a compound. Below a first attempt is made to couple these analgesic profiles to a somatosensory phenotype derived from QST testing.

MAIN OUTCOMES

In **Chapter 2-4** the analgesic potential of novel analgesic compounds was assessed. These three studies were conducted with a single-dose, double-blind, randomized, cross-over design with positive and placebo controls in healthy subjects. The PainCart was used as the multimodal test setting. The results of each study provided a comprehensive analgesic profile of the investigated drug. **Chapter 2** describes the results of two dose levels of PF-06372865, and α2/α3/α5 subtype selective γ-Aminobutyric acid ($GABA_A$) partial agonist. PF-06372865 is a potent ligand of the allosteric benzodiazepine site of the $GABA_A$ receptor, which exhibits functional selectivity for receptors containing α2/α3/α5 over those containing α1, postulating a better analgesic efficacy with fewer sedative side effects. The PainCart showed an increase in the pressure pain tolerance threshold (PTT) in both dose levels and an increase in the cold pressor PTT in the high dose level. Additionally, no sedation or other intolerable adverse events were observed which would limit its clinical use. Despite these promising results, a lack of analgesic efficacy was shown in a study with chronic lower back pain (CLBP) patients.[27] It was hypothesized that a α1-sparing, partial subtype-selective $GABA_A$ positive allosteric modulator would achieve higher receptor occupancy (RO) than a benzodiazepine without limiting adverse events (AEs) and demonstrate analgesia, Although patient benefit derived from benzodiazepines has never been established. Results from Cochrane reviews have highlighted that the evidence base for the treatment of CLBP with benzodiazepines is weak and indicates that there is insufficient evidence to recommend prescribing benzodiazepines for back pain.[37] It could be debated that CLBP patients were chosen erroneously for further exploration of the analgesic effects of PF-06372865. Nmerous high-quality randomized controlled trials have been performed on patients with CLBP and none of them exerted positive results for neuropathic CLBP syndromes.[7,74-78] It would have been worth considering to explore analgesic efficacy of PF-076372865 by linking the analgesic profile created by the PainCart to a surrogate somatosensory phenotype. Baron et al. revealed distinct phenotypes shown through QST profiling of pain patients with different aetiologies.[79] The cluster 2 and 3 phenotypes were most affected by cold and pressure pain thresholds, which were the pain models most affected by PF-06372865. Patients with post-herpetic neuralgia are mostly represented in cluster 2 and 3 (approximately 80%).[79] Perhaps it would have been more beneficial to choose these patients for a phase 2 or 3 study to evaluate efficacy. To support this hypothesis, PF-06372865 shows a similar analgesic PainCart profile to pregabalin which did not demonstrate significant pain reduction in patient with neuropathic CLBP syndromes,[76] but it did yield positive outcomes in post-herpetic neuralgia patients,[80] giving more incentive to explore efficacy via this Alternative method.

Further investigation of PF-06372865 as an analgesic was discontinued, however, it is currently being studied for its antiepileptic properties.[81]

In **Chapter 3** two dose levels of PF-06273340 were under investigation, a peripherally restricted small molecule inhibitor of tropomyosin-related kinase (Trk) A, B and C. Nerve growth factor (NGF) is a key mediator of chronic pain that signals through, among others, TrkA, TrkB and TrkC. The 400 mg dose of PF-06273340 significantly reduced the hyperalgesia seen in the UVB heat model. The lower dose level of PF-06273340 did not show an effect on any of the endpoints, suggesting that the PainCart is able to detect active dose ranges of a compound. PF-06273340 showed a similar analgesic profile of PF-06273340 compared with ibuprofen, a non-steroidal anti-inflammatory drug (NSAIDs). The analgesic

effect of the high dose is in agreement with the expected mechanism of action of this molecule. NGF is upregulated in experimental models of inflammation, including UVB sensitization,[28,29] and anti-NGF monoclonal antibodies and Trk inhibitors (including PF-06273340) have shown efficacy in nonclinical models of inflammatory pain, *e.g.*, the UVB model. Because the NGF pathway involves NGF binding to the TrkA kinase receptor, inhibition of TrkA has been clinically validated as a target for pain. In situations such as prolonged inflammatory responses, analgesics that suppress NGF/TrkA signalling might be considered to be effective therapy. Moreover, anti-NGF or TrkA inhibitors, are also candidate analgesics in the treatment of chronic pain caused by osteoarthritis (OA). Clinical studies in patients with OA might therefore have been warranted, endorsed by the pain reducing properties of the NGF neutralizing antibody tanezumab in studies with these patient population.[38-41] Before PF-06273340 will move forward in studies with OA patients, it is essential to link it to a somatosensory phenotype within this patient population. It is well-known that the pathophysiology of OA pain is complex, with significant inter-individual variability in symptomatology. QST could be used to phenotype OA patients into sub-groups which might differ in treatment response. A recent study presented QST findings of patients with knee OA and demonstrated a reduced pain thresholds for mechanical hyperalgesia, cold pressor and CPM, and an amplified temporal summation compared with healthy volunteers. After treatment with a topical NSAIDs the CPM normalized while the other paradigm remained unchanged.[82] These findings cannot directly be correlated to the analgesic profile of PF-06273340. Further research is needed, for example to treat the same patient group with an anti-NGF compound. Unfortunately, the metabolism of PF-06273340 is mediated by aldehyde oxidase, leading to reduced confidence in the prediction of human metabolic clearance and to unpredictable toxicity and clinical safety. The latter is essential as pan-Trk inhibitors require restriction to the peripheral compartment to avoid undesirable side effects associated with Trk inhibition in the central nervous system (CNS).[42] Further development of PF-06273340 was discontinued.

Discussed in **Chapter 4** are the results of PF-05089771, a small molecule inhibitor of the voltage gated sodium channel 1.7 ($Na_V1.7$). A significant body of evidence implicates sodium channels in mediating the pathophysiological components of both neuropathic and nociceptive pain.[30-32] PF-05089771 was being developed for diabetic peripheral neuropathy alone and concomitantly administered with pregabalin. The aim was to assess the efficacy in the PainCart to evaluate for future potential additional pain indications for PF-05089771 in addition to diabetic peripheral neuropathy (DPN), and also provide clinical translation from these evoked pain endpoints in healthy subjects to the future outcome in a DPN Proof of Concept (POC) study to inform of the utility of the PainCart as a translatable clinical battery. PF-05089771, alone nor administered concomitantly with pregabalin, did not demonstrate an analgesic effect in any of the end points. The same lack of results were found in the POC study in DNP patients where a modest, but not statistically significant, pain relief was observed.[43] Possible reasons for the modest pain reduction that were opted are the dose selection, the inability to access the $Na_V1.7$ receptors in the CNS, the role of the peripheral nerve terminal in nociception generation, and whether selective $Na_V1.7$ blockage is sufficient.[43] An Alternative consideration is the influence that $Na_V1.7$ inhibitors have on the activation and inactivation of the $Na_V1.7$ receptor. BIIB074 is another $Na_V1.7$-selective, state-dependent, sodium channel blocker.[44] This compound causes an acceleration in the onset of inactivation of the $Na_V1.7$ receptor and a delayed recovery from the inactivation. Moreover, it does not affect the activation of the receptor.[45] BIIB074 is currently the only $Na_V1.7$ inhibitor that has moved forward into phase 3 clinical trials for continued investigation in patients with trigeminal neuralgia.

It can be hypothesized that the wrong patient population was selected for PF-05089771 where trigeminal neuralgia would have been a better fit. Trigeminal neuralgia is an idiopathic paroxysmal pain most often characterized by episodes of spontaneous, severe shooting or jabbing pain that may feel like an electric shock, in the area innervated by the trigeminal nerve, usually triggered by innocuous stimuli. $Na_V1.7$ is preferentially expressed in peripheral neurons, including trigeminal neurons, which supports further development of BIIB074, or PF-05089771 for that matter, in trigeminal neuralgia. BIIB074 has not been evaluated with the PainCart, but most likely would have affected the electrical and mechanical stimulation paradigms as these paradigms have the closest resemblance to the clinical symptoms. It is also expected to influence pain models that modulate sodium influx in the generation of action potentials. The capsaicin model is a hyperalgesia model by activation of the transient receptor potential vanilloid 1 receptor (TRPV1). Activation of TRPV1 produces a sodium influx that ultimately results in the release of a cocktail of neuropeptides initiating and modulating neurogenic inflammation.[83] It can be postulated that a $Na_V1.7$ inhibitor, such as PF-05089771 or BIIB074, may attenuate pain thresholds in a capsaicin model. Evaluation of the capsaicin-induced hyperalgesia model was described in **Chapter 8**.

In section two of this thesis the scientific results of several studies are presented regarding validation and improvement of pain models. In **Chapter 5**

we quantified the reproducibility of the pain models included in the PainCart. Reproducibility is a long-lasting and still ongoing debate in the scientific world. In scientific research, credibility is of utmost importance. Reproducibility of methods increases the power of a scientific claim. Unfortunately, irreproducibility of major findings in high-profile journals ranges from 75% to 90%.[33,34] Basically, there are two major camps: those who are for more reproducibility (Reproducibility movement) and those who are against. The Reproducibility movement states that it is, and has always been, an essential part of science; not doing so is simply bad science. It is an important step in the 'Scientific Method' allowing science to progress by building on previous work; without it progress slows. This requires the submission of the data and computational tools used to generate the results; without it results cannot be verified and built upon. Adherence to agreed guidelines for the conduct of experimental research is necessary, as well as access to the protocol and the collected data.[35] The opposing camps debates whether or not reproducibility, at least in the form proposed, is not now, nor has it ever been, has been an essential part of science.[36] The idea of a single well-defined scientific method resulting in an incremental, and cumulative, scientific process is, at the very best, moot.[36] Requiring the submission of data will encourage a level of distrust among researchers and promote the acceptance of papers based on narrow technical criteria.[36] Misconduct has always been part of science with surprisingly little consequence. The public's distrust is likely more to with the apparent variability of scientific conclusions.[36] Consensus on this topic seems unlikely. In our study we had full access to all collected data being that all four studies were conducted at the same centre (Centre for Human Drug Research (CHDR)), giving us the opportunity to evaluate the reproducibility of the pain models. We were able to replicate the results throughout all included studies to a reasonably expected degree (an inherent variability of biological systems taken into account), increasing the robustness and generalisability of the results. Reproducible results are important to obtain since these results are the foundation to initiate trustworthy advances in a research program.

In a double-blind, double-dummy, single dose, randomized, placebo-controlled, crossover study, described in **Chapter 6**, we explored the analgesic effects of a classical (paracetamol) and a non-classical (Δ9-Tetrahydrocannabino (Δ9-THC)) analgesic. To investigate the role of sedation rather than analgesic effects of psychoactive compounds, a negative control was included in this study in the form of the H1 antihistaminergic promethazine. The lack of effect of Δ9-THC in the PainCart confirms the suspicion on the value of cannabinoids in treating patients with chronic neuropathic pain. A recent systematic review concluded

that there is a lack of high-quality evidence for the efficacy of any cannabis-based medicine in any condition with chronic neuropathic pain.[46] At best, only few patients with neuropathic pain will benefit from long-term use of cannabis-based medicines.[46] The Special Interest Group on Neuropathic Pain (NeuPSIG) for the pharmacotherapy of neuropathic pain gave a weak recommendation against the use of cannabis-based medicines.[13] The use of cannabis as an analgesic should be evaluated at a regular base by the care-taker to avoid unnecessary exposure to harm in the absence of benefit. Adverse events, such as somnolence, sedation and feeling high may attribute to pain relief indirectly, but confusion and psychosis limit the clinical usefulness of cannabis-based medicines.[46]

The Bond and Lader set of visual analogue lines are used to quantify subjective effects of sedative agents. Δ-9-THC significantly reduced subjective alertness and significantly increased calmness compared with placebo, and promethazine significantly reduced subjective alertness compared with placebo. No analgesic effects were measured (for Δ9-THC and promethazine) by the PainCart, despite the presence of sedation as established with the Bond and Lader. The results described in **Chapter 6** may have been disappointing in terms of their analgesic profile, it proved an important quality that sedation is of no influence in the PainCart.

In **Chapter 7** we present the prevalence and characteristics of patients with postinflammatory hyperpigmentation (PIH) after ultraviolet-B (UVB) irradiation being used in the UVB inflammation model, and, based on these results, we improved the pain model to minimize the risk for development of this long-term side effect. Due to a relative short-term follow-up in previous conducted studies with the PainCart, we were unable to detect long-term adverse events as a result of the UVB irradiation. Under-reporting of adverse events may lead to a false sense of safety in a study design. Guidelines for detection and reporting of harm are needed, which would benefit most from a multidisciplinary approach.[47] Subjects should have an active role in reporting adverse events. A group of multidisciplinary investigators and patients have developed a patient-reported outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE),[47] where subjects can report their adverse events after completion of the study. Additionally, investigators should report these adverse events in a standardized manner, journals should dedicate space for reports on harm, and regulatory agencies should oversee these reports.[48] Reporting of adverse events, even long-term adverse events, in clinical trials is essential to evaluate the subject's safety. It also gives the opportunity to learn from the incident and ultimately improve the design of the study in order to increase the subject's safety. Characteristics found

in the first study that were prone to the development of PIH, like UVB dosage and skin type, were adjusted in the second study. The aim was to create a pain model that is able to induce enough hyperalgesia to evaluate analgesic compounds without the high risk of developing long-lasting side effects. This study showed that irradiation with a two-fold, instead of a three-fold, of the minimal erythema dosage induced a long-lasting and stable hyperalgesia in subjects with the Fitzpatrick skin type of not higher than III. Whether or not this hyperalgesia is enough to evaluate efficacy of analgesic needs further investigation, for example, with ibuprofen, a non-steroidal anti-inflammatory drug (NSAIDs). This also gives an opportunity to compare the new results to results described in **Chapter 2-4**.

In the final chapter, **Chapter 8**, we aimed to validate – at CHDR - the capsaicin-induced hyperalgesia/allodynia model and to incorporate the pain model in the PainCart. Concerns about validity and the complexity of multimodal approach have existed for some time.[12] Validity and repeatability studies have been demonstrated for many pain models separately. Complexity of multimodal testing and their contributing factors, however, is little investigated. In the current study we were able to demonstrate that the test-rest variability was good. The study-to-study variation, in the current and four previous conducted PainCart studies without the capsaicin model, was small with consistent pain thresholds throughout all studies. The data suggest that, at least with these models, there is limited interference between the models which is essential in a multimodal model test setting. The capsaicin model is known to produce a primary mechanical allodynia/thermal hyperalgesia and a secondary mechanical allodynia.[49,50] In the current study no mechanical allodynia was induced, either on the primary or on the secondary area. A key learning from this validation study is a better understanding on the influence of the capsaicin formulation (*e.g.*, cream or ethanol solution) on mechanical stimulation. As described in this chapter, we believe that the formulation of the capsaicin might have negatively influenced the induction of secondary hyperalgesia. An Alternative consideration could be the study design where the timing and sequence of different activities and paradigms were the cause of a lack of positive results. Secondary hyperalgesia might be a short-lasting phenomenon which, in hindsight, should have been evaluated within 30-60 minutes after removal of the capsaicin cream. Further evaluation of all putative reasons should be done in order to optimize the capsaicin model to a pain model robust enough to explore analgesic profiles on candidate analgesic drugs.

FUTURE PERSPECTIVES

Despite the availability of strong analgesics, chronic pain is one of the largest unmet needs in medicine. Developing new and specific painkillers is even more important because many of the classic analgesics are highly addictive. Pain models help to predict the efficacy of a compound in the treatment of clinical pain, and may also predict in which types of pain the drug will be effective. However, development of new pain models and further refinement of existing pain models is needed.

One potential disadvantage of experimental pain models is that per definition, they only measure nociception, as affective components (fear, mood) and psychosocial factors that influence pain, are lacking. This may limit the extent to which pharmacological effects measured using pain models can be extrapolated to clinical pain. This will not, or to a lesser extent, be true for compounds that influence nociceptive processes, but will play an important role for new drugs that are expected to positively influence pain by (also) influencing affective components of pain. If a patient with moderate nociceptive pain also has a high level of anxiety, pain intensity will be importantly increased. New analgesic compounds are being developed that are expected to positively influence pain not only by decreasing pain directly, but also by decreasing the accompanying fear. At CHDR, we are currently developing a pain model in which we aim to lower pain thresholds by introducing fear of tissue damage using virtual reality (VR). The VRpain enhancement model may be able to include the affective component of pain to a (nociceptive) pain model.

It is known that non-nociceptive information regarding pain can both induce pain and modulate it,[51-53] suggesting that pain is evoked by information that must exceed a certain threshold, but not necessarily by a nociceptive stimulus. Acerra et al. demonstrated this in patients with complex regional pain syndrome (CRPS). These patients experienced pain when they were given the mere suggestion that they were touched, despite the lack of effective touch.[51] Virtual reality can be used as a supporting technique in pain relief.[54] Pain requires attention and VR may be particularly effective in distracting the patient's focus on pain during painful procedures. The virtual world generated by the computer can alleviate the pain in subjects submerged in VR. The stronger the illusion of the virtual world, the more VR will distract the patient, the more substantial the pain can be reduced.[55] Hoffman et al. showed that, compared with standard care (without VR), burn patients consistently reported a reduction in pain (> 30%) during wound care

and physical therapy.[54,56-58] SnowWorld has been designed specifically for this purpose (www.vrpain.com). We expect that this psychology can also be reversed. Additional attention to pain, together with the affective and psychosocial components (fear and anticipation), can be expected to increase the pain perception when a painful stimulus is administered. Exploring the role of VR in an experimental pain test setting may also teach us more about the role of how affective components influence pain.

Better understanding of existing pain models is an Alternative approach to increase the predictive value of analgesics. An established model to evaluate systemic inflammation is the human endotoxin model.[59] In this experimental setting, purified lipopolysaccharide (LPS) from E. coli or other Gram-negative bacteria is administered intravenously to healthy volunteers resulting in flu-like symptoms, increased production of C-reactive protein (CRP) and increased concentrations of pro- and anti-inflammatory cytokines. LPS from E. coli is predominantly used because of the high reproducibility of effects.[60-62] In various animal models and in clinical studies, the immune response induced by LPS has been demonstrated to modulate cognitive functions and nociceptive pain. LPS administration decreased pressure and heat pain thresholds, supporting a relationship between acute systemic inflammation and pain perception.[63,64] Combining the human endotoxin model with nociceptive testing could serve as a model for studying inflammatory hyperalgesia via mechanisms that are not yet covered by PainCart's UVB or capsaicin-induced heat pain model. Moreover, the model may be a good Alternative for the currently used UVB model, which produces a stable inflammatory-like hyperalgesia, but also unwanted long-lasting side effects (**Chapter 7**).

Central sensitization is an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input. While neuropathic pain conditions are predominately the result of damage to the peripheral nervous system, their persistence appears to rely on maladaptive processes within the CNS,[49] making it an area of interest in human evoked pain model research. Central sensitization is also found in subjects with sleep disturbances.[65,66] Sleep deprivation affects the perception of pain (mainly thermal pain thresholds) as well as spontaneous pain.[67,68] Impaired sleep may affect processes relevant for the development and maintenance of chronic pain such as endogenous pain inhibition.[69] Multiple studies have reported that sleep-deprived subjects respond differently to evoked pain tests in a controlled setting. However it is still a topic of discussion as other clinical studies have provided conflicting results.[68,70-73] If validated, the sleep deprivation model may be used to demonstrate effects of relatively more centrally acting analgesic compounds. It may therefore serve as an Alternative to the capsaicin model (**Chapter 8**), where we were unable to demonstrate central sensitization using a mechanical stimulation paradigm.

The work presented in this thesis adds to the search of pain models with a high resemblance of clinical pain and gives incentive to investigate the translation of analgesic profiles deducted from multimodal pain model testing to somatosensory phenotyping by means of QST research. We evaluated existing pain models for their reliability and reproducibility in a multimodal model setting, we improved models which shifted to a negative risk-benefit assessment, and we incorporated a new pain model to the PainCart to increase its reach in the complex field of pain research.

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NEDERLANDSE SAMENVATTING

Pijnbestrijding is een van de grootste uitdagingen voor de moderne geneeskunde. De International Association for the Study of Pain (IASP) heeft geschat dat de prevalentie van chronische pijn kan oplopen tot 35%. Dit betekent dat 1 op de 3 mensen last heeft van chronische pijn op een bepaald moment in hun leven, en deze aantallen nemen eerder toe dan af. Potente analgetica zoals opioïden hebben serieuze bijwerkingen en chronische behandeling met opioïden kan leiden tot tolerantie en verslaving. Er blijft een grote medische vraag naar nieuwe, verbeterde behandelingen van chronische pijn. De ontwikkeling van betere en meer specifieke pijnstillende geneesmiddelen blijft daarom essentieel.

Met een toenemende vraag om farmacodynamische effecten van nieuwe middelen zo vroeg mogelijk aan te tonen, is het belang van de nociceptieve modellen in gezonde proefpersonen nu groter dan ooit. Nociceptieve modellen zijn modellen waar een pijnlijke stimulus gegeven wordt die vervolgens gekwantificeerd kan worden. Er bestaan verschillende methoden om bij mensen pijn op te wekken, zoals mechanische, thermische, elektrische en chemische stimulatie. Stimuli kunnen worden gegeven op verschillende weefseltypen, bijvoorbeeld huid, spieren of ingewanden. Factoren zoals stimulusintensiteit, frequentie, duur en locatie kunnen worden gestandaardiseerd. Farmacodynamische evaluatie door middel van nociceptieve modellen biedt de mogelijkheid om te meten voor welke modaliteit van pijn een nieuw middel het meest geschikt is (nociceptief, neuropathisch of inflammatoir), en welke andere effecten bijdragen aan de werking ervan (bijv. sedatie, tolerantie). Het biedt daarnaast de mogelijkheid om het dosisbereik te bepalen waarop nieuwe analgetica hun farmacologische effect uitoefenen. Nociceptieve modellen kunnen ook helpen bij de keuze van de doelpopulatie voor vervolgstudies. Het gebruik van nociceptieve modellen bij gezonde vrijwilligers heeft voordelen boven het beoordelen van de effecten van nieuwe geneesmiddelen bij patiënten met pijn; de pijn die veroorzaakt wordt in nociceptieve modellen is voorspelbaar in zijn intensiteit, terwijl klinische pijn van nature fluctueert. Daarnaast kunnen de pijnstillende eigenschappen worden onderzocht zonder de invloed van begeleidende symptomen die vaak worden gezien bij patiënten met pijn, zoals depressiviteit, angst, slaapgebrek, etc. Een nadeel van nociceptieve modellen is dat de pijn stimulus kortdurend is -in tegenstelling tot het chronische karakter van de meeste klinische pijn- en dat het alleen in staat is om een enkel positief somatosensorisch symptoom te induceren. Hierdoor is het wel geschikt is om verschijnselen zoals nociceptieve en inflammatoire pijn, maar, in mindere mate, hyperalgesie en allodynie te onderzoeken. Daarnaast spelen factoren als cognitie, emotie en gedragsmatige reacties op pijn geen rol in een nociceptief model in gezonde proefpersonen. Multimodaal testen geeft de mogelijkheid om meerdere mechanismen betrokken bij pijn te onderzoeken. Door verschillende pijnmodaliteiten te combineren kan in zekere zin een profiel van een pijngeneesmiddel ontwikkeld worden. Dit pijn profiel weerspiegelt de effecten van een pijnstiller op mechanismen die betrokken zijn bij klinische pijn. Op deze manier kan multimodaal pijnonderzoek helpen bij het bepalen van de geschikte doelpopulatie voor nieuwe pijnstillende middelen op basis van hun profiel van effecten op een verscheidenheid aan pijnmechanismen , afhankelijk van de bijdrage van elk van deze mechanismen aan klinische pijnfenotypes. Hoofdstuk 1 richt zich op de verschillende modellen die worden gebruikt om pijn bij gezonde proefpersonen op te wekken en de methoden om de pijn stimulus te beoordelen. Nociceptieve modellen zijn onderverdeeld in het energiedomein (d.w.z. mechanische, thermische, elektrische en chemische inductie) en zijn verder onderverdeeld in een stimulatiegebied (d.w.z. huid, spieren en ingewanden) dat wordt gestimuleerd.

SECTIE I: EFFECTIVITEIT VAN VERSCHILLENDE (NIEUWE) ANALGETICA MET BEHULP VAN DE PAINCART

In studies beschreven in hoofdstuk 2-4 werd het analgetische potentieel van een aantal nieuwe analgetische middelen beoordeeld. Dit werd gedaan met behulp van een door het CHDR ontwikkelde multimodale testbatterij die de “PainCart” wordt genoemd. De resultaten van elke studie toonden het analgetische profiel van het in het desbetreffende hoofdstuk onderzochte middel dat verkregen werd door middel van de PainCart. In hoofdstuk 2 werden de analgetische effecten van twee doseer sterktes (15 en 65 mg) van PF-06372865 gepresenteerd, een nieuwe α2/α3/α5 subtype selectieve γ-aminoboterzuur ($GABA_A$) partiele agonist. PF-06372865 heeft een hogere selectiviteit voor receptoren die de α2,α3 ofα5 bevatten ten opzichte van de receptoren die een α1 bevatten, waardoor het verwacht kan worden een analgetische werkzaamheid te hebben, maar minder sedatieve bijwerkingen, die vooral veroorzaakt worden door de alpha1 subunit van de $GABA_A$ receptor. De PainCart vertoonde een verhoging van de pijn tolerantiegrens (PTT) bij de druk stimulatie test in beide doseersterktes en een toename van de PTT van de koude water test bij de hoge doseersterkte. Bovendien werd er geen sedatie waargenomen. We concludeerden dat PF-06372865 analgetische effecten heeft bij doseringen die geen significante sedatie veroorzaken die het klinische gebruik ervan zou beperken. Bovendien bevestigde de huidige studie de rol van de PainCart als een hulpmiddel bij de ontwikkeling van pijnstillers met een nieuw

werkingsmechanisme, voor de behandeling van verschillende pijntoestanden, en om een proof-of-concept (POC) aan te tonen.

In hoofdstuk 3 wordt een fase I-studie beschreven van het nieuwe onderzoeksmiddel PF-06273340, een remmer van de tropomyosine-gerelateerd kinase (Trk) A, B en C receptoren. Zenuwgroeifactor (NGF) is een belangrijke mediator van chronische pijn. NGF is een lid van de neurotrofine-familie die signalen afgeeft via tyrosinekinase-receptoren van de Trk-familie, bij voorkeur TrkA. Hiervan is bekend dat het zowel acute als chronische regulatie van pijnsignalering induceert door fosforylatie-afhankelijke regulatie van ionkanalen die betrokken zijn bij pijnoverdracht en bij de opregulatie van pijngerelateerde genen. 20 gezonde vrijwilligers namen deel aan de studie en kregen vijf middelen toegediend in een cross-over design: PF-06273340 50 mg, PF-06273340 400 mg, pregabaline 300 mg, ibuprofen 600 mg en placebo. De hoge dosis PF-06273340 liet een verhoogde pijn detectiegrens (PDT) zien voor het ultraviolet B (UVB) model. De lage dosis liet geen duidelijke pijnstilling zien, wat suggereert dat de PainCart in staat is te detecteren bij welke dosis een nieuwe stof analgetische effecten begint te hebben. PF-06273340 vertoonde een vergelijkbaar analgetisch profiel vergeleken met ibuprofen, een niet-steroïde ontstekingsremmer (NSAID). Het analgetische effect van de hoge dosis en het profiel van effecten op de PainCart is in overeenstemming met het verwachte werkingsmechanisme van dit molecuul. NGF wordt opgereguleerd in experimentele modellen van ontsteking, waaronder bij het UVB model.[1,2] Deze studie demonstreerde voor het eerst de pijnstillende effecten van een pan-Trk-remmer bij de mens.

Hoofdstuk 4 beschrijft een studie waar de analgetische potentie van een krachtige selectieve $Na_V1.7$ natriumkanaalblokker, PF-05089771, werd onderzocht. Natriumkanaalblokkers worden al langere tijd gebruikt voor de behandeling van pijn, maar dit wordt beperkt door het gebrek aan selectiviteit voor verschillende natriumkanaalsubtypen die kunnen leiden tot centraal zenuwstelsel (CZS) en cardiovasculaire bijwerkingen. Als zodanig is er speciale belangstelling voor het $Na_V1.7$-subtype, dat voornamelijk tot uitdrukking komt in nociceptieve en sympathische neuronen. Toediening van PF-05089771 leidde niet tot significant andere effecten op de pijn detectie of pijn tolerantie drempels dan placebo toediening. Negatieve resultaten werden ook gevonden in de PoC-studie bij patienten met diabetische pijnlijke neuropathie waarin een bescheiden, niet-statistisch significante, pijnverlichting werd waargenomen.[3] Mogelijke redenen voor deze bescheiden pijnreductie werden besproken, zoals de doseersterkte, het onvermogen om ook in het CZS te komen, de rol van de perifere zenuwterminal bij het genereren van nociceptie en of selectieve $Na_V1.7$-blokkering voldoende is [3].

SECTIE II:
VALIDATIE EN VERBETERING VAN NOCICEPTIEVE MODELLEN

Hoofdstukken 5-8 van dit proefschrift richten zich op de validatie of verbetering van nociceptieve modellen. In hoofdstuk 5 wordt de PainCart gevalideerd door de reproduceerbaarheid te evalueren. Hoewel reproduceerbaarheid als essentieel wordt beschouwd voor elke methode die wordt gebruikt in wetenschappelijk onderzoek, wordt deze slechts zelden onderzocht. Er is ook opvallend weinig gepubliceerd over de reproduceerbaarheid van nociceptieve modellen. In dit hoofdstuk onderzochten we de reproduceerbaarheid van de PainCart door de pijnstillende effecten van pregabaline en ibuprofen te meten in vier al eerder uitgevoerde onderzoeken op het CHDR. In totaal namen 81 gezonde proefpersonen deel aan de vier onderzoeken. De variabiliteit tussen alle nociceptieve modellen liep uiteen van 2,2% tot 30,6%, wat als gering beschouwd kan worden. De vier onderzoeken met een vergelijkbaar ontwerp toonden reproduceerbaarheid met betrekking tot de opgenomen nociceptieve modellen. De relatief hoge consistentie en reproduceerbaarheid van twee analgetica, bij doseringen waarvan bekend is dat ze effectief zijn bij het behandelen van klinische pijn, ondersteunen de validiteit van het gebruik van de PainCart om de analgetische activiteit te onderzoeken en de actieve dosering van vermoedelijke analgetische middelen in vroege klinische ontwikkeling te bepalen.

Hoofdstuk 6 richt zich op de validatie van de PainCart door bestaande geneesmiddelen met verschillende farmacologische werkingsmechanismen te onderzoeken. Het doel was om de analgetische effecten van klassieke (paracetamol) en niet-klassieke analgetica (Δ9-THC) te onderzoeken in vergelijking met een negatieve controle met een sederende werking (promethazine). Paracetamol vertoonde geen significante vermindering van pijnsensatie of op subjectief cognitief functioneren in vergelijking met placebo. Promethazine induceerde een statistisch significante vermindering van PTT voor de koude water test en de druk stimulatie test. Bovendien werd een verminderde subjectieve alertheid waargenomen. Δ9-THC vertoonde een statistisch significante afname in PTT voor elektrische stimulatie test en de druk stimulatie test. Δ9-THC toonde ook subjectieve effecten, waaronder veranderingen in alertheid en kalmte. Deze studie vond geen effect op pijndrempels of zelfs een verminderde pijntolerantie als gevolg van Δ9-THC en promethazine, , met behulp van de PainCart. Pijndrempels na toediening van paracetamol werden niet significant beïnvloed, wat mogelijk te wijten was aan onvoldoende statistische kracht. Wij hebben aangetoond dat het onwaarschijnlijk is dat sedatie op zichzelf pijndrempels kan verhogen. Dit is van

belang bij de interpretatie van de resultaten van onderzoeken met nieuwe pijn geneesmiddelen die als bijwerkingen tot sedatie leiden.

Een evaluatie en verbetering van het UVB model werd in hoofdstuk 7 gepresenteerd. Het UVB model is een model voor inflammatoire pijn waarbij de huid met UVB bestraald wordt om een tijdelijke overgevoeligheid voor warmte te induceren. Het UVB model werd in zes verschillende studies op het CHDR gebruikt om farmacodynamische effecten te meten van potentiele analgetica. Het mag echter geen weefselschade veroorzaken of schadelijke bijwerkingen op de lange termijn hebben. De huidige studie werd opgezet nadat meerdere proefpersonen over postinflammatoire hyperpigmentatie (PIH) rapporteerden die langer duurde dan verwacht. PIH is een donkere verkleuring op de huid door een eerdere ontsteking of beschadiging van de huid, in dit geval de UVB bestraling. In het eerste deel van de studie werden alle 142 proefpersonen die eerder waren blootgesteld aan de UVB bestraling uitgenodigd voor een klinische evaluatie van PIH. In totaal reageerden 78 van de 142 proefpersonen. De prevalentie van PIH onder de proefpersonen die reageerden was 53,8%. In het tweede deel van het onderzoek werden 18 gezonde proefpersonen blootgesteld aan een (relatief) lagere dosering UVB bestraling en werd de PDT voor het hitte pijn model en de PIH geëvalueerd. We vonden een significant en stabiel verschil in PDT tussen de huid blootgesteld aan UVB en de controlehuid met een gemiddeld verschil in PDT van -2,6 °C tot -4,5 °C. Daarnaast zagen we een lagere prevalentie van PIH in deze groep. De prevalentie 6 maanden na de bestraling was 27,8%. Hieruit konden we concluderen dat het oorspronkelijke UVB model geassocieerd is met een relatief hoge prevalentie van langdurige PIH. Een lagere dosering van de UVB bestraling daarentegen produceert een stabiele hyperalgesie en heeft een lager risico op PIH.

Er wordt continu onderzoek gedaan om nociceptieve modellen te verbeteren, bijvoorbeeld door simulatie van klinische pijnsymptomen, zoals hyperalgesie. Een veel gebruikt hyperalgesie model is het capsaïcine model, waarbij applicatie van het extract van de rode chilipeper een tijdelijke hyperalgesie induceert, resulterend in een lagere pijn drempel voor warmte en een tijdelijke mechanische allodynie. Een studie die als doel had om dit hyperalgesie model te standaardiseren wordt gepresenteerd in hoofdstuk 8. Capsaïcine is een zeer selectieve agonist voor de transient receptor potential vanilloid 1 (TRPV1). TRPV1-kanalen zijn transducers van mechanische en chemische stimuli. Thermische primaire en secundaire hyperalgesie, na toepassing van capsaïcine, kunnen worden geëvalueerd door het gebruik van laser-evoked potentials (LEP) gemeten met behulp van elektro-encefalografie (EEG). Gecombineerde LEP en EEG-registratie kan de

pijnprikkel op een meer objectieve manier kwantificeren. Dit was een tweedelig onderzoek. Deel A was een validatiestudie van het capsaïcine model. Deel B was een dubbelblind, enkelvoudige dosis, gerandomiseerde, placebo-gecontroleerde, 3-weg cross-over studie. De laatste studie was opgezet om aan te tonen dat het capsaïcine model geïncorporeerd in de PainCart ook geschikt was voor het kwantificeren van farmacodynamische effecten van pijngeneesmiddelen duloxetine en tramadol. Van zowel tramadol als duloxetine is bekend dat ze effectief zijn bij de behandeling van neuropathische pijn en deze middelen worden in de kliniek ook voor deze indicatie gebruikt als respectievelijk eerstelijns en tweedelijns behandeling. In totaal werden 20 gezonde mannelijke proefpersonen in Deel A opgenomen en 18 gezonde mannelijke proefpersonen in Deel B., Applicatie van de capsaïcine 1% crème leidde tot een stabiele warmte allodynie in het gebied waar capsaicine crème werd aangebracht (primaire gebied), maar leidde niet tot een stabiele allodynie voor hitte of mechanische stimulatie in het gebied er om heen lag (secundaire gebied). Juist mechanische allodynie in het secundaire gebied wordt beschouwd als een teken van sensitisatie op dorsaalhoorn niveau, wat ook optreedt bij neuropathische pijn. Het capsaïcine model leek niet te interfereren met de andere nociceptieve modellen in de PainCart, waardoor het gebruikt kan worden in de multimodale testsetting.

In hoofdstuk 9 wordt een eerste poging gedaan om het unieke analgetische profiel van de onderzochte middelen met de PainCart te koppelen aan een somatosensorisch fenotype. Historisch gezien worden patiënten met neuropathische pijn in fase II/III-geneesmiddelenonderzoeken geïdentificeerd op basis van etiologie. Echter vertonen patiënten met eenzelfde etiologie niet noodzakelijkerwijs eenzelfde reactie op analgetica. Vooral bij patiënten met neuropathische pijn wordt een hoge mate van variabiliteit in pijnverlichting waargenomen. Deze hoge interpatiënt variabiliteit zou kunnen komen doordat patiënten met eenzelfde etiologie van de pijn niet per se eenzelfde onderliggende pathofysiologie vertonen. Wat daarvoor pleit is dat verschillende patiënten vaak ieder een unieke set aan positieve, negatieve (bijv. hypesthesie of hypo-algesie) en spontane somatosensorische symptomen (bijv. paresthesie) vertonen. Andersom wordt dit ook waargenomen waarbij een vergelijkbare set aan somatosensorische symptomen en tekenen voorkomen bij patiënten met verschillende etiologieën. Het begint duidelijk te worden dat de klassieke benadering voor het classificeren van pijnpatiënten mogelijk niet toereikend is, maar dat stratificatie op basis van somatosensorische fenotypen de uitkomst van fase II/III-patiëntenonderzoeken kan verbeteren. Deze hypothese wordt ondersteund door de verkregen resultaten in onderzoeken waarbij veelbelovende kandidaat-analgetica geen bevredigende

pijnvermindering hebben opgeleverd bij patiënten met eenzelfde etiologie,[4–6] en op basis van andere onderzoeken waar minder variabiliteit in effectiviteit werd geobserveerd wanneer er geselecteerd werd op somatosensorische fenotypes[7–9]. Onze hypothese is dat een indeling van patiënten met neuropathische pijn op basis van hun symptomen beter aansluit op de onderliggende pathofysiologie en dat de onderliggende pathofysiologie beter aansluit op de farmacologie van pijngeneesmiddelen. Op een vergelijkbare manier onderzoeken wij met de PainCart de effecten van pijngeneesmiddelen op verschillende mechanismen die betrokken zijn bij (neuropathische) pijn. Wij veronderstellen dat er in de toekomst op basis van het PainCart profiel van een nieuw pijngeneesmiddel een voorspelling gedaan kan worden ten aanzien van de fenotypische subgroep van patiënten met neuropathische pijn waarin het middel het beste werkzaam zal zijn.

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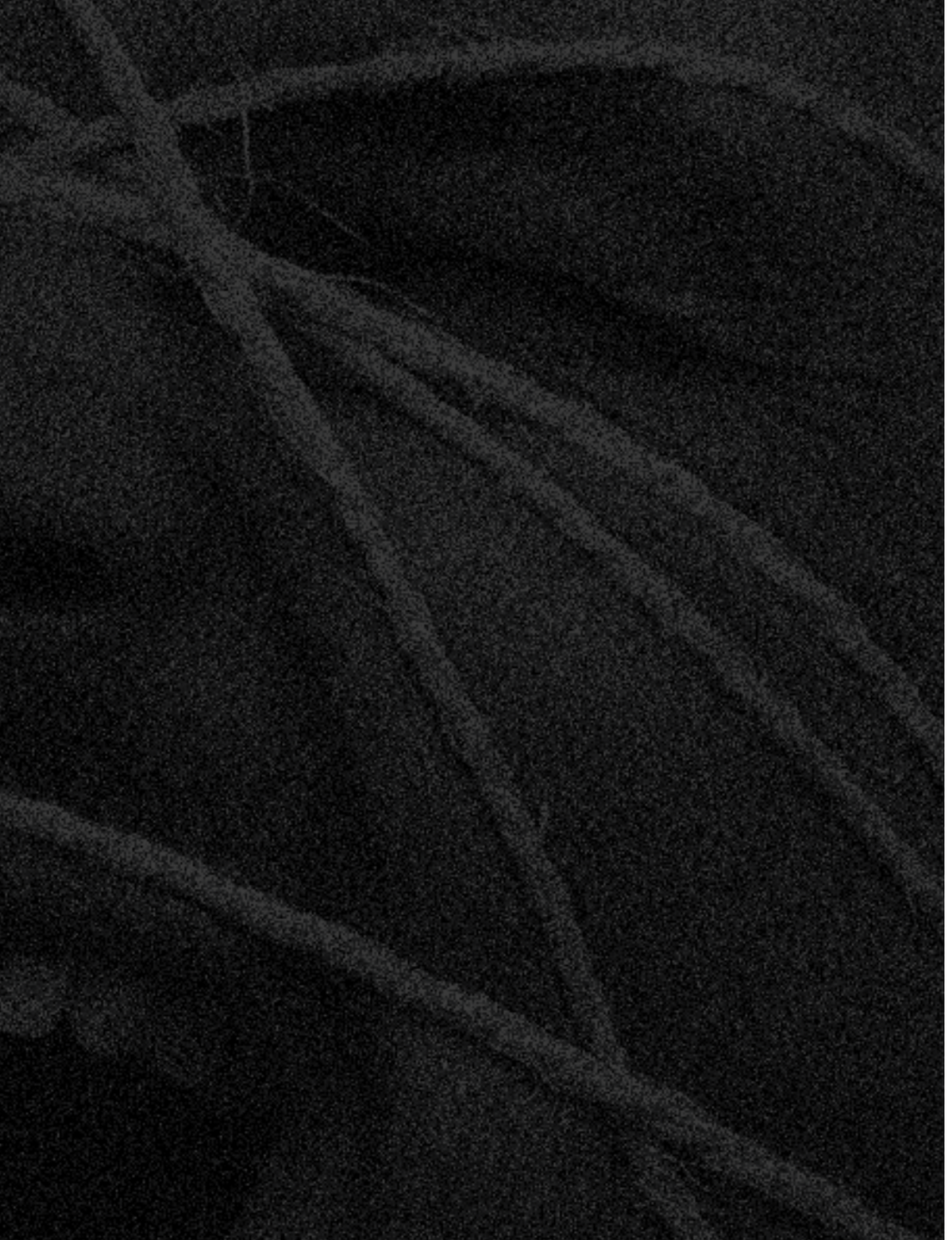

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CURRICULUM VITAE

Pieter Sjoerd Siebenga was born on October 30, 1984 on a dairy farm near Lelystad. After graduation from secondary school in 1996 (Almere College, Dronten/Kampen, The Netherlands), he moved to Santa Barbara (California, USA) to study abroad for one year. He then studied Medicine at the University of Amsterdam, from which he graduated in 2012. He started his professional career as a physician at the Department of Surgery at the Reinier de Graaf Gasthuis in Delft and Medisch Centrum Alkmaar in Alkmaar.

From 2014 through 2018, Mr. Siebenga was appointed as a research physician in the Neurology and Pain group at the Centre for Human Drug Research in Leiden, where all research was conducted, which culminated in this thesis. He focused on the efficacy of different (novel) analgesics by using the PainCart and the validation and improvement of human evoked pain models, supervised by prof. dr. A.F. Cohen and dr. G.J. Groeneveld. Whilst working as a research physician at CHDR, he was trained as a clinical pharmacologist and obtained his degree in clinical pharmacology in 2020.

Mr. Siebenga returned to clinical practice in 2018, starting the training for cosmetic medicine. He is currently working in a private clinic, UMA Institute, in Amsterdam under the supervision of dr. J. van Loghem.

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