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THE USE OF A BATTERY OF EVOKED PAIN MODELS IN EARLY PHASE DRUG DEVELOPMENT

PIETER OKKERSE

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INTRODUCTION

Acute pain, defined as short-term pain of less than 12 weeks duration, is part of normal life and experienced regularly by almost everyone. Studies on the prevalence of chronic pain in adults show that in Western Countries 19-31% of the population suffers from chronic pain, increasing with age and more common in women versus men.[1,2]

Effective treatment of pain consists of a broad range of therapies, such as paracetamol, nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. Other treatment options also include antidepressants and antiepileptics.[2,3] Due to their side effects and sometimes insufficient efficacy there is still a need for new analgesic drugs.

New analgesic drugs

Targets of novel analgesic drugs fall into three main classes: (1) incremental improvement on an existing drug mechanism, (2) a novel selective mechanism arising from better understanding of the mechanism of an existing analgesic drug and (3) a completely novel mechanism arising from basic biological studies or from human pathophysiological or genomic studies.[4] Fifty nine drugs identified as analgesics were introduced in 1960-2009,[5] however there is still an ongoing search for analgesics.

Many drugs, that are promising in preclinical research, fail to show success during their development process. In general, approximately 11% of the drugs that enter phase I of clinical development get approved.[6] In 2011-2012 there were a total of 148 failures between phase II and submission. In the failures in which reason for failure was reported; 56% failed due to lack of efficacy.[7] One of the tools that can aid in the reduction of attrition rates of new compounds is the use of biomarkers. A biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention'.[8] Biomarkers intended to measure pharmacological activity can be used to assess if a specific molecular target is reached and affected as intended and what the range of concentrations and dose levels is at which pharmacological activity is exerted. Using biomarkers in early phase human trials has the advantage of showing proof-of-concept in an early phase and reduce attrition in a later phase due to lack of efficacy. It can also show lack of efficacy in an early phase and allow attrition to occur in an earlier phase of drug development.[6]

Human pain models

A case-in-point is the use of human pain models. In contrast to clinical pain, which is normally confounded by emotional, psychological and cognitive factors caused by the underlying disease, pain models have the advantage that they are less confounded by these factors.[9] A human pain test consists of two parts; an external stimulus needs to be applied to evoke pain and this pain response needs to be measured.[10] Several methods exist for the induction of evoked pain in humans, such as mechanical, thermal, electrical and chemical stimulation. A stimulus can be phasic (lasting for milliseconds to seconds) or tonic (lasting for minutes). Stimuli can be applied to different tissue types for instance skin, muscles or viscera.[11] Possible read-outs for evoked pain can be divided into several categories; psychophysical read-outs, electrophysiological read-outs or imaging read-outs. The psychophysical readouts can be subdivided into response-dependent methods, stimulus dependent methods and a combination of both.[11] In the response-dependent method the subjects rate the intensity of a given stimulus (for instance using a visual analogue scale). For the stimulus-dependent threshold, the stimulus increases until a certain threshold (pain detection threshold, pain tolerance threshold) is reached.[10] Electrophysiological readouts include microneurography, somatosensory evoked potentials and electroencephalography (EEG). Imaging readouts include functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). These methods provide a more objective measurement of pain. However, they have a larger variation in outcome measurements and are technically more difficult to perform in a large group of subjects.[11–13]

The advantages of using human pain models versus clinical pain according to Arendt Nielsen et al.[11] are:

- Experimental stimulus intensity, duration and modality are controlled and do not vary over time.
- Differentiated responses to different standardised stimulus modalities.
- The response can be assessed quantitatively and compared over time.
- Pain sensitivity can be compared quantitatively between various normal/affected/treated regions.
- Experimental models of pathological conditions can be studied and the effects of drugs on such mechanisms quantified.

Another advantage is that the evoked pain models can be easily performed in healthy subjects, who are easier to recruit into clinical studies compared

to patients, have no concomitant diseases and don't use concomitant pain medication.

A disadvantage of human models is that the applied pain stimuli are short lasting and therefore do not mimic clinical pain. 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. We hypothesise that a multimodal approach in which multiple receptors and pathways are stimulated can be expected to resemble clinical pain to a higher degree.[9]

Clinical pain versus evoked pain

Pain in patients 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 effects of drugs on pain.[14] Evoked pain models can control some of the influencing factors and is therefore sometimes more suited to investigate the analgesic effects of drugs.[14] However, evoked pain is mostly short lasting and most stimuli are applied exogenously and are focused on skin nociceptor activation. In contrast to natural occurring pain which is mostly caused by endogenous factors, longer lasting and influenced by complex emotions.[15] The advantages of the use of evoked pain models are its performance in a controlled, standardised environment and it reproducibility, however one should always ask if there is any relevance to natural occurring pain.[15] Moore and colleagues investigated which natural occurring pain was physiologically most in agreement with evoking a pain response causing the same type of pain. For instance, intramuscular electrical stimulation closely matched clinical acute musculoskeletal pain.[15]
Another approach was taken by Oertel and Lotsch to evaluate the differences between human pain models and clinical efficacy. First they looked at which drugs were effective for different pain conditions (e.g. NSAIDs were effective for inflammatory arthritis). They also investigated which drugs were effective for which pain model (e.g. NSAIDs influence pain response in laser evoked pain). If a certain drug was both effective in the model and in the particular clinical setting the model might be predictive for the clinical setting. Agreement for a large number of pain models with clinical efficacy was observed.[16] 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.[17]

Measuring pain in patients

Accurate measurement of pain in patients is important to (1) determine pain intensity, quality, and duration, (2) aid in diagnosis, (3) help decide on the choice of therapy, (4) evaluate the effectiveness of therapy and (5) study the mechanisms of pain and analgesia.[4,18] Measuring pain intensity in patients can be performed by using visual analogue scales (VAS), verbal rating scale (VRS) and numerical rating scales (NRS).[19] The main disadvantage of these scales is that they only measure one aspect (i.e. intensity) of the pain, while pain consists of much more qualities. The McGill Pain Questionnaire was developed to address this issue; it not only measures pain intensity but also the sensory and affective qualities of pain.[18,20]
Other measurements of pain include observational tools, which are used in patients who are not able to rate their pain themselves (e.g. neonates and critically ill or sedated patients).[21] More objective measures to measure nociception and pain include monitoring changes in the autonomic nervous system (e.g. heart rate/blood pressure changes and pupillometry), biopotentials (e.g. electro-encephalography (EEG) or electromyography) and neuroimaging (magnetic resonance imaging (MRI) and positron emission tomography (PET)).[22]
In studies reporting analgesic interventions, efficacy can be reported as change in the patient's report of pain or as in changes in any of the above mentioned outcome measures.[23] As part of analgesic drug development, human pain models can be a valuable addition to analgesic trials in pain patients. If no positive evidence for the efficacy of a drug in the chosen target patient population can be found, the use of one or more human pain models can provide information on the possible effect of the compound for the treatment of pain with another etiology. Also, in several chronic pain populations, such as chronic whiplash and associated disorders, rheumatoid arthritis, vulvodynia and fibromyalgia, changes in pain tolerance levels, pain modulation and augmented brain responses and altered responses to analgesics have been found.[24–26] Using evoked pain in these patients can provide insight into the analgesic mechanisms -or lack thereof- in these altered pain states.[14]

Pain models in drug development

Evoked pain tests have been used before in combination with existing analgesic compounds. Several papers have been published reviewing the evidence for several analgesic–pain model combinations.[14,16,27,28]

An overview of the most relevant previous studies reporting on analgesic-pain model combination for this thesis is provided in Table 1.

Some human pharmacology studies have used novel analgesics in combination with pain models. CHF3381, an NMDA receptor agonist and monoamine oxidase inhibitor, attenuated secondary hyperalgesia in heat and capsaicin sensitised skin.[33] However, later in a phase IIb trial for the treatment of diabetic neuropathy, a 25% reduction in pain scores compared to baseline was observed. There was also a marked reduction in pain scores in placebo-treated subjects, consequently no significant differences were found between treatment groups.[34]

The analgesic efficacy of AZD1940, a cannabinoid agonist, was investigated by using intra epidermal capsaicin injections. AZD1940 did not significantly attenuate pain or primary and secondary hyperalgesia.[35] Later, development of this drug was discontinued.[36] Another clinical study showed that treatment with this compound was not effective in reducing pain after third molar extraction at doses exerting cannabinoid-like pharmacodynamic effects.[37] These two examples demonstrate how the use of pain models in early phase drug trials can aid in the decision-making in later stages of analgesic compound development. In the AZD1940 trials negative results in clinical pain were preceded by negative results in pain models. In the CHF3381 trials, a reduction in pain scores in a clinical study was preceded by positive results in human pain models.

In the AZD1940 trial in healthy subjects, 2 methods of capsaicin administration were used to assess analgesic potency of AZD1940. No other pain models were included.[35] Possibly using other or a combination of pain models would have shown analgesic properties of this new compound. This emphasises the importance of the use of multimodal testing.

The PainCart

The PainCart described in this thesis is a multimodal battery of pain models (Figure 1). Multimodal testing with a battery of different pain models has been performed previously.[9,31,38] The batteries have in common that they induce pain via different modalities and in different tissues. The batteries differ in the individual pain models that are included.
The pain models in the PainCart have already been extensively used in previous research. A unique aspect of the PainCart, however, is that it allows the different measurements to be performed in a combined manner and in large numbers of subjects in parallel. The individual models were chosen, based on the ability to induce pain via different modalities (electrical, mechanical, thermal), in different structures (superficial and deep) and with different duration (phasic, tonic). The PainCart mainly consists of nociceptive and inflammatory pain models. It uses the following pain induction methods; electrical stimulation task, pressure stimulation task, cold pressor task, thermal stimulation (with and without ultra-violet inflammation).[39] A typical order of PainCart measurements during a clinical trial is shown in Figure 2.

ELECTRICAL STIMULATION TASK:

For cutaneous electrical pain, electrodes are placed on the skin. A stimulator device is used to deliver an electric current. Electrical stimulation activates all nerve fiber populations. Advantages of this method is that it is widely used and that the stimuli are easy to control. However, electric current is not specific for a certain nerve; activation of certain fiber types depends on the intensity of the stimulus. Another limitation of electrical stimulation is that the stimulation directly stimulates the nerve and bypasses the sensory nerve endings.[11,14,40] In this thesis two methodologies for applying electrical current are described. A single stimulus method in which intensity of a current gradually increases.[41,42] The repeated stimulus method, adapted from methods previously described,[43] in which each single stimulus pulse is repeated 5 times with a frequency of 2 Hz at the same current intensity and the repeated stimulus intensity increases gradually. This repeated application of a stimulus over time induces an integrated and more painful response, known as temporal summation. It is suggested that temporal summation might act as a biomarker of drug effects on neuropathic pain.[43]

PRESSURE STIMULATION TASK:

For the pressure stimulation task, a tourniquet cuff is placed over the calf muscle (Figure 3). The pneumatic pressure is gradually increased until the subject indicates their pain tolerance. This method of mechanical pressure pain induction is based on methods previously described,[44,45] and has shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors. Although, setup of this computer controlled technique is more complex than using handheld pressure algometry devices, the advantage of this technique is that the test can be executed in a more standardised fashion which increases reliability and sensitivity.[14]

COLD PRESSOR TASK:

The method for cold stimulation is the immersion of a hand in a cold water bath with a temperature of ~1.0°C for 2 min or until the pain tolerance threshold is reached (Figure 4). The method of cold pressor pain is based on the methods previously described.[46,47] Cold sensation is probably mediated by activity of Aδ-fibers (cold sensation) and C-fibers (cold pain).[14]

CONDITIONED PAIN MODULATION:

The cold pressor task is also used to induce conditioned pain modulation (CPM, previously known as 'diffuse noxious inhibitory control'). CPM is quantified by comparing the electrical pain detection and tolerance thresholds before and within 5 min after the cold pressor task. CPM reflects the principle that noxious stimulation to one part of the body inhibits nociceptive neurons innervating other body parts. It is a measurement of the activation of the pain modulatory mechanism, as part of the descending endogenous analgesia system.[48,49]

THERMAL STIMULATION:

Contact heat pain is induced by a 3x3 cm thermode placed on the skin of the subject's back which gradually increases in temperature. The pain response is characterised by 'first pain response' in which Aδ fibers are activated, followed by a second pain response which is mediated by C-fibers.[14]
In addition to contact heat pain on normal skin, a 3x3 cm area of the skin is irradiated by UV light. This UV irradiation produces a discernible erythema (sunburn), which causes hyperalgesia and a decrease in heat pain thresholds. This UVB model acts as a model for inflammatory pain.[50,51]

This thesis describes the validation of this battery of pain models, the use of this battery in different populations, and the application of this battery in the development of new compounds. The main question in this thesis is if this battery of pain models can be used as biomarker for clinical efficacy the development of new analgesic drugs.

OUTLINE OF THIS THESIS

The methodology of the individual tests included in the battery of nociceptive tests (PainCart) is described in **Chapter 2**. In **Chapter 3** a study is described in which the PainCart is used in combination with a set of known analgesics.
Xen2174 is norepinephrine transporter inhibitor, developed for the treatment of acute postoperative pain and neuropathic pain. This compound was administered intrathecally in healthy subjects. Pharmacodynamic measurements in this study were performed using the PainCart. This is described in **Chapter 4**.

Animal studies suggest that serotonin/noradrenalin re-uptake inhibitors co-administered with opioids have a synergistic effect. **Chapter 5** describes a study which investigated the synergistic effects of milnacipran and buprenorphine in healthy subjects using the PainCart.

Chapters 2 to 5 describe the use of the PainCart to measure pain in healthy adult subjects. **Chapter 6** investigates if pain research using the PainCart is feasible and acceptable to healthy adolescents after the administration of paracetamol.

The PainCart can be used in healthy subjects, but also in patients. **Chapter 7** describes a clinical study of a novel neurotrophic factor developed for the treatment of neuropathic pain. This study was mainly set up to investigate the pharmacokinetics and the safety of BG00010. As exploratory endpoints, PainCart measurements were performed in sciatica patients to assess the feasibility to perform these tests in patients.

In another study, PainCart measurements were also performed in patients with diabetes mellitus, patient with painful diabetic neuropathy (PDN) and patients with chronic idiopathic axonal polyneuropathy (CIAP). Possible differences between healthy subjects and patients may be important in the design of early phase clinical drug studies in which multi-modal pain testing is considered. **Chapter 8** describes the main differences between performing the PainCart measurements in healthy volunteers versus populations with a chronic pain condition.

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TABLE 1

Overview of different analgesic groups and their effect on different pain models.

Drug	Pain model				
	Electrical pain	**Cold pressor**	**Pressure pain**	**UVB and thermal**	**Thermal grill**
Strong opioids	Positive evidence[16]	Positive evidence[16]	Mixed evidence[16]	Positive evidence[16]	Positive evidence[29]
NMDA antagonist	Positive evidence[27]	Unknown	Positive evidence[16]	Mixed evidence[27]	Positive evidence[30]
NSAIDs	Positive evidence[27]	Mixed evidence[16]	Negative evidence[16]	Positive evidence[16]	Unknown
TCAs	Positive evidence[27]	Mixed evidence[27]	Positive evidence[31]	Unknown	Unknown
Sodium channel blocker	Unknown	Positive evidence[32]	Unknown	Unknown	Unknown
A2δ ligands	Positive evidence[27]	Negative evidence[27]	Unknown	Positive evidence[27]	Unknown

UVB indicates ultraviolet B; NSAID, nonsteroidal anti-inflammatory drug; TCA, tricyclic antidepressant and NMDA, N-methyl-D-aspartate.

FIGURE 1

The PainCart

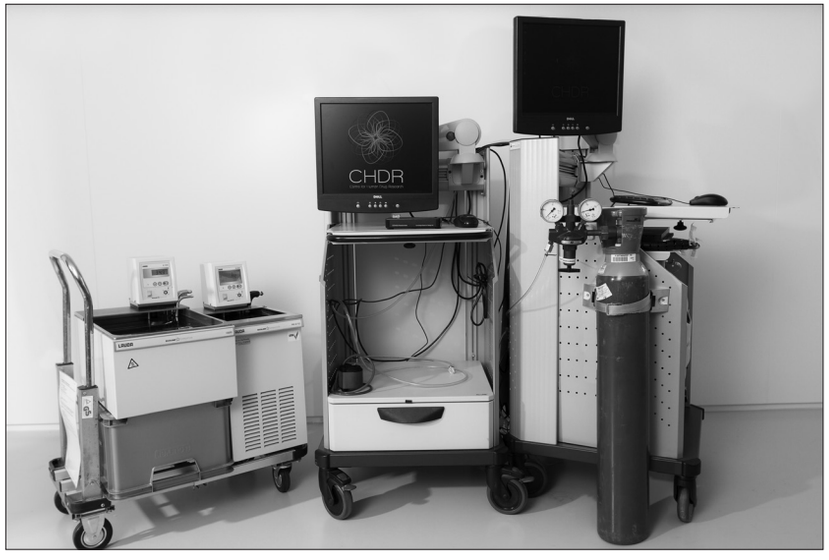
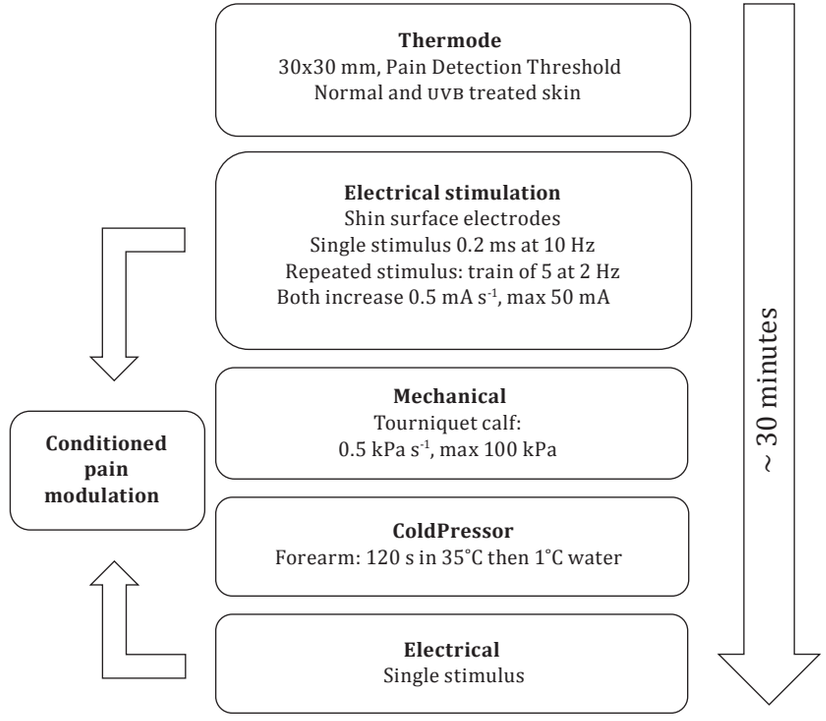


FIGURE 2

Overview and sequence of pharmacodynamic tests.



°C, degree Celsius; Hz, hertz; kPa, kilopascal; ms, millisecond; mA, milliampere; UVB, ultraviolet B

FIGURE 3
Pressure stimulation task

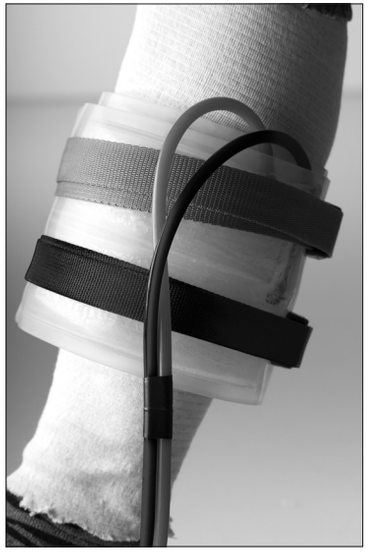


FIGURE 4
Cold pressor task

CHAPTER II

Determining pain detection and tolerance thresholds using an integrated, multi-modal pain task battery

Journal of Visualized Experiments, 2016 Apr 14;(110)

This manuscript was originally published as video article. The video component of this article can be found at http://www.jove.com/video/53800/

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ABSTRACT

Human pain models are useful in the assessing the analgesic effect of drugs, providing information about a drug's pharmacology and identify potentially suitable therapeutic populations. The need to use a comprehensive battery of pain models is highlighted by studies whereby only a single pain model, thought to relate to the clinical situation, demonstrates lack of efficacy. No single experimental model can mimic the complex nature of clinical pain. The integrated, multi-modal pain task battery presented here encompasses the electrical stimulation task, pressure stimulation task, cold pressor task, the UVB inflammatory model which includes a thermal task and a paradigm for inhibitory conditioned pain modulation. These human pain models have been tested for predicative validity and reliability both in their own right and in combination, and can be used repeatedly, quickly, in short succession, with minimum burden for the subject and with a modest quantity of equipment. This allows a drug to be fully characterised and profiled for analgesic effect which is especially useful for drugs with a novel or untested mechanism of action.

INTRODUCTION

Human pain models are useful in the evaluation of analgesics, providing information about a drug's pharmacology and identifying potentially suitable therapeutic populations. Yet the field is plagued by studies yielding inconsistent findings. The reason for these differences has been put down to the use of different pain assessment methods and different subject populations.[1] To correctly predict clinical analgesia, the right pain model is needed.[2,3] Nevertheless, mechanism-based pain model selection has led to many failures in predicting clinical efficacy.[4]

The need to use a comprehensive battery of pain models is highlighted by studies whereby only a single pain model, thought to relate to the clinical situation, demonstrates lack of efficacy. No single experimental model can replicate the complex nature of clinical pain. Therefore, one pain model cannot be used exclusively to screen the pharmacological mechanism of action of a compound intended to treat clinical pain. Furthermore, the use of a panel of pain models allows a drug to be fully characterised and profiled. This is especially useful for drugs that have a novel or untested mechanism of action.

There are various paradigms for assessing validity of animal or human models of disease such as investigating the predictive, construct, concurrent or convergent, discriminant, etiological, and face validity of a model.[5] A pain model can be considered of higher value and more relevant to human disease the more criteria it satisfies. However, a more simple measure of validity is to evaluate a model's predictive validity and reliability.[6]

With early phase drug development there are also other considerations that need to be taken into account to assess the value of a pharmacodynamic measurement. The assessment should not be too burdensome, should not take too long, and the results should be quickly evaluable, automated and secure data collection is desirable. Also the ability to test several subjects concurrently requires equipment that is technically standardised and well characterised.[7]

While other evoked pain batteries exist, their objective is more directed towards the classification of pain and for assessing pathophysiological pain mechanisms.[8] Yet other batteries aim to represent a broad range of pathophysiology including pain models for muscle and visceral pain.[9] While suitable for testing in acute situations, their invasive nature do not make them suitable for testing repeatedly for longer periods.

The pain models presented here satisfy many of the above mentioned criteria making them especially useful for clinical studies in both healthy

subjects and patients. The multi-modal pain task battery that is presented here encompasses the electrical stimulation task, pressure stimulation task, cold pressor task, UVB inflammatory model that includes a thermal task and a inhibitory conditioned pain modulation (iCPM) paradigm that takes advantage of interactions between the tasks The human pain models presented here have been tested for predicative validity and reliability both in their own right and in combination.

Protocol

Ethics statement: Procedures involving human subjects have been approved in numerous studies by an independent ethics committee the Stichting Beoordeling Ethiek Biomedisch Onderzoek (Foundation BEBO) and the Leiden University Medical Center.

1 INTEGRATED PAIN ASSESSMENT TASKS

NOTE: The task administration and interface is based on Spike2 software and an analogue-to-digital converter that performs the conversions needed for stimulus triggering and signal recording. This ensures uniform task administration, data capture, handling and storage, and standardising the delivery of tasks by controlling the stimulus generation equipment while presenting instructions to the subject and feedback on slider position via a second monitor.
NOTE: Perform the tasks in short succession and in the order presented. The duration of performing all the tasks is approximately 30 min.

1. PAIN SCORING

NOTE: For most tasks, stimuli of progressively increasing intensity are presented.

» Prior to the task, present the subject with an electronic visual analogue scale (eVAS) slider.
» Instruct the subject to indicate the intensity of their pain on a scale from 0 (none) to 100 (intolerable pain) by moving the slider from left to right.
» During training, and when necessary, provide the subjects with standardised definitions (Table 1) and instructions.
» Inform the subject that moving the slider all the way to the left ends the administration of the painful stimulus.
» Record when stimulus becomes painful (eVAS > 0), corresponding to the pain detection threshold. Record when the pain is no longer tolerable to the subject (eVAS = 100), corresponding to the pain tolerance level of the subject; and the area under the stimulus-response curve (AUC).

NOTE: During training, it is beneficial to provide subjects with a context of pain intensity. Following each task assess the maximal pain intensity using a 100 mm eVAS, with 0 and 100 defined as 'no pain' and 'worst pain imaginable', respectively (Table 1).

2 ELECTRICAL STIMULATION TASK

NOTE: The task has been shown to primarily assess nociception generated from the Aδ and C sensory afferent fibers, which pass nociceptive signals from the periphery to the spinal cord. The Aδ fibers conduct the signal relatively rapidly, causing the sharp localization of pain and the rapid spinal response which is perceived during a transcutaneous electrical stimulus.[10] The method of electrical stimulation is based on methods described previously.[11]

» Clean an area of skin with skin preparation gel overlying the tibial bone, 100 mm distal from the caudal end of the patella. If required, shave the area beforehand.
» Place two Ag-AgCl electrodes on the skin. Place the middle of the first electrode (anode) 100 mm distal to the caudal end of the patella. Place the middle of the second electrode (cathode) directly (±135 mm) underneath the first.
» Record the resistance of the 2 electrodes using an ohmmeter. Ensure it is <2 kΩ. Optionally, remove the electrodes and re-cleanse the skin with skin preparation gel. Instruct the subject to sit comfortably with their foot flat on the floor.
» Connect the electrodes to a constant current stimulator and apply a tetanic pulse from 0 mA in steps of 0.5 mA/s (cutoff 50 mA), with a frequency of 10 Hz with a duration of 0.2 ms.

3 PRESSURE STIMULATION TASK

NOTE: This method of pressure pain induction has been shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors[12] and is based on methods previously described.[13]

» Place an 11 cm wide tourniquet cuff over the gastrocnemius muscle. Instruct the subject to sit comfortably with their foot flat on the floor. Inflate with a constant pressure rate increase of 0.5 kPa/s up to 100 kPa. Control the pressure with an electro-pneumatic regulator.

4 COLD PRESSOR TASK

NOTE: The cold pressor task involves the submersion of an extremity (generally a hand) into cold water. It is used in clinical studies to investigate cardiovascular responses and nociception. It is also a method to induce iCPM (formerly known as diffuse noxious inhibitory control (DNIC)-like effect).[14] The method of cold pressor pain is based on the methods previously described.[15,16]

» Prepare two thermostat-controlled, circulating water baths set at 35.0 ± 0.5°C and 1.0 ± 0.5°C.
» Place a 35 cm tourniquet on the subject's non-dominant upper-arm. During hand immersion, either regulate the blood pressure manually using a sphygmomanometer or by using a custom built electro-pneumatic regulator.
» Instruct the subject to sit comfortably with their palm flat, fingers spread wide without touching the bath and rate their pain intensity using the eVAS.
» Instruct the subject to place their non-dominant hand into a warm water bath for 2 min.
» At 1 min 45 s inflate the blood pressure cuff on their upper-arm to 20 mmHg below resting diastolic blood pressure.
» At 2 min instruct the subject to move their hand from the warm water bath, directly placing their hand into the cold water bath to similar depth.
» After reaching pain tolerance, or after reaching a time limit (120 s), instruct the subject to remove their arm from the water. At this point deflate the blood pressure cuff and give the subject a towel to dry their forearm.

5 CONDITIONED PAIN MODULATION PARADIGM

NOTE: iCPM is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[14] The degree of iCPM is assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and after the cold pressor task.

» Repeat the electrical stimulation task (section 2) within 5 min after the end of the cold pressor task.

6 ULTRA-VIOLET INFLAMMATION MODEL

NOTE: The UVB "sun burn" model is a pain model in which erythema is induced on the skin by exposing the skin to UVB light in a well-controlled and reproducible manner. This exposure causes changes to the skin which leads to pain perception being intensified in the affected area (primary hyperalgesia) and is used as biomarker for inflammatory pain. This inflammation model is based on the methods previously described.[9] Inform subjects that the UVB exposure may leave long-lasting (6-12 months) skin marking/tanning and that exposure to UVB in general has been linked to premature skin aging and skin cancer.

1 DETERMINING A SUBJECT'S MINIMAL ERYTHEMIC DOSE (MED)

» Turn on the UVB lamp and allow it to warm up for at least 10 min prior to use. Replace the fluorescent tubes once output is < 3.0 mW/cm2 (after approximately 50-100 working h).
» Instruct the subject to stand with their right hand holding their left shoulder. Place the UVB lamp on the right upper back/shoulder of the subject, in direct contact with the skin. Only induce the erythema on even-toned healthy skin; moles, tattoos, nevus and acne must be avoided.
» Apply the UVB exposure at the screening visit in ascending doses (see Table 2) to 6 different 1 x 1 cm areas of skin on the back to determine the individual UVB dose that produces the first clearly discernible erythema (minimal erythemic dose (MED).
» Assess the erythemic response 24 h (± 2 h) after the exposure of the 6 doses. Determine the MED visually, by means of consensus of two observers with good colour vision, by observing which dose produces the first clearly discernible erythema. Choose the 3rd UVB dose to approximate the mean MED for the respective skin type.[17]

2 UVB EXPOSURE

» Apply a 3 x 3 cm UVB exposure equivalent to the subject's 3-fold individual MED. Apply this UVB exposure to the subject's back 24 h prior to the first battery of tasks/dosing. Ensure the UVB exposure produces a homogeneous, well-demarcated area of skin erythema and hyperalgesia.

3 ASSESSMENT OF SKIN THERMAL DETECTION THRESHOLD

» Using a 3x3 cm thermode measure the thermal pain detection threshold on normal skin contralateral to the site of UVB irradiation followed by UVB irradiated skin. Set the temperature initially to 34°C, then ramp up by 0.5°C/s. Record the average pain detection threshold of 3 stimuli.

7 SUBJECTS

NOTE: In addition to standard selection criteria and to ensure a reasonably homogeneous subject population the following exclusion criteria should be considered. Exclude subjects who meet the following criteria.

- Indicate nociceptive tasks are intolerable at screening.
- Achieve tolerance of >80% of maximum input intensity for cold, pressure and electrical tasks (to exclude pain tolerant individuals which may obfuscate an analgesic effect).
- Have any current, clinically significant, known medical condition, particularly any existing conditions that would affect sensitivity to cold (such as atherosclerosis, Raynaud's disease, urticaria, hypothyroidism) or pain (parasthesia etc.). Use healthy subjects only.
- Use prescription or nonprescription drugs (especially analgesics) and dietary/herbal supplements within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study treatment.
- Have dark skin (Fitzpatrick skin type V or VI), widespread acne, tattoos or scarring on back (due to interference with the UVB model).
- Sunbathe or have used sunbeds in the 6 months prior to screening or are unable to not be exposed to excessive sunlight or to sunbathe for the duration of the study. Skin coloration due to sunlight and sunburn affect the UVB study endpoints.

NOTE: Unless contraindicated, women should be included and where possible menstrual cycle should be either monitored or controlled for (e.g., testing only during luteal phase).

Representative results

The primary outcome variable of interest is the PTT for the electrical stimuli, pressure and cold pressor tasks, and the PDT for the thermal (heat) stimuli on normal and UVB-exposed skin (Table 3). Data collected from the pain model assessments should be summarised descriptively (absolute values and change from baseline) by time and treatment. In addition, plots showing the mean (95% confidence interval (CI)) result and the mean change from baseline (95% CI) at each time point by treatment should be presented (see Figures 1 and 2). Results following placebo treatment should be relatively stable throughout the study day (Figures 1 and 2). Analgesic responses i.e., increases in the PDT or PTT, should reflect the pharmacokinetic properties of the drug. For the cold pressor task, the relatively rapid onset of action and short half-life of fentanyl and ketamine are reflected in the increase in PTT times (Figure 1). In contrast, the increase in PTT following pregabalin administration mirrors the pharmacokinetics of this drug which has a longer tmax and half-life (Figure 2). The known insensitivity of the cold pressor task for other analgesics are shown by there being little change from placebo (Figures 1 and 2). Nonetheless, the other tasks in this battery are sensitive to these drugs e.g., the UVB model captures the analgesic properties of the NSAID ibuprofen – allowing for drugs to be fully characterised.

Depending on the ultimate design of the study, analyse the endpoints with a mixed model analysis of variance (ANOVA) 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 with the average baseline measurement as covariate.

Discussion

For novel and established analgesics alike, a profiling approach is proposed that utilizes reliable and predictive multi-modal pain models. In contrast to other more onerous pain tasks, such as chemical (e.g., capsaicin, nerve growth factor) hyperalgesia or visceral pain models, the pain tasks mentioned in this protocol can be used repeatedly, quickly, in short succession, with minimum burden for the subject and with a modest quantity of equipment. By using a battery of pain biomarkers such as the one mentioned in this protocol, (plasma) concentration-effect relationships can be established leading to better estimation of a drug's pharmacological activity. Thereby, more rational choices can be made regarding the therapeutic effect of a drug rather than simply using animal data and the maximum tolerated dose derived from adverse events.[7]

The design of a clinical study utilizing these pain models needs careful consideration. While the aforementioned pain models provide a suitable basis for screening potentially analgesic drugs, other factors need to be considered, especially taking into account the pharmacological mechanisms of the drug and its pharmacokinetics.[18] Standard practice for researching analgesics should be applied, including the use of positive controls and designing studies that are randomised (balanced, where applicable), placebo-controlled, and double-blind. Furthermore, it is critical that pain tasks are performed consistently between subjects, with standardised instructions and environmental conditions. While there is risk of an interaction

between the tasks e.g., sensitization or additive effects, careful study design and consistent delivery of the tasks minimizes this. In fact, one of these interactions is taken advantage of in this battery by incorporating the ICPM paradigm.

When deciding to include pain tasks in a study, the overall burden of the study should be considered as this may restrict the number of treatment arms or the number of times a task is repeated. If other tasks are used, e.g., measures of sedation or alertness, this may limit the total number of tasks a subject can perform within a study day; this is especially true if populations other than healthy adult subjects are included *e.g.* adolescents or chronic pain patients.

A series of validated pain tasks early in drug development is crucial to bridge findings in the laboratory and those in the clinical situation, provide valuable information in regard to the mechanism of action of a new drug, choose the most applicable patient population to be studied; and ascertain the most relevant nociceptive test for more intensive PK/PD modelling. PK/PD modelling can be used to identify responders and non-responders, better estimate the time-course of analgesia or aid in the development of different formulations.[19] By characterizing analgesics in both healthy subjects and patients, a translational connection between early phase development and the clinic can be established. It may also be used to provide information on the pain physiology and pathophysiology in these populations.[20] Eventually, the ability to link the efficacy profile of a drug to the pain profile of a patient could help guide individualised treatments in the future.[21]

Human pain models are valuable tools used to assess the analgesic potential of novel compounds and predict their clinical efficacy. While the implementation of these models can be complex and multifaceted, with proper execution, these pain models can provide predictive and reliable results.

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TABLE 1

Standard definitions of VAS anchor-points.

Threshold	Verbal instructions to subject (during training and as a reminder)	Resulting eVAS scores
PDT (Pain Detection Threshold)	"Start moving the eVAS-slider when first change in sensation from non-painful to ainful is felt "	> 0 (= 1)
PTT (Pain Tolerance Threshold)	"When pain intensity is no longer tolerable"	= 100 (intolerable pain)
Post-task VAS	"An example of the worst pain imaginable could be a surgical treatment without anesthetic"*	max 100 (worst imaginable pain)

*Pain is a unique personal experience; this definition is provided only to provide a consistent (nociceptive) frame of reference and is chosen as it somewhat negates experiences of loss, psychological suffering, and vicarious pain.[22]

TABLE 2

UVB dose regiment per skin type (mJ cm^{-2}).

Skin type	I	II	III	IV
Dose				
#1	64	126	176	234
#2	91	177	248	330
#3	128	251	351	467
#4	181	355	496	660
#5	256	502	702	934
#6	362	710	993	1321

TABLE 3

The outcome variables (endpoints) defined for a study.

Task	Endpoints
Primary Endpoints	
Thermal Task (Normal Skin)	PDT
Thermal Task (UVB Skin)	PDT
Electrical Task (pre-cold pressor)	PTT
Pressure Task	PTT
Cold Pressor Task	PTT
Secondary Endpoints	
Electrical Task (pre-cold pressor)	PDT, AUC, and post-test VAS
Pressure Task	PDT, AUC, and post-test VAS
Cold Pressor Task	PDT, AUC, and post-test VAS
Conditioned Pain Modulation Response (change from electrical pre- and post-cold pressor)	PDT, AUC, and post-test VAS

Pain Detection Threshold (PDT), Area Under the Visual Analogue Scale (VAS) pain Curve (AUC), and post-test VAS.

FIGURE 1

Effect of intravenous analgesics on cold pressor pain tolerance thresholds. Example time course of the mean change from baseline profile in least squares means (95% CI error bars) for the pain tolerance threshold for cold pressor task after 30 min intravenous administration of placebo (circle), (S)-ketamine 10 mg (triangle), fentanyl 3 mcg/kg (square), and phenytoin 300 mg (diamond).

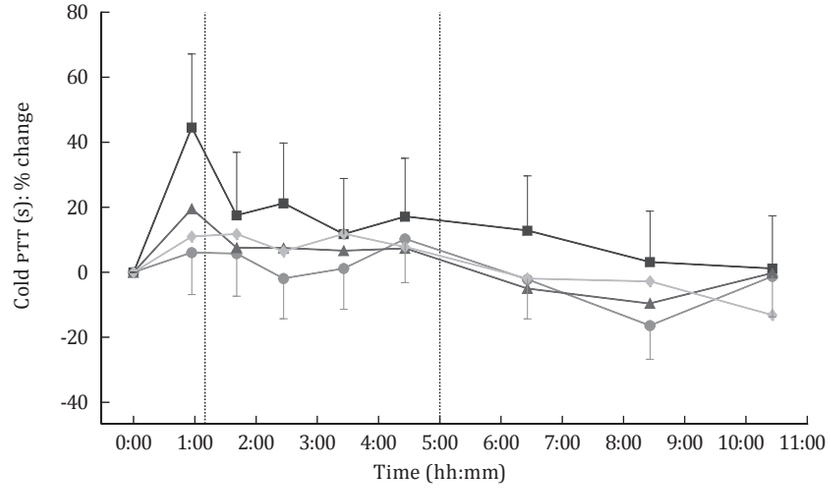
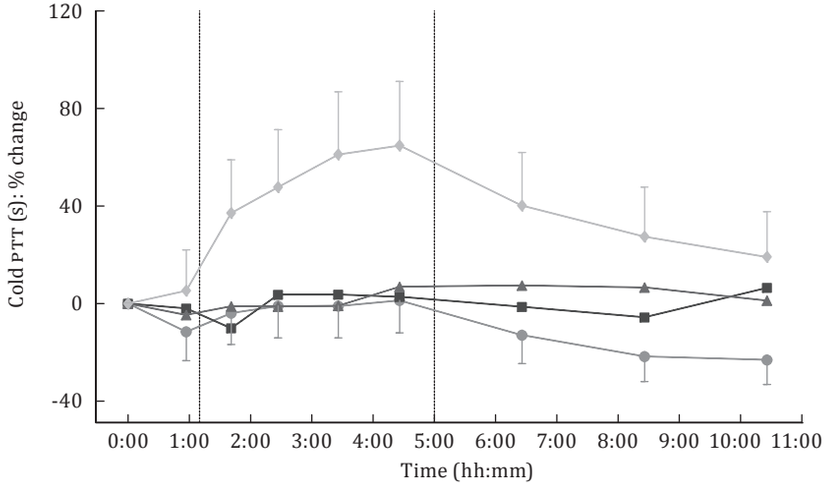


FIGURE 2

Effect of oral analgesics on cold pressor pain tolerance thresholds. Example time course of the mean change from baseline profile in least squares means (95% CI error bars) for the pain tolerance threshold for cold pressor task after oral administration of placebo (circle), imipramine 100 mg (triangle), ibuprofen 600 mg (square), and pregabalin 300 mg (diamond).



CHAPTER III

The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study

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ABSTRACT

The aim was to investigate the ability of a battery of pain models to detect analgesic properties of commonly used analgesics in healthy subjects. The battery consisted of tests eliciting electrical, mechanical and thermal (contact heat and cold pressor)-pain and included a UVB model, the thermal grill illusion and a paradigm of conditioned pain modulation. Subjects were administered fentanyl 3 μg kg^{-1}, phenytoin 300 mg, (S)-ketamine 10 mg and placebo (part I), or imipramine 100 mg, pregabalin 300 mg, ibuprofen 600 mg and placebo (Part II). Pain measurements were performed at baseline and up to 10 h post-dose. Endpoints were analysed using a mixed model analysis of variance. Sixteen subjects (8 female) completed each part. The pain tolerance threshold (PTT) for electrical stimulation was increased (all $P<0.05$) compared to placebo for (S)-ketamine (+10.1%), phenytoin (+8.5%), and pregabalin (+10.8%). The PTT for mechanical pain was increased by pregabalin (+14.1%). The cold pressor PTT was increased by fentanyl (+17.1%) and pregabalin (+46.4%). Normal skin heat pain detection threshold was increased by (S)-ketamine (+3.3%), fentanyl (+2.8%) and pregabalin (+4.1%). UVB treated skin pain detection threshold was increased by fentanyl (+2.6%) and ibuprofen (+4.0%). No differences in conditioned pain modulation were observed. This study shows that these pain models are able to detect changes in pain thresholds after administration of different classes of analgesics in healthy subjects. The analgesic compounds all showed a unique profile in their effects on the pain tasks administered.

INTRODUCTION

Pharmaceutical science continues to search for suitable biomarkers that can assist in predicting the therapeutic potential of analgesic medication and, therefore, its efficacy in the target population. Data intensive, early-phase studies provide a valuable opportunity that can offer this translational information.[1] A series of nociceptive pain tests used early in drug development could bridge preclinical findings and those in the clinic to provide valuable information about the mechanism of action of a new drug and to benchmark new drugs to existing analgesics. The need to use a comprehensive battery of pain models is highlighted by studies in which only a single pain model, thought to relate to the clinical situation, demonstrates lack of efficacy.[2,3] A single evoked model cannot replicate the complex nature of clinical pain. Therefore, one evoked pain model cannot be used exclusively to screen the pharmacological mechanism of action of a new compound, for which this mechanism has not been demonstrated earlier. The aim of this study was to pharmacologically validate an integrated range of human pain models that can be used as a combined screening tool for early stage clinical drug development.

Each pain model in this battery has been used before.[4–7] However, the integrated execution of these tests has not yet been investigated, and it is mostly unclear how well-known and frequently used analgesic compounds influence the pain tests when used in this integrated manner. Data obtained from early phase clinical studies may be used for the determination or confirmation of a drug's mechanism of action. Furthermore, results obtained from pain models could be useful for the prediction of the efficacy of the drug in future clinical populations or potential disease states.[8] This battery of tests should be able to help establish whether a drug is acting centrally or peripherally, whether it is more suitable for a particular modality of pain (nociceptive, neuropathic or inflammatory), and which other effects contribute to its mode of action (sedation, tolerance, etc.). Nociceptive tests, when used in combination with pharmacokinetic (PK) parameters, can be used to provide information regarding future dose selection of new drugs. Particularly if used in combination with pharmacokinetic-pharmacodynamic (PK/PD) modelling and simulation techniques, the establishment of a threshold of pharmacological activity may be determined and used for dose prediction.[9]

The models in this study were chosen to represent a broad range of pain modalities and nociceptor function, combined with the possibility

to perform these pain tests in a standardised setting in clinical studies. Regarding the choice of compounds, a selection was made of distinctly different, relevant, targets of analgesia. The analgesic mechanism of action of these compounds was compared using the existing literature.[4–6,10–13] Specific compounds, representative of a range of mechanistic classes, were chosen if they showed analgesic efficacy in previous pain models in humans or if their efficacy in pain models was expected but yet unknown. It was hypothesised that the battery of pain models would show distinct response patterns for the different analgesic classes.

METHODS

Subject and study design

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Healthy male and female subjects between 18 and 45 years with a body mass index of 18-30 kg m^{-2} were enrolled. All subjects gave written informed consent. The subjects underwent a full medical screening, including taking medical history, a physical examination, blood chemistry and haematology, urinalysis, electrocardiogram 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, wide-spread acne, tattoos or scarring on the back were excluded due to the inability to assess MED accurately. Also, subjects who were regular users of any illicit drugs, had a history of drug abuse or a positive drug screen at screening were excluded. Smoking and the use of xanthine-containing products was not allowed during dosing days. Alcohol was not allowed at least 24 h before each scheduled visit or during the stay in the research unit. Except for contraception, subjects were not allowed to use prescription medications within 7 days and over-the-counter analgesics within 3 days of nociceptive assessments. Female subjects were required to have an intrauterine device, a contraceptive implant or were willing to continuously use oral contraceptives (i.e. skip their menstruation) during the study period, to prevent influences of menstrual phase.[14]

This was a two-part, randomised, double blind, placebo-controlled, four-way crossover, single dose study. The total number of planned subjects was 16 in each part. In part I subjects received the study drug or placebo intravenously over a 30 min time period in the antecubital vein. Treatment consisted of fentanyl 3 μg kg^{-1} (Hameln Pharmaceuticals GmbH, Hameln, Germany), phenytoin 300 mg (Diphantoïne, Apotex Europe Ltd, Leiden, The Netherlands), (S)-ketamine 10 mg (Ketanest-S, Eurocept BV, Ankeveen, The Netherlands) and sodium chloride 0.9% (placebo). In Part II, subjects received the over encapsulated study drug or placebo orally with 150 mL of still water. Treatment consisted of imipramine hydrochloride 100 mg (Centrafarm BV, Etten-Leur, The Netherlands), pregabalin 300 mg (Lyrica, Pfizer Limited, Kent, UK), ibuprofen 600 mg (Nurofen oval tablet, Reckitt Benckiser Healthcare BV, Hoofddorp, The Netherlands) and placebo tablets (lactose monohydrate with 1% magnesium stearate). Subjects participated in either part I or Part II in which they received all four treatments. The study treatments were randomly allocated based on a 4 x 4 William's square. The randomisation code was generated by a study-independent statistician using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Each treatment period consisted of two study visits to the clinical research unit. During the first visit UVB erythema was induced. On the morning of the next day, subjects received the study treatment after which the PD and PK assessments were performed (Figure 1). Subjects were discharged at the end of the study day. There was a 1-week washout period between treatment periods.

PD assessments

Nociceptive (pain) detection and tolerance thresholds were measured using a battery of human pain models. The battery is an integrated range of tests for measuring different modalities of nociception and takes approximately 30 min to complete (Figure 1).[15] It aims to assess as objectively as possible the levels of pain induced by several noxious mechanisms in human subjects. A training session was included as part of the screening examination to reduce learning effects during the study. All tests have previously been shown to be sensitive to the effects of analgesics in healthy adults. All measurements were performed in a quiet room with ambient illumination. Per session, there was only one subject in the same room.

For the electrical stimulation tests, the pressure stimulation test and the cold pressor test, pain intensity was measured continuously (beginning

from when the first stimulus was applied until the predetermined 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 pain intensity reaches the maximum possible score. For each test the pain detection threshold (PDT), pain tolerance threshold (PTT) and area under the curve (AUC) were determined. The AUC was calculated as the surface under the pain intensity-stimulation (-time for cold pressor) curve.

Thermal grill — The thermal grill consisted of a set of eight juxtaposed bars of cold and warm innocuous temperatures (18°C and 42°C) on which the subject placed their dominant hand for 20 s. During this time, the subject rated unpleasantness, pain sensation and thermal sensation using the eVAS-slider.

Thermode testing and UVB model — The method of UVB irradiation was based on methods previously described.[16] UVB irradiation (TL01[narrow-band], Phillips, Amsterdam, The Netherlands) was applied at the screening visit in ascending doses to determine the individual UVB dose that produced the first clearly discernible erythema. The threefold individual MED of UVB was applied 24 h 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 x 3 cm. Subsequently, a 3x3 cm thermode (TSA-II, Medoc Ltd., St. Ramat Yishai, Israel) was used to measure pain detection thresholds (initially 34°C, ramp 0.5°C s^{-1}, average of three stimuli) on the normal skin contralateral to the site of UVB irradiation and on the UVB irradiated skin (cut-off 50°C).

Electrical stimulation test — For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™) were placed on cleaned, scrubbed, and if required, shaved skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be <2 kW. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5, Digitimer, Cambridge, UK).

For the single stimulus, adapted from methods previously described[17,18] (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 mA s^{-1} (cut-off 50 mA).

For the repeated stimulus, adapted from methods previously described,[19] each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3-8 s between the repetitions. Current intensity increased from 0 mA in steps of 0.5 mA s^{-1} (cut-off 50 mA). Pain detection threshold was taken as the value (mA) whereby a subject indicated either: all 5 stimuli were painful, or the train of 5 stimuli started feeling nonpainful but ended feeling painful (VAS > 0). The pain intensity for each stimulation was measured using the eVAS slider, until pain tolerance threshold or a maximum of 50 mA was reached.

Pressure stimulation test — The method of mechanical pressure pain induction was based on methods previously described, and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.[20,21] Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa s^{-1}. 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, at which point the device released pressure to the cuff.

Cold pressor test — The method of cold pressor pain was based on the methods previously described[22,23] and is the most commonly used test to induce conditioned pain modulation (CPM, previously known as 'diffuse noxious inhibitory control').[24] Subjects placed their nondominant hand into a water bath at 35 ± 0.5°C for 2 min. At 1 min 45 s a blood pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min 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.

CPM — CPM is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[24] The degree of CPM was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the cold pressor test.

Measurements of drug concentrations in plasma — Samples for determination of compounds in plasma were obtained at baseline, 0.5, 1, 2, 3, 4, 6, 8 and

10 h after the start of administration. Samples were collected in 6 mL K_2EDTA tubes. Plasma was separated within 30 min of blood collection by centrifugation at 2000 g for 10 min. All samples were stored in an upright position at – 40°C. Drug concentrations in plasma were determined using Liquid Chromatography–Mass Spectrometry (LC-MS/MS). The analytical range was 0.200-50.0 ng ml^{-1} for fentanyl, 1.00-200 ng ml^{-1} for (S)-ketamine, 0.500-100 ng ml^{-1} for norketamine, 20.0-10 000 ng ml^{-1} for phenytoin, 0.5 -100 ng ml^{-1} for imipramine and desipramine, 20.0-20 000 ng ml^{-1} for pregabalin and 100-100 000 ng ml^{-1} for ibuprofen. Quality control for the analytical performance of the assays for all compounds showed acceptable performance. Standard curves were linear for the ranges tested (R>0.99 for all compounds). Control runs were performed in low, medium and high concentrations of each compound. Coefficients of variation varied from 1.5% to 7.9%.

Statistical analysis

The sample size calculation was based on previous studies performed in our centre. The detectable effect sizes using a paired t-test with a 0.050 two-sided significance level and 16 subjects were as follows (standard deviations [SDs] are rounded): electrical stimulation repeated stimulus AUC 225 (6%; assuming an SD of 300), electrical stimulation single stimulus AUC 450 (16%; assuming an SD of 600), pressure stimulation AUC 525 (9%; assuming an SD of 700), cold pressor Area Above the Curve 337 (17%; assuming an SD of 450).

PK analysis was performed using noncompartmental analysis. The peak concentration and the time to the peak concentration were recorded as observed. In addition, the terminal half-life, the area under the plasma concentration-time curve from time zero to the time of the last sample (AUC_{0-last}) and from time zero to infinity (AUC_{0-inf}), the volume of distribution (V_d) and the clearance were determined for all compounds. AUC's were calculated using the linear trapezoidal method. Calculations were performed using R v2.12.0 (R Foundation for Statistical Computing, Vienna, Austria).

PDT and PTT variables follow a log-normal distribution and were therefore log-transformed before analysis. Transformed parameters were back-transformed after 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 squares 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. The contrasts for the relevant time periods based on the PK profiles of the compounds (0-1 h for (S)-ketamine, 0-5 h for fentanyl ibuprofen and pregabalin, 0-10 h for phenytoin and imipramine) are presented. 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.

RESULTS

A total of 39 subjects, of whom 18 were female, were randomised by treatment (Figure 2); subjects had a mean age of 22.5 ± 2.8 years and had a mean body mass index of 21.8 ± 1.7 kg m^{-2}. In part I where we studied the effects of intravenous analgesics, 18 subjects received placebo treatment, 17 fentanyl, 17 (S)-ketamine and 20 phenytoin. In one subject the dose administration was prematurely stopped due to an adverse event (syncope) during phenytoin administration. In the oral Part II, 16 subjects received placebo, 17 ibuprofen, 17 imipramine and 16 pregabalin. In both parts 16 subjects completed all four study periods.

An overview of the pharmacodynamic output variables is provided in Table 1 (part I), Table 2 (Part II), Figure 3 and Figure 4. Differences compared to placebo for the cold pressor test were observed after administration of fentanyl (pain tolerance threshold, PTT; estimate of difference[95% confidence interval]/17.1%[2.3%-33.9%]) and pregabalin (pain detection threshold, PDT and PTT; 36.8%[5.9%-76.8%]/46.4%[27.1%-68.6%]). Electrical stimulation single stimulus parameters changed after administration of (S)-ketamine (PTT; 10.1%[0.2%-20.9%]), phenytoin (PDT and PTT; 31.5%[10.3%-56.8%]/8.5%[1.4%-16.1%]), and pregabalin (PTT; 10.8%[2.4%-19.9%]). The PTT for pressure pain was only increased by pregabalin (14.1%[4.3%-24.9%]). The normal skin heat PDT increased after administration of (S)-ketamine (3.3%[1.1%-5.6%]), fentanyl (2.8% [1.1%-4.5%])

and pregabalin (4.1%[1.3%-7.0%]). UVB-treated skin PDT increased after administration of fentanyl (2.6%[1.2%-4.1%]) and ibuprofen (4.0%[1.8%-6.3%]). Thermal grill maximum unpleasantness was not influenced by any of the compounds administered. After administration of ibuprofen, an increase was observed for the thermal grill pain intensity (1.25[0.25-2.25]). Inhibitory conditioned pain modulation was influenced by administration of imipramine and pregabalin. These compounds also caused an increase in the difference between pre- and post-cold pressor electrical stimulation PDT (0.88[0.06-1.70]/1.95[0.84-3.06]). The effect sizes for the compounds during the relevant analysis period compared to placebo for the different pain models are shown in Figure 5.

The observed PK parameters for the compounds and their active metabolites are listed inTable 3.

All subjects experienced at least one adverse event (AE) during their participation. In part I, the incidence of AEs was 100% in the active treatment groups (fentanyl, (S)-ketamine and phenytoin) compared to 33% in the placebo group. In Part II, 100% of the subjects receiving imipramine, 87.5% of the subject receiving pregabalin, 41.2% of the subjects receiving ibuprofen and 50% of the subjects receiving placebo tablets reported AEs. In part I, the most reported AEs were dizziness (82%), nausea (65%) and feeling hot (53%) for fentanyl; dizziness (82%), nausea (35%) and feeling abnormal (29%) for (S)-ketamine and pain in extremity at administration site (60%), dizziness (55%) and nausea (30%) for phenytoin. In Part II, the most reported AEs were nausea (12%), fatigue (12%) and dizziness (12%) for ibuprofen; somnolence (65%), nausea (59%) and dizziness (29%) for imipramine and dizziness (56%), somnolence (31%) and nausea (31%) for pregabalin. All AEs were mild or moderate in severity.

DISCUSSION

The main objective of this study was to investigate the ability of a battery of pain models to detect analgesic properties of commonly used analgesics in healthy subjects. A biomarker can be defined as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.".[25] This battery of different pain models was able to detect differences in pharmacological and analgesic properties, consistent with the PK properties of each individual compound. Each compound tested in this study demonstrated its own profile of effects on evoked pain in the different models included in the pain test battery. Most of these effects were in line with earlier described literature and with the expected PD and PK profile of the drugs. The drugs and doses used had already proven to be efficacious analgesics in clinical practice in either acute pain or in neuropathic pain. This battery of pain models can be used as biomarker to assess the PD responses of analgesic drugs.

Strong opioids previously showed effects on electrical pain, cold pressor, thermal pain and the thermal grill.[4,11] In this study fentanyl affected pain thresholds in the cold pressor test and thermal testing. No effects were observed on the electrical pain tests, or the pressure pain paradigm. The effect of fentanyl on a broad range of pain tests corresponds with the many types of clinical pain that respond to strong opioids. Previous reports have shown decreases in pain intensity and unpleasantness after morphine administration on the thermal grill.[11] Here, maximum unpleasantness and pain intensity did not change after fentanyl administration.

(S)-ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, showed effects on the cold pressor test, electrical stimulation (both single and repeated stimulus) and thermal heat pain. The effects of (S)-ketamine on the cold pressor test have not been reported before. In a study previously performed,[26] the cold pressor test was used in combination with (S)-ketamine, but only in order to induce a conditioned pain modulation (CPM) response. In a previous review,[5] no differences were observed in PDT during heat skin stimulation. In the current study, we found an increase in heat PDT on the normal skin in the first hour after dosing. Heat PDT in the UVB treated skin did not differ compared to placebo.

An effect of (S)-ketamine on the thermal grill pain and unpleasantness was expected, as these effects were shown previously.[10] Here, however, (S)-ketamine did not result in a decrease in unpleasantness or pain sensation in the thermal grill paradigm. An explanation for these differences could be our method of dosing, where the bolus administration of (S)-ketamine was not followed by a continuous infusion as described in other studies.[10]

There is limited literature available about the effect of sodium channel blockers on human pain models. One study has been published in which the effects of phenytoin and lamotrigine on cold pressor pain were investigated;[12] both phenytoin and lamotrigine reduced pain scores in healthy subjects. In the current study we only observed an increase in PDT and PTT in the electrical stimulation single stimulus paradigm. The therapeutic range for phenytoin in epilepsy is between 8 and 25 μg ml^{-1} in plasma.[27]

The observed C_{max} in the study was 8.3 μg ml^{-1}, which is at the lower end of the therapeutic range. Higher doses or repeated dosing may lead to a more pronounced effect on the pain models.

In Part II, pregabalin showed positive effects on cold pressor (PDT and PTT), electrical single stimulus (PTT), CPM (PDT) and thermal heat pain in normal skin. Alpha-2δ ligands have previously been shown to affect pain in human pain models; gabapentin showed positive effects on pain in an electrical hyperalgesia model in healthy subjects.[28] Conversely, gabapentin failed to show effects on heat PDT in healthy subjects.[29] Pregabalin has not been investigated in pain models in healthy subjects but in in patients with painful chronic pancreatitis, pregabalin attenuated visceral pain.[30] Of all compounds administered pregabalin showed the largest effect on most of the pain paradigms (heat, cold, pressure and electrical pain). This might be due to the relatively large dose of pregabalin that was used. However the same single dose of 300 mg was also used in other studies in which dosages of pregabalin of ≥300 mg showed a significant opioid sparing effect.[31] Further studies are needed to show if these large effects can be replicated. Currently pregabalin is mainly used in the treatment of neuropathic pain[32] and its use in acute postoperative pain is under investigation.[31] The positive effects of pregabalin on several (acute) nociceptive pain models in this study may be an argument for its potential use also in the treatment of acute nociceptive pain.

Ibuprofen increased the heat PDT in UVB treated skin. These effects were also previously shown by others.[4] Ibuprofen was the only compound administered that only increased heat PDT in UVB treated skin but not in normal skin. This in contrast with (S)-ketamine and pregabalin (increased heat PDT in normal skin, but not in UVB treated skin) and fentanyl (increased heat PDT in normal and UVB treated skin). These effects of ibuprofen were expected and reflect its inhibition of cyclooxygenase by this nonsteroidal anti-inflammatory drug, given the inflammatory type of pain that is caused in the UVB hyperalgesia model.

Imipramine only increased CPM, but did not affect other outcome measures. In previous research, imipramine increased acute pain tolerance after electrical stimulation, pressure pain and visceral pain.[5,13] Compared to the other compounds in this study, imipramine and its active metabolite desipramine have a relatively long half-life of 6.54 h and 56.2 h, respectively. We only performed measurements up to 10 h after dose administration, which may partially explain the negative findings in this study. In favour of this argument is that an increasing trend could still be observed at the last measurements in the electrical repeat stimulation paradigm PTT. Furthermore, in the clinical setting a titration period of several weeks is needed for imipramine before its efficacy can be assessed.[32] Here, we only administered a single dose. Imipramine was used as the tricyclic antidepressant of choice in this study because of previous positive results in human pain models. However, a recent meta-analysis showed that there is only limited evidence for the use of imipramine in neuropathic pain.[33]

In part I of the study, no effect was observed on CPM by either (S)-ketamine, fentanyl or phenytoin. High variability in CPM measurements was observed throughout the study, for all delta electrical stimulation parameters (PDT, PTT and AUC). Previous research conducted has shown a potentiation of CPM after administration of strong opioids.[34] Others observed no CPM response after ketamine treatment in healthy volunteers.[26]

In Part II of the study, both imipramine and pregabalin increased the difference in pain detection threshold after *vs* before the cold pressor (delta PDT), which may be indicative for an increase in CPM. A study performed in patients with pancreatitis did not show changes in CPM responses after administration of pregabalin. To our knowledge no studies are published in which the CPM responses in healthy subjects after administration of α2δ ligands or tricyclic antidepressants were measured. The noradrenergic system plays an important role in central pain modulation;[35] so the increase in delta PDT observed after administration of imipramine is likely to be explained by the enhancement of the inhibitory effect on noradrenaline reuptake.

No decrease on thermal grill maximum unpleasantness or maximum pain ratings could be observed in this study. However, overall, most subjects did not experience the thermal grill as unpleasant or painful, as reflected by the low scores on the VAS for pain and unpleasantness, which resulted in a non-normal distribution of the data, making them difficult to analyse.

Previous studies in which the thermal grill was used applied a range of combinations of warm and cold stimuli to assess relationships between painful and nonpainful sensations.[11,36] In the current study a fixed temperature of the warm and cold bars was used. Furthermore, the occurrence of paradoxical pain elicited by the thermal grill illusion can be variable. A study by Bouhassiara and colleagues reported a large subpopulation of subjects who only reported paradoxical pain when large cold-warm differentials were applied[37] . Due to the apparent necessity to tailor this method to each individual subject, it is difficult to standardise this method and incorporate it in a battery of pain models.

Multimodal testing with different pain models has been performed previously; with and without the administration of analgesic compounds.[3,13,38] Here we combined both the execution of a broad range of human pain models and the administration of analgesic compounds with different mechanisms of action. An advantage of the battery of pain models was that the tests could be executed repeatedly in a relatively short time (~30 min) in a standardised fashion.

By repeatedly administering these pain tests in one day this battery was able to determine time-effect profiles of the drugs. Small individual differences between different compounds could be assessed. Although PK/PD modelling was not performed in this study, study designs using repeated application of this battery of pain models can be used to assess PK/PD relationships.

Overall PK parameters measured in this study were reasonably consistent with the known PK data for these analgesics. Fentanyl's terminal half-life and volume of distribution were somewhat lower compared to values reported in literature.[39] Phenytoin, (S)-ketamine and its active metabolite norketamine showed kinetics that were consistent with the literature.[27,40] The t_{max} of imipramine was as expected. The terminal half-life was shorter, but this could have been related to the relatively short sampling period; the half-life of its active metabolite desipramine was longer than expected.[41] Ibuprofen and pregabalin showed PK that were consistent with the literature.[42,43]

A large number of pain models were used in this study. This yielded an even greater number of outcome variables. No correction for multiple testing was applied. Therefore, this multimodal test battery should be considered as a screening tool for analgesic properties of compounds in development for the treatment of pain, and not as a way to definitively prove effects on a specific evoked pain model with statistical significance. When the analgesic effect of a new drug on a certain pain mechanism has already been established, predefining a primary outcome measure would prevent the need to correct for multiple testing. Maximum effect sizes differed for the pain models used. For instance after pregabalin administration the contrast compared to placebo for heat PDT was 4.1%, while the contrast for cold PTT was 46.4%. Variability for these tests was also markedly different, with the coefficient of variation for the heat PTT being much lower than for the cold PTT. To account for this variation, studies using this battery of pain models need to be adequately powered. Only one (expected analgesic) dose of each compound was used in our study. Therefore, one-to-one comparisons between different compounds cannot be made on individual pain models. However, the pharmacodynamic profiles of these single doses matched the plasma profile of the compounds used. Reproducibility of the pharmacological effects of the compounds on the pain models was not directly assessed in this study. We were able to replicate effects of different analgesics on individual pain tests as described before,[4–6] however future studies are needed to investigate the reproducibility of the effect profiles that we observed.

One session of the battery of pain models lasted approximately 30 min. During one study period, 10 sessions were performed. This might have led to fatigue and diminishing concentration during the tests. This is also shown in Figures 3 and 4, where variation in the placebo group is observed between measurements during the day. In order to correct for these unavoidable effects, a crossover design with a placebo arm included was used. Somnolence was observed by 31% and dizziness by 56% of the subjects receiving pregabalin. Oral doses of imipramine also caused similar AEs, however imipramine did not show effects on the pain tasks administered. Other substances that are known to have strong sedating effects on the central nervous system also do not influence evoked pain tests. For instance cannabinoids and benzodiazepines have limited effects on pain thresholds.[5,44] Therefore, we believe that the somnolence and the dizziness caused by the pregabalin is not responsible for the effects on the pain tasks administered.

Several drugs acting at different targets are currently under clinical development for the treatment of acute and neuropathic pain. These drugs are in different stages of the clinical development. Examples are selective sodium channel blockers, nerve growth factor antagonists and fatty acid amide hydrolase inhibitors.[45–47] A recent review suggested that a limited set of human pain models could be sufficient to predict analgesic efficacy.[2] With our integrated battery of pain models it is possible to profile new compounds against currently existing analgesic compounds to predict their potential clinical use.

In conclusion, it was shown that this battery of pain models is able to detect changes in pain detection and pain tolerance thresholds after administration of different classes of analgesic compounds in healthy male and female subjects. The analgesic compounds all showed a unique profile in their effects on the pain tests administered. These profiles were in most cases compatible with the expected pharmacology. The knowledge of these profiles can be used to benchmark analgesic properties of these new drugs against established analgesics in early phase clinical studies in healthy subjects.

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TABLE 1

Least squares means for pharmacodynamic outcome measures and estimates of difference, 95% confidence intervals and P-values for main contrasts for part I.

	LS Means				Contrasts		
Parameter	**Placebo** (0-1 h) (0-5 h) (0-10 h)	**Fentanyl 3 µg kg^{-1}** (0-5 h)	**(s)-ketamine 10 mg** (0-1 h)	**Phenytoin 300 mg** (0-10 h)	**Fentanyl 3 µg kg^{-1} Placebo** up to 5 hours	**(s)-ketamine 10 mg Placebo** up to 1 hour	**Phenytoin 300 mg Placebo** up to 10 hours
Cold PDT (s)	4.3 4.5 4.4	4.4	6.1	5.2	-3.6% (-34.2%, 41.0%) P=0.8435	39.7% (-14.1%, 127.1%) P=0.1758	18.8% (-16.0%, 67.9%) P=0.3143
Cold PTT (s)	22.0 21.6 20.7	25.3	24.8	21.4	17.1% (2.3%, 33.9%) P=0.0230	12.7% (-5.5%, 34.4%) P=0.1820	3.6% (-8.2%, 17.0%) P=0.5562
Electrical Repeat PDT (mA)	2.7 2.7 2.7	2.8	2.6	2.9	5.0% (-15.4%, 30.2%) P=0.6523	-1.9% (-27.0%, 31.7%) P=0.8957	4.3% (-14.4%, 27.2%) P=0.6692
Electrical Repeat PTT (mA)	10.3 10.4 10.5	11.2	11.6	10.8	8.3% (-1.2%, 18.8%) P=0.0871	12.7% (-0.2%, 27.3%) P=0.0533	3.7% (-4.8%, 12.9%) P=0.3934
Electrical Single PDT (mA)	6.7 7.2 7.3	7.9	8.0	9.6	9.8% (-9.4%, 33.0%) P=0.3324	18.3% (-11.4%, 57.9%) P=0.2525	31.5% (10.3%, 56.8%) P=0.0032
Electrical Single PTT (mA)	21.4 22.1 22.3	23.5	23.6	24.2	6.7% (-0.7%, 14.7%) P=0.0770	10.1% (0.2%, 20.9%) P=0.0447	8.5% (1.4%, 16.1%) P=0.0193
CPM PDT (mA)	0.50 0.37 0.68	1.18	-0.11	0.37	0.811 (-0.319, 1.941) P=0.1576	-0.611 (-2.913, 1.692) P=0.6022	-0.305 (-1.196, 0.585) P=0.4925
CPM PTT (mA)	0.84 0.69 0.74	0.62	0.62	1.13	-0.074 (-0.938, 0.789) P=0.8648	-0.215 (-1.880, 1.449) P=0.7994	0.394 (-0.306, 1.095) P=0.2622
Pressure PDT (kPa)	15.0 12.8 12.7	13.0	14.9	14.9	1.6% (-15.2%, 21.6%) P=0.8636	-0.8% (-24.2%, 29.7%) P=0.9527	16.9% (-0.5%, 37.4%) P=0.0572
Pressure PTT (kPa)	42.2 42.7 41.8	45.8	45.5	42.3	7.2% (-0.2%, 15.1%) P=0.0571	7.9% (-3.4%, 20.5%) P=0.1765	1.1% (-5.0%, 7.6%) P=0.7291
Normal skin-heat PDT (°C)	43.88 44.18 44.13	45.41	45.33	44.61	2.8% (1.1%, 4.5%) P=0.0018	3.3% (1.1%, 5.6%) P=0.0034	1.1% (-0.4%, 2.6%) P=0.1508
UVB skin-heat PDT (°C)	39.11 38.94 38.93	39.95	39.44	38.93	2.6% (1.2%, 4.1%) P=0.0006	0.8% (-1.1%, 2.9%) P=0.4075	-0.0% (-1.2%, 1.2%) P=0.9813
Grill Un-pleasantness VAS Max (mm)	9.0 8.5 8.2	6.2	7.8	9.3	-2.33 (-5.06, 0.40) P=0.0923	-1.22 (-4.92, 2.47) P=0.5140	1.13 (-1.40, 3.65) P=0.3709
Grill Pain intensity VAS (mm)	5.6 4.4 4.5	4.4	7.3	5.6	-0.03 (-1.51, 1.44) P=0.9661	1.77 (0.85, 4.38) P=0.1844	1.11 (-0.15, 2.36) P=0.0824

CPM, conditioned pain modulation; LS, least squares; PDT, pain detection threshold; PTT, pain tolerance threshold; VAS, visual analogue scale

TABLE 2

Least squares means for pharmacodynamic outcome measures and estimates of difference, 95% confidence intervals and P-values for main contrasts for Part II.

	LS Means				Contrasts		
Parameter	**Placebo** (0-5 h) (0-10 h)	**Ibuprofen 600 mg** (0-5 h)	**Imipramine 100 mg** (0-10 h)	**Pregabalin 300 mg** (0-5 h)	**Ibuprofen 600 mg Placebo** up to 5 hours	**Imipramine 100 mg Placebo** up to 10 hours	**Pregabalin 300 mg Placebo** up to 5 hours
Cold PDT (s)	4.1 3.7	3.8	3.6	5.6	-8.3% (-29.5%, 19.3%) P=0.5109	-4.7% (-24.3%, 19.9%) P=0.6698	36.8% (5.9%, 76.8%) P=0.0174
Cold PTT (s)	17.2 16.0	17.7	18.1	25.1	2.9% (-10.7%, 18.7%) P=0.6850	12.6% (-1.3%, 28.6%) P=0.0769	46.4% (27.1%, 68.6%) P=<.0001
Electrical Repeat PDT (mA)	2.1 2.0	2.3	2.3	2.0	13.1% (-20.0%, 60.1%) P=0.4779	16.6% (-16.0%, 61.8%) P=0.3497	-0.6% (-30.5%, 42.0%) P=0.9719
Electrical Repeat PTT (mA)	8.9 8.8	9.9	9.3	10.1	11.3% (-1.1%, 25.4%) P=0.0749	5.1% (-6.2%, 17.7%) P=0.3808	13.1% (-0.3%, 28.4%) P=0.0561
Electrical Single PDT (mA)	7.5 7.3	7.6	8.0	7.3	0.6% (-19.4%, 25.6%) P=0.9565	9.4% (-10.4%, 33.5%) P=0.3673	-2.7% (-22.3%, 22.0%) P=0.8112
Electrical Single PTT (mA)	19.9 19.7	20.1	20.5	22.0	1.2% (-6.2%, 9.2%) P=0.7525	3.9% (-3.3%, 11.6%) P=0.2887	10.8% (2.4%, 19.9%) P=0.0121
CPM PDT (mA)	-0.19 0.31	0.70	1.19	1.76	0.895 (-0.213, 2.003) P=0.1122	0.879 (0.060, 1.699) P=0.0364	1.950 (0.840, 3.061) P=0.0007
CPM PTT (mA)	0.56 0.76	1.08	0.88	1.21	0.519 (-0.160, 1.198) P=0.1319	0.117 (-0.452, 0.685) P=0.6795	0.644 (-0.036, 1.323) P=0.0630
Pressure PDT (kPa)	14.5 14.2	14.4	13.4	13.9	-0.7% (-22.2%, 26.7%) P=0.9528	-5.6% (-24.8%, 18.5%) P=0.6062	-4.6% (-25.3%, 21.9%) P=0.6998
Pressure PTT (kPa)	41.1 41.7	44.0	44.3	47.6	5.3% (-3.9%, 15.4%) P=0.2576	7.7% (-0.9%, 17.0%) P=0.0773	14.1% (4.3%, 24.9%) P=0.0052
Normal skin-heat PDT (°C)	43.32 43.25	44.08	43.62	45.09	1.7% (-1.0%, 4.5%) P=0.2080	0.9% (-1.7%, 3.5%) P=0.5108	4.1% (1.3%, 7.0%) P=0.0049
UVB skin-heat PDT (°C)	38.63 38.49	40.17	38.92	39.08	4.0% (1.8%, 6.3%) P=0.0006	1.1% (-0.9%, 3.1%) P=0.2589	1.2% (-0.9%, 3.3%) P=0.2671
Grill Un-pleasantness VAS Max (mm)	1.4 1.7	3.0	2.6	2.3	1.57 (-0.41, 3.55) P=0.1177	0.90 (-0.97, 2.78) P=0.3357	0.89 (-1.09, 2.86) P=0.3698
Grill Pain intensity VAS (mm)	1.0 1.2	2.3	1.0	1.7	1.25 (0.25, 2.25) P=0.0151	-0.16 (-1.10, 0.77) P=0.7250	0.73 (-0.25, 1.71) P=0.1425

CPM, conditioned pain modulation; LS, least squares; PDT, pain detection threshold; PTT, pain tolerance threshold; VAS, visual analogue scale

TABLE 3

Pharmacokinetic parameters for administered compounds. Data are means ± standard deviation and median (range) for T_{max}.

	Fentanyl	(S)-ketamine	Norketamine	Phenytoin	Ibuprofen	Imipramine	Desipramine	Pregabalin
C_{max} (ng ml^{-1})	1.23 ± 0.66	52.4 ± 15.7	23.2 ± 5.7	8,280 ± 1,740	53,300 ± 9,360	77.2 ± 21.1	25.4 ± 14.6	9,650 ± 1,880
T_{max} (h)	0.50 (0.50-1.22)	0.50 (0.50-2.33)	1.10 (1.05-3.05)	0.55 (0.50-2.13)	2.02 (1.08-3.00)	3.12 (1.08-6.00)	6.00 (2.07-10.00)	1.56 (1.08-5.00)
$t_{½}$ (h)	2.52 ± 1.08	3.12 ± 1.30	4.89 ± 1.14	12.50 ± 2.86	1.80 ± 0.29	6.54 ± 1.96	56.2 ± 8.7	5.30 ± 0.58
AUC_{0-last} (ng h ml^{-1})	2.21 ± 0.72	102 ± 18.6	129 ± 35.7	54,500 ± 7,980	174,000 ± 30,200	441 ± 128	163 ± 99.2	49,700 ± 7,340
AUC_{0-inf} (ng h ml^{-1})	3.10 ± 0.84	111 ± 22.2	180 ± 54.6	135,000 ± 34,800	182,000 ± 35,600	782 ± 193	517 ± 100	71,200 ± 9,860
Clearance (l h^{-1})	1.05 ± 0.32	93.6 ± 20.1	60.2 ± 17.7	2.36 ± 0.54	N.C.	N.C.	N.C.	N.C.
V_d (l)	3.56 ± 1.29	410 ± 153	411 ± 106	40.60 ± 4.97	N.C.	N.C.	N.C.	N.C.

AUC_{0-inf}, estimated area under the plasma concentration-time curve from time of dosing to infinity; AUC_{0-last}, area under the plasma concentration-time curve from time of dosing to the last observation; C_{max}, maximum concentration; T_{max}, time at which C_{max} was observed; $t_{½}$, apparent terminal half-life; V_d, apparent volume of distribution of the drug; NC, not calculated.

FIGURE 1

Overview study design

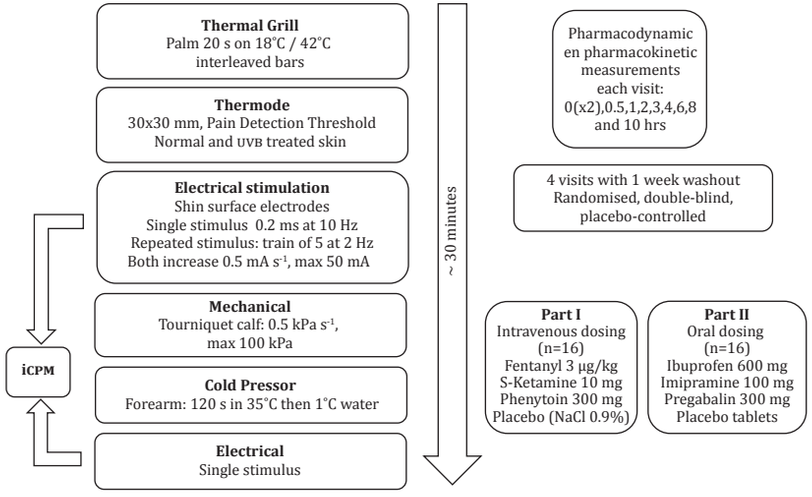


FIGURE 2

Disposition of Subjects

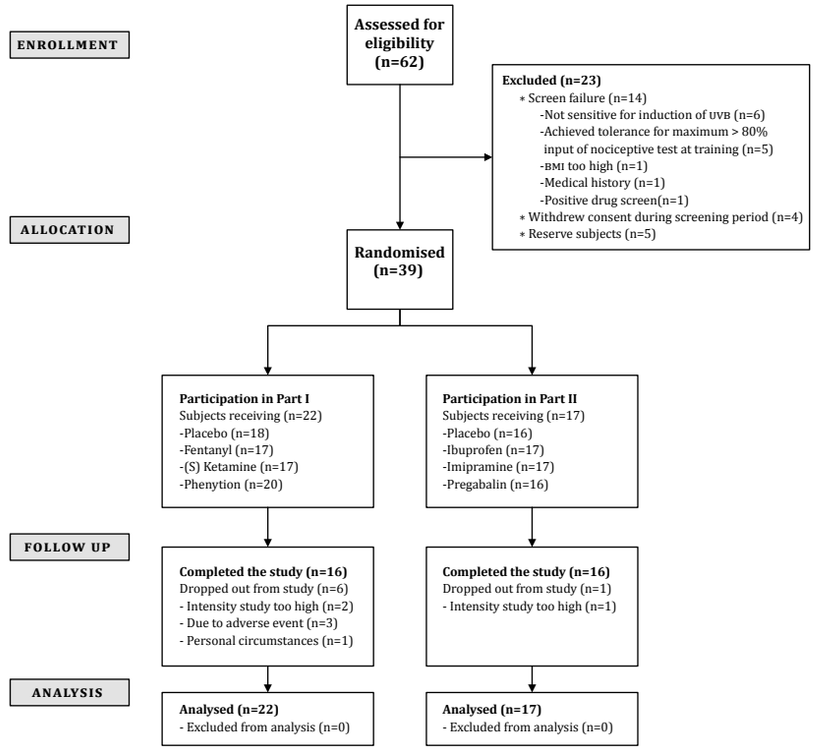


FIGURE 3

Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for cold pressor (A/B), electrical stimulation (C/D: repeated stimulus), and (E/F: single stimulus) and the electrical stimulation (single stimulus) delta pain detection threshold (G/H) after administration of the different compounds in Part I (A, C, E, G) and Part II (B, D, F, H).

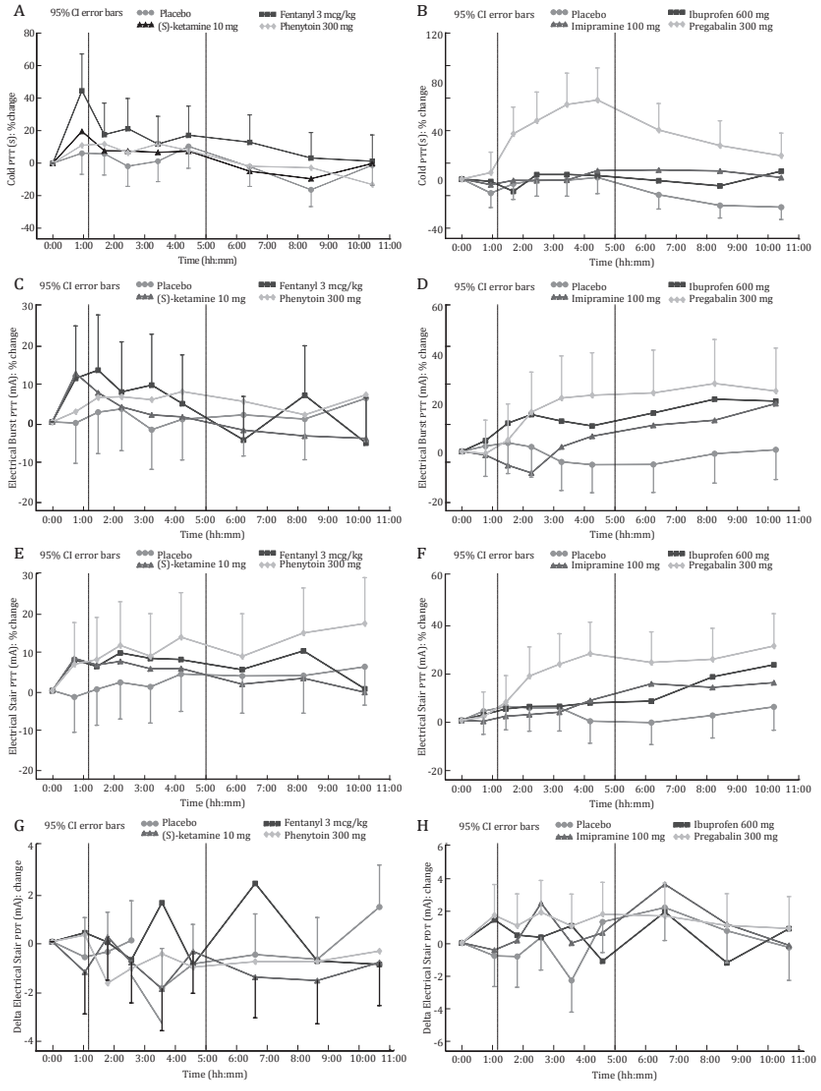


FIGURE 4

Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for pressure stimulation (A/B), the heat pain detection threshold for thermal testing on normal skin (C/D), and UVB-irradiated skin (E/F) and the thermal grill maximum unpleasantness VAS (G/H) after administration of the different compounds in Part I (A, C, E, G) and Part II (B, D, F, H).

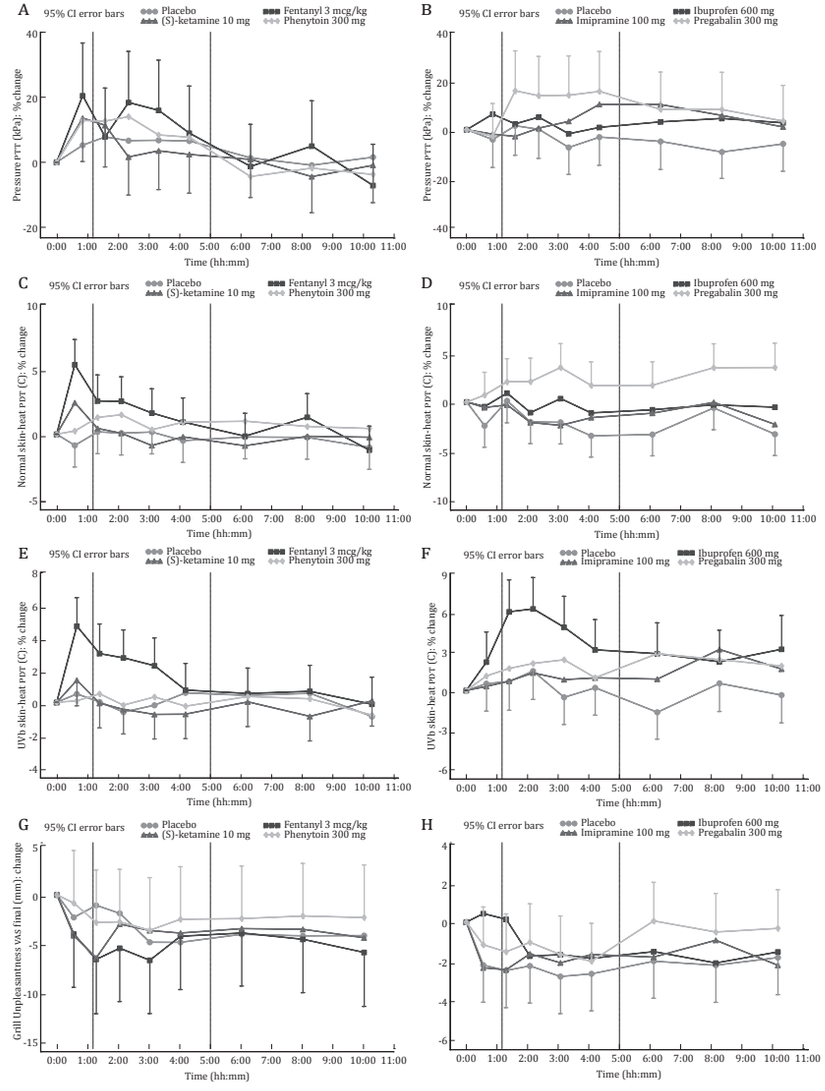
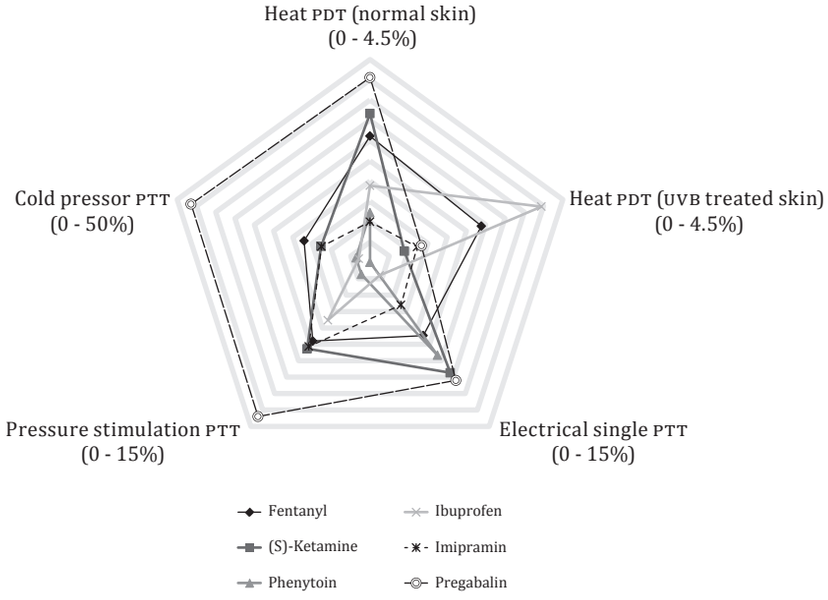


FIGURE 5

Radar chart of effect sizes of the compounds used. Effect sizes are given as the contrast between the different compounds and placebo.



CHAPTER IV

Pharmacokinetics and pharmacodynamics of intrathecally administered Xen2174, a synthetic conopeptide with norepinephrine reuptake inhibitor and analgesic properties

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ABSTRACT

Xen2174 is a synthetic 13-amino acid peptide that binds specifically to the norepinephrine transporter, which results in inhibition of norepinephrine uptake. It is being developed as a possible treatment for moderate to severe pain and is delivered intrathecally. The current study was performed to assess the pharmacodynamics (PD) and the cerebrospinal fluid (CSF) pharmacokinetics (PK) of Xen2174 in healthy subjects. This was a randomised, blinded, placebo-controlled study in healthy subjects. The study was divided into three treatment arms. Each group consisted of eight subjects on active treatment and two or three subjects on placebo. The CSF was sampled for 32 h using an intrathecal catheter. PD assessments were performed using a battery of nociceptive tasks (electrical pain, pressure pain and cold pressor tasks). Twenty-five subjects were administered Xen2174. CSF PK analysis showed a higher area under the CSF concentration-time curve of Xen2174 in the highest dose group than allowed by the predefined safety margin based on nonclinical data. The most common adverse event was post lumbar puncture syndrome, with no difference in incidence between treatment groups. Although no statistically significant differences were observed in the PD assessments between the different dosages of Xen2174 and placebo, pain tolerability in the highest dose group was higher than in the placebo group (contrast least squares mean pressure pain tolerance threshold of Xen2174 2.5 mg–placebo[95% confidence interval],/22.2%[-5.0%, 57.1%], P=0.1131). At the Xen2174 dose level of 2.5 mg, CSF concentrations exceeded the prespecified exposure limit based on the nonclinical safety margin. No statistically significant effects on evoked pain tests were observed.

INTRODUCTION

The majority of patients undergoing surgery experience moderate to severe pain in the postoperative period.[1] Treatment consists of multiple pain relief agents and strategies. Significant side effects may occur with the use of opioids.[2] Nonsteroidal anti-inflammatory drugs (NSAIDS) and paracetamol are not sufficiently effective against moderate to severe postoperative pain and should be administered in combination with opioids.[3,4] Thus, there remains a clinical need for the development of new efficacious therapies with a beneficial side effect profile.

The venom of the marine cone snail genus *Conus* provides a rich source of pharmacologically active compounds.[5] The peptide Mr1A, identified in the venom of *Conus marmoreus,* causes inhibition of norepinephrine (NE) uptake by the NE transporter (NET) in a selective, noncompetitive manner.[6,7] Mr1A showed an antinociceptive effect after intrathecal administration in mice.[8,9] This peptide has a relatively poor chemical stability in solution. To overcome this, Xen2174, modelled on Mr1A, was developed. Xen2174 is a synthetic 13-amino acid peptide that does not cross the blood-brain barrier and is being developed for the intrathecal treatment of moderate to severe pain. *In vitro* pharmacology studies have demonstrated that Xen2174 binds specifically to the NET, but not to other central nervous system molecular targets, resulting in selective inhibition of NE uptake by NET in a noncompetitive manner.[10] Tricyclic antidepressants are also potent NE reuptake inhibitors (NRIS), but their poor specificity relative to other monoamine transporters and various G-protein-coupled receptors, results in dose-limiting side effects in clinical use.[9,11] *In vivo* pharmacology studies in rat models of neuropathic pain have demonstrated that intrathecal administration of Xen2174 produces rapid and long-lasting anti-allodynic effects, which were found to be greater in magnitude and duration than those of intrathecal morphine.[9] Additional pharmacology studies have demonstrated that Xen2174 also provides long lasting antinociception in a rat model of postsurgical pain.[12] In an inflammatory pain model in rats (inflammation induced by injecting Freund's Complete Adjuvant) Xen2174 did not relieve pain after thermal latency or paw pressure tests (Investigator's brochure Xen2174. Xenome Ltd., unpublished). Toxicology studies have shown that Xen2174 causes convulsions and seizures when administered at high doses in rats and dogs. In a beagle dog study in which Xen2174 was administered intrathecally at doses of 0, 1, 2, 4 and 8 mg (5 animals/gender/dose), seizures were observed in three dogs; one in the 1 mg and two in the 2 mg dose

group. In follow up dog study in which 24 animals were treated, no seizures or changes on EEG were observed after administration of 1, 2, 4 and 8 mg/animal. The no-observed-adverse-effect level (NOAEL) in dogs was 1.0 mg/animal (Investigator's brochure Xen2174. Xenome Ltd., unpublished).

Xen2174 has previously been administered to humans in four clinical studies. However, only limited data have been available on the pharmacokinetic (PK) profile of Xen2174 in cerebrospinal fluid (CSF) and no conclusive data have been available on its analgesic properties in humans (Table 1). The aim of the current study was to assess the PK profile of Xen2174 in plasma and CSF when administered intrathecally to healthy subjects, and to assess which modalities of pain were affected by treatment with Xen2174, using evoked pain tasks.

MATERIALS AND METHODS

The study was approved by the Medical Ethics Committee of the BEBO Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Subjects

Healthy male and female subjects between 18 and 45 years with a body mass index (BMI) of 18 to 30 kg m^{-2} were enrolled. All subjects gave written informed consent. The subjects underwent a full medical screening to assess eligibility. Subjects with an abnormal electroencephalogram (EEG) at screening, a (family) history of epilepsy, a history of seizures, complaints of low back pain, regular user of any illicit drugs or history of drug abuse, a positive drug screen or other clinical significant abnormalities were excluded. Use of xanthine-containing products and alcohol was not allowed from 1 day prior to admission to the clinical research unit and during the stay at the research unit. Subjects were not allowed to use any medications from 2 weeks prior to the start of the study days.

Experimental design

This was a randomised, double-blind, placebo-controlled, serial-cohort, single ascending dose study of Xen2174 or placebo, administered intrathecally to healthy volunteers. At each dose stage, subjects were randomised to Xen2174 or placebo. Cohorts 1 and 2 consisted of eight subjects administered Xen2174 and three subjects receiving placebo. Cohort 3 consisted of eight subjects administered Xen2174 and two administered placebo. The three ascending doses of Xen2174 were 0.5 mg (cohort 1), 1.0 mg (cohort 2) and 2.5 mg (cohort 3). The maximum dose of 2.5 mg was chosen in order to have a threefold safety margin in the dose per kg body weight compared with the NOAEL in dogs. The lower dose of 0.5 mg was chosen based on the human equivalent dose of the median effective dose (ED_{50}) in rats exposed to the Brennan model of postsurgical pain.

Subjects arrived at the clinical research unit on the day before dosing and remained in-house for at least 56 h after study drug administration. The study drug was administered via a spinal needle at the L3-L4 or L4-L5 interspace, using a median approach. After administration, an intrathecal sampling catheter was left in place for the following 32 h. Subjects were asked to stay in bed in either a recumbent or supine position as much as possible during the period that the spinal catheter was in place, and up to 12 h after the spinal catheter had been removed.

Safety assessments were performed at specified time points and the occurrence of general symptoms was monitored continuously. The computer-generated randomization list was prepared by the statistician prior to the start of the study. Doses were prepared by a pharmacist/technician not involved in any of the study procedures.

Study drug

Xen2174 in glucose 5% was given intrathecally as bolus injection of 3 ml. Glucose 5% was used as placebo. Before drug administration, the skin on the lower back was anesthetised locally with 1-2 ml lidocaine. All intrathecal injections of the study drug were carried out by an experienced anaesthesiologist under aseptic conditions using a spinal catheter set. Owing to difficulties with CSF sampling, different spinal catheter sets were used during the course of the study: a Sprotte Special 21G needle with a 25G catheter (cohort 1) (Pajunk, Geisingen, Germany), a 19G needle with a 23G catheter (five subjects in cohort 2) (Pajunk, Geisingen, Germany) and a Spinocath 22G catheter (six subjects in cohort 2 and 10 in cohort 3) (B Braun, Melsungen, Germany). With the Sprotte Special cannula catheter set, the study drug was administered using the Sprotte needle (epidural introducer with an atraumatic modified pencil point) after which the sampling

catheter was left in place. The Sprotte needle had a directional bevel which was directed cranially. The study drug was administered directly through the epidural introducer. The catheter was placed after drug administration at the same level via the introducer. For the Spinocath set, first an introducer was inserted into the epidural space. After that, the study drug was administered into the intrathecal space using a 25G/27G pencil point needle. Thereafter, the sampling catheter was inserted into the intrathecal space through the epidural introducer. With both catheter sets, the sampling catheter was inserted 2-5 cm into the intrathecal space and left in place for the following 32 h. The Pajunk catheter had three lateral orifices at the distal end of the catheter. The Spinocath catheter had a central and lateral opening on the catheter tip. The intrathecal needle was placed with the subject in the sitting position. After insertion of the spinal catheter, the catheter was secured and subjects were placed directly in supine position afterwards. They were asked to stay in the supine or recumbent position while the catheter was in place.

Study assessments

The primary objectives of the study were to evaluate the effects of Xen2174 on evoked pain tasks and to assess the PK profile of Xen2174 in plasma and CSF. Nociceptive (pain) detection and tolerance thresholds were measured using a battery of evoked pain tasks. The battery takes approximately 25 min to complete. The evoked pain tasks (electrical pain, pressure pain and cold pressor tasks) were performed predose (twice) and 2, 4, 6, 8, 10, 48, 72 and 96 h after study drug administration. A training session was included as part of the screening examination to reduce learning effects during the study. All tests had previously been shown to be sensitive to the effects of analgesics in healthy adults.

Pain intensity was measured continuously for each nociceptive task using an electronic visual analogue scale (eVAS) scale ranging from 0 (no pain) to 100 (most intense pain tolerable). The equipment was programmed to cease giving stimuli if pain intensity reached the maximum possible score. For each task, the pain detection threshold (PDT), pain tolerance threshold (PTT) and area under the pain intensity-stimulation (-time for cold pressor) curve (AUC) were calculated.

Electrical stimulation task

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™, 3M Europe, Diegem, Belgium) were placed on the skin, 10 cm distal from the patella overlying the tibia. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5, Digitimer, Cambridge, UK). For the single stimulus, adapted from methods described previously[13,14] (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 $mA \cdot s^{-1}$ (cut-off 50 mA). For the repeated stimulus, adapted from methods described previously,[15] each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3-8 s between the repetitions. Current intensity increased from 0 mA in steps of 0.5 mA (cutoff 50 mA). The pain detection threshold was taken as the value (mA) when a subject indicated either that all five stimuli were painful or that the train of five stimuli, having started as feeling nonpainful became painful (VAS > 0). The pain intensity for each stimulation was measured using the eVAS slider, until the PTT was reached or a maximum of 50 mA was reached.

Pressure stimulation task

The method for inducing mechanical pressure pain was based on methods described previously, and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.[16,17] Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 $kPa \cdot s^{-1}$. 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, at which point the device released pressure to the cuff.

Cold pressor task

The method of cold pressor pain was based on the methods described previously[18,19] and is the most commonly used test to induce inhibitory conditioned pain modulation (iCPM, also known as 'diffuse noxious inhibitory control').[20] Subjects placed their nondominant hand into a water bath (minimal depth 200 mm) at 35 ± 0.5°C for 2 min. At 1 min 45 s a blood

pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min the subject moved that hand from the warm water bath, directly into a similar sized bath at 1.0 ± 0.5°C. The subjects were instructed to indicate when the PDT was reached as well as the pain intensity, by moving the eVAS slider. When the PTT or a time limit (120 s) was reached, subjects were instructed to remove their hand from the water.

Conditioned pain modulation

Conditioned pain modulation is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[20] The degree of iCPM was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the cold pressor task.

Measurements of drug concentrations in plasma and CSF

Samples for the determination of Xen2174 in the plasma were obtained at baseline, and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48 and 72 h postdose. CSF samples were obtained using the intrathecal catheter at baseline, and at 0.5, 1, 2, 4, 8, 12, 24, and 32 h postdose. The potential for Xen2174 to adhere to components of the sampling material was tested prior to study execution. Acceptable recovery was obtained. First 0.2 ml CSF, representing the catheter dead-space, was sampled and discarded. Subsequently 0.3 ml was sampled with a new syringe, divided into two cryotubes and frozen at -70°C within 30 min of collection. Plasma was separated within 20 min of blood collection by centrifugation at 2000 g for 10 min. Samples were stored at -70°C until analysis. Plasma and CSF concentrations of Xen2174 were measured via high-performance liquid chromatography with tandem mass spectrometry detection. The lower limits of quantification were 1.0 ng ml^{-1} and 10 ng ml^{-1} for the concentrations of Xen2174 in plasma and CSF respectively. Sample analysis was performed by Pharmaceutical Product Development, Inc., Richmond, VA, USA.

EEG

All subjects received a standard 21-lead clinical EEG at the screening visit. The 1-h EEG recording was performed to detect subjects with abnormal EEG activity or with preseizure activity when stressed, through hyperventilation (for at least 3 min) and photic stimulation. Study EEG recording was initiated 1 h predose, and continued until 24 h postdose. Any change from the baseline EEG observed after dosing and interpreted in a blinded fashion by the clinical neurophysiologist as clinically significant, was reported as an adverse event (AE).

Statistics

No formal power analysis was performed. However, a previous study in which the electrical stimulation task was performed and where analgesia could be measured in healthy subjects used similar group sizes.[13] The statistical analysis plan was part of the study protocol. For Xen2174 all PK parameters were analysed by noncompartmental methods. Summary statistics for each PK parameter were calculated for each dose group. The individual and median concentrations were plotted *vs.* time both on a linear and a logarithmic scale. Dose proportionality was assessed from dose-normalised AUC.

Residual Q-Q plots were produced to check the assumption of normality of the error term in the mixed effects models. This was done by visual inspection, the Shapiro-Wilk test statistic and the p-value for the test of normality. All PDT and PTT variables followed a log-normal distribution and were therefore log-transformed before analysis. Transformed parameters were back-transformed after analysis.

To assess the interaction effect of Xen2174 on nociceptive variables, the (transformed) variables were analysed with a mixed model analysis of variance, with treatment, time and treatment by time as fixed factor, subject as random factor and the (average) predose value as covariate. The contrasts calculated within the model were between the placebo and active treatments. Contrasts within the overall treatment effect and the time effect were estimated and reported, along with 95% confidence intervals. Subjects assigned to placebo within each cohort were treated as a single group. All calculations were performed using SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

A total of 33 healthy subjects (four females) participated in the study (Figure 1); subjects were aged 18-43 years (mean age 25.6 years) and had a BMI of 19-30 kg m^{-2}(mean 24.4 kg m^{-2}). The clinical phase of the study

started on 28 December 2011, and the last study visit was on 18 June 2012. One subject in cohort 3, in whom CSF sampling was not possible was replaced. The replacement subject was dosed in an unblinded fashion. Only PK assessments were performed in this subject.

Owing to sampling problems with the spinal catheter the study was amended. During the cohort 1 treatment the diameter of the spinal catheter was increased, and during the cohort 2 treatment, the type of spinal catheter was changed. Owing to a high incidence of postlumbar puncture syndrome in cohort 1, only male subjects with a BMI above 23 kg m^{-2} were recruited in cohorts 2 and 3.

A large number of AEs was observed in this study (Table 2). There was no clear difference in the severity or duration of AEs between the different dosing groups and placebo. The most commonly reported AE was post lumbar puncture syndrome (25 out of 33 subjects). This AE was reported in all dose groups. In the majority of the subjects, complaints of headache as presentation of postlumbar puncture syndrome started after removal of the spinal sampling catheter. In two subjects, the severity of these complaints was mild, in 16 subjects moderate and in seven subjects severe. Subjects experiencing these complaints were treated with paracetamol and caffeine. Because of inadequate treatment response, 11 subjects were treated with an epidural blood patch; one subject was treated with two epidural blood patches. Evoked pain tasks were not performed subsequent to analgesic dosing for postlumbar puncture syndrome.

Other commonly reported AEs were catheter site related reaction and back pain. This included a bruised feeling on the back, irritation, pain and stiffness. Paraesthesia was experienced by six subjects; in two during administration, and in four during the period when the catheter was in place. All these complaints were mild, and resolved shortly after spinal catheter removal.

One subject experienced a serious AE during the study. This subject continued to have headache complaints after treatment with the epidural blood patch. He was evaluated at the emergency room of the local university hospital to exclude severe pathology. No abnormalities were found on a computed tomography scan of the head, and the subject was discharged from the hospital the next morning. The headache complaints resolved without sequelae.

One subject reported persistent tinnitus after participation in the study, which persisted beyond the end of the clinical phase of the study. This subject was referred to an otolaryngologist for follow up.

No consistent clinically relevant abnormalities in vital signs, chemistry and haematology blood results, urinalysis, electrocardiograms or 24-h EEG registrations were observed.

Evoked pain tasks

The mean changes in the least squares means from baseline over 96 h following Xen2174/placebo administration for the different evoked pain task variables (AUC, PDT, PTT) were evaluated. The summary statistics of the PTT are provided in Table 3. The time course for the mean change in the PTT from baseline in the first 48 h following Xen214/placebo administration for the different evoked pain tasks is shown in Figure 2.

Following treatment with Xen2174 2.50 mg, we observed an increase in the PTT over a prolonged period of time for the electrical stimulation tasks (single (overall treatment p-value, contrast least squares mean of the PTT Xen2174 2.5 mg – placebo[95% confidence interval], contrast P-value/ P=0.1801, 17.1% [-10.4%, 53.2%], P=0.2372) and repeated stimulation (P=0.0713, 28.9% [-3.3%, 71.7%], P=0.0811)) and the pressure stimulation task (P=0.0328, 22.2% [-5.0%, 57.1%], P=0.1131). No clear differences in PTT between the different dose groups could be observed for ICPM (P=0.7615, 0.68 [-1.48, 2.84], P=0.5253) or the cold pressor task (P=0.5419, -3.4% [-27.8%, 29.2%], P=0.8091). AUCs and PDTs for the different pain tasks did not show any significant results. Seventeen subjects missed one or more nociceptive tests because of concurrent postlumbar puncture headache and treatments.

Drug concentrations in CSF and plasma

The mean PK concentration-time profiles and the corresponding PK variables of Xen2174 in CSF are shown in Figure 3 and Table 4, respectively. The mean half-life ranged between 4.27 h and 7.14 h in CSF. The AUC (concentration-time) from time zero to infinity ($AUC_{0-\infty}$) values increased more than proportionally with dose in all dose groups.

The PK concentration-time profiles and variables of Xen2174 in plasma are shown in Table 5 and Figure 4. In general, concentrations were approximately 500- to 2000-fold lower in plasma than in CSF. Average plasma peak maximum concentration (C_{max}) increased from 5.49 ng ml^{-1} at the 0.5 mg dose level to 9.75 ng ml^{-1} at 1 mg and 15.4 ng ml^{-1} at the 2.5 mg dose level. C_{max} appeared to increase slightly less than proportionally with dose

between 0.5 and 2.5 mg. The average time to reach the plasma C_{max} (T_{max}) was 1.94, 3.69 and 6.89 h, for the 0.5, 1, and 2.5 mg doses, respectively. $AUC_{0-\infty}$ increased proportionally to dose.

DISCUSSION

The present study showed that the 2.5 mg dose of Xen2174 administered intrathecally was able to influence pain thresholds in several evoked pain tasks. The pain tasks showed an increase in PTTs for the electrical pain tasks and the pressure pain task in favour of the highest dose of Xen2174 tested, although statistical significance was not reached.

In nonclinical experiments, intrathecal administration of Xen2174 produced anti-allodynic and antinociceptive effects in rats.[9,12] The chronic constriction injury (CCI) model and the L5/L6 ligation model used in the study by Nielsen et al. are both models for neuropathic pain, while Obata and colleagues used a model of postincisional pain. The models used in the present study were mainly for acute nociceptive pain. Owing to the differences in etiology in these models, no direct translation can be made between the results in nonclinical results and the results in humans. Dosages in the present study were based on nonclinical data. The half maximal effective concentration (EC_{50}) in a functional assay for the binding of Xen2174 to the NET, resulting in the inhibition of NE uptake by the transporter, was 183 nM, which corresponds to a concentration of 0.26 mg l^{-1}. The median effective dose (ED_{50}) concentration in CSF for antinociception in the Brennan model for postoperative pain in rats was 0.86 µg intrathecally (hypothetical concentration in CSF 3.2 mg l^{-1}). The ED_{50} for anti-allodynia in the CCI model in rats was 15.7 nmol (22.1 µg, leading to a hypothetical CSF concentration of 81.9 mg l^{-1}).[9] It was expected that dosages in the range of 1.0-2.5 mg would lead to CSF concentrations above the observed EC_{50} and ED_{50}, and thus induce nociceptive effects. The observed C_{max} (after administration of 2.5 mg of Xen2174) in CSF of 33.2 mg l^{-1} was above the ED_{50} for the antinociception in the Brennan model, but below the ED_{50} for anti-allodynia in the CCI model.

The Xen2174 1.0 mg intrathecal dose in dogs was determined as the NOAEL in dogs in nonclinical studies. The ratio of the $AUC_{0-\infty}$ measured in CSF in the Xen2174 2.5 mg dose group in humans compared with that in dogs after a 1 mg intrathecal injection was 1.43 (unpublished data). A preferred and expected safety margin for this $AUC_{0-\infty}$ ratio for single intrathecal doses of Xen2174 in dogs (expected ratio to be at least 10) was not reached, leading the sponsor to discontinue further development of this compound.

Xen2174 is one of a novel class of NRIs for the treatment of pain. It has been shown to exert its effects via spinal activation of α_2-adrenoceptors subsequent to NE reuptake inhibition.[9] Other NRIs include tricyclic antidepressants and tapentadol. The tricyclic antidepressant imipramine increases the PTT for pressure pain and for electrical stimulation.[21] Tapentadol combines opioidergic activity with noradrenergic activity with both mechanisms accounting for the analgesic effects. It is efficacious in the treatment of moderate to severe acute pain compared with placebo.[22] Furthermore, tapentadol caused activation of conditioned pain modulation in patients with diabetes in an experimental setting.[23]

Several polymorphisms are known for the NET gene (SLC6A2). Patients carrying the homozygous SNP2 G/G variant of this gene reported a longer analgesic onset time after medication administration than heterozygous and A/A homozygous patients.[24] Hypothetically, a larger overall analgesic effect could have been observed if SNP2 G/G subjects had been excluded from the study. An equipotent analgesic effect might have been achieved with lower CSF concentrations. Unfortunately, no genotyping for polymorphisms was performed in the present study.

In addition to local anaesthetics, which are used for spinal anaesthesia, there are several analgesic compounds that are intrathecally administered. Clonidine, an α_2-adrenergic receptor agonist, showed analgesic action after intrathecal and epidural administration.[25,26] Ziconotide, a synthetic equivalent of the venom of a marine snail, exerts its effect by binding and blocking voltage-sensitive calcium channels.[27] Opioids show postoperative analgesia when administered intrathecally.[28] Intrathecal NSAIDs have been tested for their analgesic efficacy in patients but are not used in current clinical practice.[29] Only two studies have reported the use of evoked pain models after intrathecal drug administration.[26,30] Intrathecal ketorolac, an NSAID, was tested in a study in healthy volunteers but did not show an effect on pain from acute heat stimuli.[30] Clonidine caused an increase in heat pain tolerance after intrathecal administration.[26] In the current study we confirmed that intrathecal drug administration in combination with performing a battery of evoked pain tasks is feasible, even with concurrent CSF sampling.

An increase of 28.9% in least squares mean (electrical repeat PTT) and 22.2 % (pressure PTT) was observed after administration of Xen2174 compared with placebo. Similar effect sizes for electrical pain (42%) and pressure pain (22%) testing were observed after administration of an analgesic dose of alfentanil in previous research,[31] suggesting that the difference we observed in pain tolerance was clinically relevant. The observed increase in

PTTs lasts for a long period (Figure 2), whereas the CSF concentration steadily drops (Figure 3). The prolonged analgesic effect cannot be explained by the CSF concentrations but it should be noted that such a measure is a surrogate for tissue concentration and receptor binding, and therefore may reflect a similar distribution to the effect site (reflected by half-life for equilibration, $t_{½,ke0}$) to that observed with other analgesics and consequential clearance from the effect site.[32] Although no mechanistic validation can be provided, the long duration of action has already been observed in nonclinical experiments, in which doses of intrathecal Xen2174 provided longer relief of tactile allodynia in CCI rats compared with morphine.[9]

An increase in pain tolerance was observed in the electrical pain tasks and the pressure pain task, but no differences were observed in the cold pressor task. Earlier research with a centrally acting NRI, imipramine, also did not show an effect on the cold pressor task.[21] The lack of effect on this task could suggest that administration of Xen2174 has only local effects, at and below the level of administration, but no effects at higher levels – for example, at the level of the brainstem. There was a difference in the level of administration of the study drug (L4-L5) and the dermatomes in which the cold pressor task was performed (C6-C8). In a study in which several amide local anaesthetics were compared, drug administration was performed at the second or third lumbar interspace, and the maximum level of sensory block to pinprick was level T2 in all dose groups.[33] This might also be why no effects of Xen2174 on ICPM could be observed. The centrally acting NRI tapentadol has been shown to increase ICPM.[23] Other explanations for the conflicting outcomes might include the fact that different methods were used to measure ICPM, or differences in patient populations.

Many studies employ evoked pain tasks to assess the analgesic effects of new drugs in healthy human subjects. Most of these studies test only one or two modalities of pain.[34] The advantage of the method that was used in the current study was the combination of the different pain tasks in a standardised way. Earlier research has shown the advantages of multi-modal pain testing.[35] Different evoked pain tests have different sensitivities for different analgesics.[34] Using only one pain task could lead to a negative trial, while using a broad set of pain tasks could give a better understanding of how the different mechanisms that play a role in evoked pain tests are influenced, and therefore of the different pharmacological properties of a new compound. The models used in the present study represent only acute nociceptive pain models. No spontaneous, chronic or neuropathic pain was investigated. Therefore, caution should be exercised when interpreting our results.

While it has been shown that many different analgesics that are known to be effective in clinical acute and chronic pain management can affect the different tests that were used in this pain battery,[34,36,37] the acute responses tested in the current study are not necessarily good models of chronic pain. Given the mode of action of Xen2174 to enhance descending inhibition, these acute measures may not adequately assess efficacy in clinical settings of chronic pain.

The limitation of multi-modal testing is the large number of different outcome variables. In the present study five PD tests, yielding 15 different variables were analysed without applying a correction for multiple testing. Only a weak signal for a dose-response relationship was observed in the study. Therefore, the multi-modal battery of pain tasks should be considered as a first screening tool for studying the analgesic properties of pain compounds in development. When the analgesic effect of a new drug on a certain pain mechanism has been established, predefining a primary outcome measure would prevent the need to correct for multiple testing. Furthermore, the present study was not formally powered for analgesic efficacy on the evoked pain tasks.

CSF sampling was limited in cohorts 1 and 2 because of catheter sampling difficulties. The introduction of a different type of intrathecal catheter improved the sampling success rate in the second part of cohort 2 treatment and in cohort 3. The total volume of CSF in humans is approximately 170 ml.[38] Administration of 2.5 mg Xen2174 intrathecally would theoretically lead to a C_{max} of 14,705 ng ml^{-1}. We found a C_{max} of 33,200 ng ml^{-1} after administration of 2.5 mg of Xen2174. This may suggest that the study drug was not completely mixed throughout the CSF at T_{max}. Alternatively, the CSF volume in which the drug can freely diffuse, even if proper mixing had occurred, was overestimated for yet unknown reasons. Describing the PK in CSF is different to that in plasma. Drugs administered intravenously are rapidly distributed within the central distribution volume. The PK of drugs administered in less 'well-stirred', oscillating fluid systems, like the CSF, is more difficult to predict;[38,39] as such, it is difficult to predict drug concentrations at a particular level in the spinal column or intracranially. However, describing the dose-response relationship is more feasible if the site of injection of a drug is directly at the target site,[38] which was the case in the present study.

No PK or PK/PD modelling was performed on the data. As discussed previously, the site of administration was the same as that of sampling. As a consequence, the drug concentrations of the CSF samples may have

been the sum of the concentration in CSF and that of the drug solution that had not yet fully distributed throughout the CSF, for which we could not quantitatively correct. The development of a PK model on these CSF data would have resulted in high uncertainty in parameter estimates and large values for variability, also contributed by to the limited number of subjects. As a result, the parameter estimates were not expected to have physiological meaning, but merely to describe the observations in the lower spine. Moreover, Xen2174 has a high molecular weight and is therefore not expected to passively cross the blood-brain barrier to a large extent, apart from leakage. Finally, using the PK models that describe the CSF concentrations in the lower spine as the driving force for the PD would also have resulted in parameter estimates with high levels of uncertainty and large between subject variability – in our view, parameter estimates that have limited physiological meaning. The purpose of measuring CSF and plasma samples was to provide quantitative evidence of CNS exposure and limited plasma exposure which in our view, is sufficiently supported by the non-compartmental analysis. Given the lack of real physiological meaning that PK parameter estimates would have had, it was decided not to develop a PK model; similarly, the development of a PK/PD model would not have been logical.

Based on the literature, the incidence of postlumbar puncture syndrome was higher than expected. In a study in which the same intrathecal catheter was used, one out of eight subjects reported headaches.[40] A possible explanation for this difference might be the age difference (63.3 years *vs.* 25.6 years in our study). Younger age is an established risk factor for the occurrence of postlumbar puncture headache. Other reported risk factors are a low BMI and female gender.[41] Nonetheless, the exclusion of women and subjects with a BMI below 23 kg m^{-2} from cohorts 2 and 3 did not reduce the incidence or severity of this AE.

In the present study, there was a weak signal that Xen2174, at a dose of 2.5 mg increased the PTT for pressure pain. However, at the highest dose level tested, CSF Xen2174 concentrations exceeded the required exposure limit based on the nonclinical safety margins, which makes it unlikely that the compound can be used in practice for the treatment of acute pain.

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TABLE 1

Summary of previous clinical studies with Xen2174.

Study	Number (n) of subjects	Dose	Outcomes	Serious adverse events*
Phase 1 study of Xen2174 administered intravenously in healthy male subjects	n=16 treated with Xen2174; n= 4 placebo	10-200 $\mu g\ kg^{-1}$	No effects on nociceptive testing.	None
Phase 1-2 open-label study of Xen2174 administered intrathecally in oncology patients with chronic pain	n=36 treated with Xen2174	0.025-40 mg	No definitive conclusions regarding clinical benefit due to small number of patients per dose group and variation in type of pain. Each cohort contained at least one patient with >90% reduction in pain scores.	Confusion and dysphasia (0.25 mg), apnea, unresponsiveness, grand mal seizure (40 mg), aseptic drug-induced meningitis (40 mg)
Phase 2 study of Xen2174 administered intrathecally in adults prior to bunionectomy surgery (partially completed)	n=13 treated with Xen2174; n=3 placebo	1.0 mg	No final conclusion regarding clinical efficacy.	None
Phase 1 EEG safety study of Xen2174 administered in healthy male and female subjects	n=28 treated with Xen2174; n=7 placebo	0.1-2.5 mg	No apparent effects on EEG.	None

*Considered related to the study drug. EEG, electroencephalogram

TABLE 2

Summary of treatment emergent adverse events (AEs) by frequency [n(%)]. AEs occurring more than once within one treatment are reported.

Treatment	Placebo (N=8)	Xen2174 0.5 mg (N=8)	Xen2174 1.0 mg (N=8)	Xen2174 2.5 mg (N=9)
Subjects with ≥1 AE	8 (100)	8 (100)	8 (100)	9 (100)
Number of different AEs	16	15	13	17
Post Lumbar Puncture Syndrome	5 (62.5)	5 (62.5)	7 (87.5)	8 (88.9)
Catheter Site Related Reaction	7 (87.5)	3 (37.5)	4 (50.0)	6 (66.7)
Back Pain	3 (37.5)	2 (25.0)	4 (50.0)	5 (55.6)
Headache	5 (62.5)	3 (37.5)	4 (50.0)	2 (22.2)
Paraesthesia	3 (37.5)	1 (12.5)	1 (12.5)	1 (11.1)
Dizziness	-	3 (37.5)	-	1 (11.1)
Fatigue	1 (12.5)	-	1 (12.5)	2 (22.2)
Musculoskeletal Stiffness	-	-	-	3 (33.3)
Presyncope	2 (25.0)	-	1 (12.5)	-
Somnolence	-	1 (12.5)	-	2 (22.2)

TABLE 3

Least squares means for the pain tolerance thresholds and estimates of difference, 95% confidence intervals and p-values for main contrasts.

	LS Means					Contrast		
Parameter	**Placebo**	**Xen2174 0.5 mg**	**Xen2174 1.0 mg**	**Xen2174 2.5 mg**	**Treatment p-value**	**Xen2174 0.5 mg - Placebo**	**Xen2174 1.0 mg – Placebo**	**Xen2174 2.5 mg – Placebo**
Cold PTT (s)	39.94	33.18	34.84	38.58	0.5419	-16.9% (-38.1%, 11.5%) P=0.2072	-12.8% (-35.1%, 17.2%) P=0.3502	-3.4% (-27.8%, 29.2%) P=0.8091
Electrical repeated PTT (mA)	11.01	10.06	10.39	14.19	0.0713	-8.7% (-33.4%, 25.2%) P=0.5610	-5.7% (-30.3%, 27.8%) P=0.6967	28.9% (-3.3%, 71.7%) P=0.0811
Electrical single PTT (mA)	25.54	22.85	22.52	29.92	0.1801	-10.5% (-33.2%, 19.8%) P=0.4406	-11.8% (-34.0%, 17.8%) P=0.3805	17.1% (-10.4%, 53.2%) P=0.2372
ICPM: Delta Electrical Stair PTT (mA)	-1.88	-1.05	-0.83	-1.20	0.7615	0.83 (-1.29, 2.96) P=0.4294	1.05 (-1.06, 3.16) P=0.3162	0.68 (-1.48, 2.84) P=0.5253
Pressure PTT (kPa)	60.52	54.36	53.68	73.95	0.0328	-10.2% (-30.1%, 15.5%) P=0.3888	-11.3% (-30.7%, 13.6%) P=0.3285	22.2% (-5.0%, 57.1%) P=0.1131

ICPM, inhibitory conditioned pain modulation; kPa, kilopascal; LS, least squares; mA, milliampere; PTT, pain tolerance threshold; s, seconds

TABLE 4

Cerebrospinal fluid (CSF) pharmacokinetic parameters for Xen2174.

Dose Xen2174 (mg)		**C_{max} (ng ml^{-1})**	**T_{max} (h)**	**$t_{½}$ (h)**	**AUC_{last} (h ng ml^{-1})**	**$AUC_{0-\infty}$ (h ng ml^{-1})**
Xen2174 0.5 mg	Mean	4080	0.50	7.14	6532	8081
	SD	4090	0.00	4.69	5717	7930
Xen2174 1.0 mg	Mean	5600	1.40	4.27	29655	29912
	SD	3340	1.47	0.790	15348	15443
Xen2174 2.5 mg	Mean	33200	0.56	4.83	157594	159146
	SD	16600	0.18	0.843	63810	64089

$AUC_{0-\infty}$, The area under the curve from time zero to infinity; AUC_{last}, The area under the curve from time zero to the last measurable concentration; C_{max}, peak concentration; SD, standard deviation; T_{max}, time to reach C_{max}; $t_{½}$, half-life

TABLE 5

Plasma pharmacokinetic parameters for Xen2174.

Dose Xen2174 (mg)		**C_{max} (ng ml^{-1})**	**T_{max} (h)**	**$t_{½}$ (h)**	**AUC_{last} (h ng ml^{-1})**	**$AUC_{0-\infty}$ (h ng ml^{-1})**
Xen2174 0.5 mg	Mean	5.49	1.94	5.79	34.2	45.3
	SD	3.20	1.15	2.38	12.4	10.9
Xen2174 1.0 mg	Mean	9.75	3.69	5.96	69.7	87.5
	SD	3.49	2.89	3.28	15.9	14.5
Xen2174 2.5 mg	Mean	15.4	6.89	8.62	200	221
	SD	5.83	3.14	1.41	39.7	39.3

$AUC_{0-\infty}$, The area under the curve from time zero to infinity; AUC_{last}, The area under the curve from time zero to the last measurable concentration; C_{max}, peak concentration; SD, standard deviation; T_{max}, time to reach C_{max}; $t_{½}$, half-life

FIGURE 1

Disposition of subjects

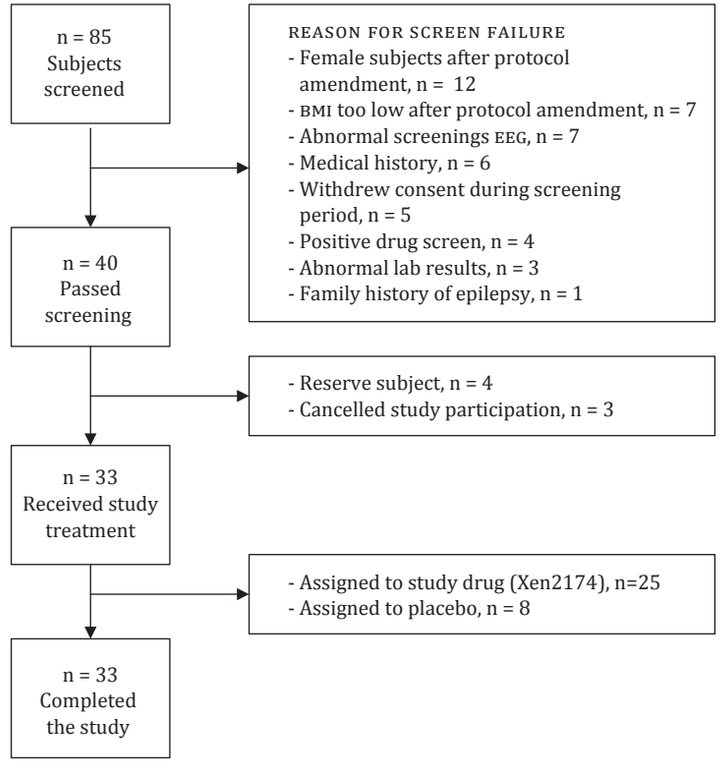


FIGURE 2

Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for electrical stimulation tasks (single [A] and repeated stimulus [B]), cold pressor task [C] and the pressure stimulation task [D] after administration of single doses of Xen2174 (0.5, 1.0 or 2.5 mg) or placebo. Vertical lines represent the 95% confidence intervals.

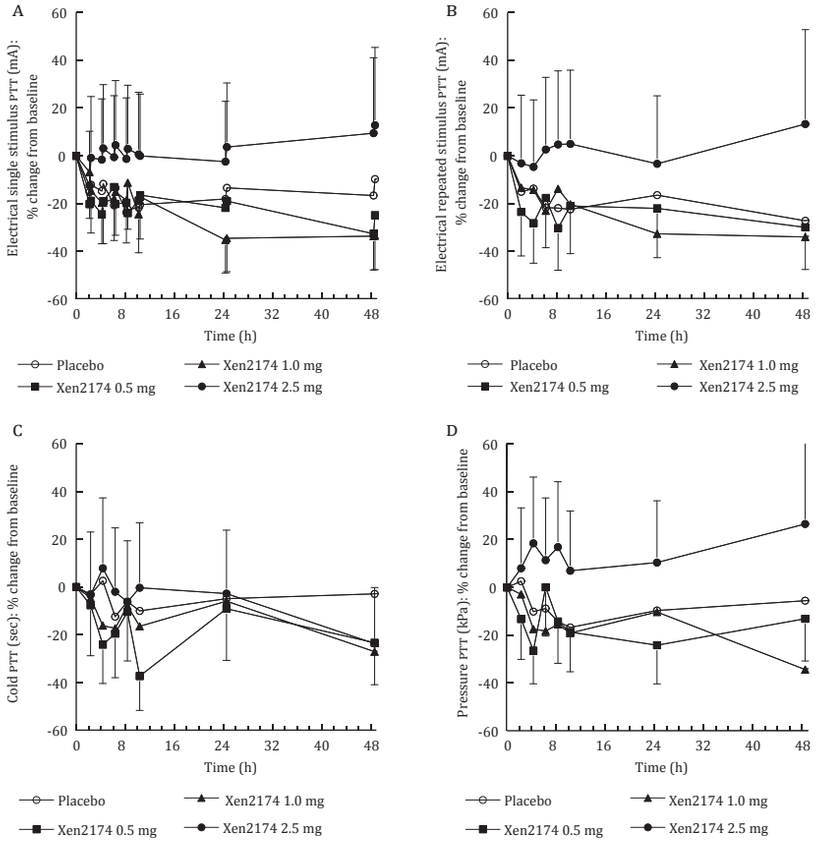


FIGURE 3

Mean CSF Xen2174 concentration-time by cohort. Vertical lines represent the standard deviation.

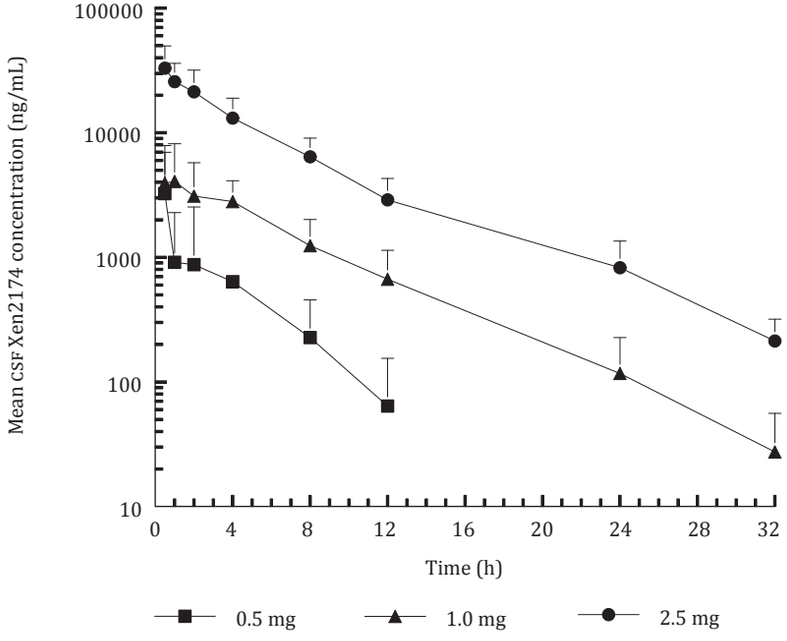
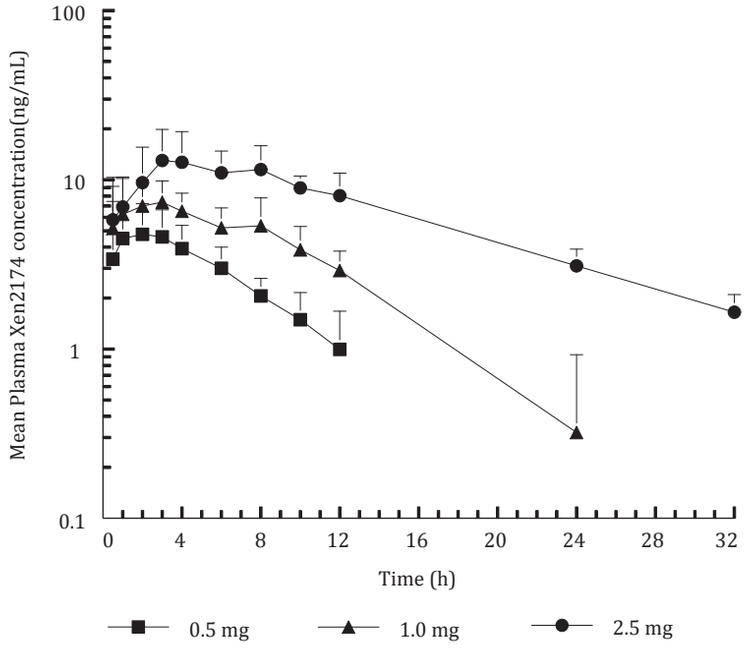


FIGURE 4

Mean Plasma Xen2174 concentration-time by cohort. Vertical lines represent the standard deviation.



CHAPTER V

No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery

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ABSTRACT

Serotonin–norepinephrine reuptake inhibitors inhibit the reuptake of serotonin and noradrenalin and are used in the treatment of neuropathic pain. Animal studies suggest that milnacipran co-administered with opioids may potentiate the analgesic effect of μ-opioid receptor agonists. This study hypothesised that co-administration of milnacipran and buprenorphine would have a synergistic effect in evoked pain models in healthy subjects. This was a randomised double-blinded, placebo-controlled, four-way cross-over, multiple dose clinical trial to investigate the analgesic effects of buprenorphine (placebo, 0.5, 1 and 3 μg kg^{-1}) in combination with milnacipran (placebo, 25 and 50 mg) in healthy subjects. 11 healthy men were enrolled in the study. Buprenorphine alone showed a dose-response relationship indicative of anti-nociception in the pain tests. Following milnacipran administration no changes were seen in the pharmacodynamic measurements for pain, psychomotor function, body stability or eye movements. For the electrical tests, cold pressor test and pressure pain test, buprenorphine alone was superior when compared with buprenorphine plus milnacipran. No differences in pharmacodynamic variables, besides an increase in pupil/iris ratio, were observed after repeated administration of milnacipran 50 mg. Single and multiple doses of 25 or 50 mg milnacipran did not further potentiate the anti-nociceptive effects of buprenorphine. Buprenorphine showed dose-dependent effects consistent with its pharmacological profile. Milnacipran alone did not affect any of the pain variables. The combination of both buprenorphine and milnacipran did not potentiate or show a synergistic effect on the pain models used in this study.

INTRODUCTION

Severe pain represents an important challenge for the clinician. Guidelines for moderate to severe pain treatment recommend the use of opioids. However, these can lead to dose-dependent side effects such as constipation, nausea, vomiting and sedation.[1]

Current strategies in opioid use in the clinic include administering the lowest dose possible with still an adequate analgesic effect. An alternative strategy is to combine opioids with other drugs that might have a synergistic effect, which could thus lead to lower opioid dosages and therefore fewer side effects. Some suggested combinations are opioids in combination with norepinephrine transporter modulators, calcium channel alpha-2 delta ligands or local anesthetics.[2]

It has been demonstrated that milnacipran, a serotonin-noradrenaline reuptake inhibitor (SNRI), inhibits C-fibre-mediated nociceptive synaptic transmission in the spinal dorsal horn after the establishment of spinal long term potentiation in a neuropathic pain model, by activating both spinal serotonergic and noradrenergic systems.[3] The inhibition of the C-fibre-mediated transmission by milnacipran could provide new evidence regarding the analgesic mechanism of SNRIs in chronic pain. Currently, milnacipran is available as a pharmacological intervention to treat chronic neuropathic pain and fibromyalgia; however its effectiveness in the treatment of pain is limited.[4,5]

There is nonclinical evidence that milnacipran potentiates the antihyperalgesic effects of opioids such as tramadol.[6] Furthermore, in animal studies the antihyperalgesic effects of milnacipran can be blocked by naloxone, an opioid receptor antagonist, suggesting a possible opioidergic mechanism of action of this SNRI.[7] This is supported by findings that show that noradrenergic, serotonergic and endogenous opioidergic systems are essential for milnacipran to reduce mechanical hyperalgesia.[6]

Serotonin-norepinephrine reuptake inhibitors (SNRIS) have shown effectiveness in human evoked pain models (single electrical stimulation and repetitive electrical stimulation) with venlafaxine, even with a short period of time (h) between the administration and the measured effect.[8] Numerous human evoked pain models are sensitive to the effects of opioids[9] . Currently tapentadol, a drug that is both a μ-opioid receptor agonist and a noradrenaline reuptake inhibitor, is marketed for the treatment of severe acute and chronic neuropathic pain. Synergy between the dual mechanism of action of tapentadol has been demonstrated in several preclinical studies.[10]

This study aimed to evaluate the potential synergy and potentiation – as shown in preclinical studies – of milnacipran, when co-administered with a potent μ-opioid receptor partial agonist, buprenorphine, in evoked pain models in healthy subjects. This study aimed to investigate whether a subtherapeutic dose of buprenorphine could become therapeutic through co-medication with single or multiple doses of milnacipran and to determine whether the analgesic effect of buprenorphine at *therapeutic* dose levels could be enhanced by milnacipran.

METHODS

The study was approved by the Medical Ethics Committee of the BEBO Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2012-002302-43).

Subjects

Healthy male subjects between 18 and 45 years with a body mass index of 18-30 kg m^{-2} were to be enrolled after having given written informed consent. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and haematology, urinalysis and electrocardiogram (ECG) to assess eligibility. Key exclusion criteria were as follows: clinical significant abnormalities during screening, regular user of any illicit drugs or history of drug abuse, a positive drug screen at screening or smoking within 3 months prior to screening. Use of xanthine-containing products and alcohol was not allowed during the stay at the research unit. Subjects were not allowed to use any medication from one week prior to the start of the study days.

Study design and treatments

This was a randomised, double-blind, placebo-controlled, four-way crossover study with three different doses of buprenorphine or placebo in combination with milnacipran or placebo. The total number of planned subjects was 10.

The four treatment arms were as follows: buprenorphine active treatment in combination with milnacipran 25 mg (BUP+MIL-25), buprenorphine active treatment in combination with milnacipran 50 mg (BUP+MIL-50), buprenorphine-placebo in combination with milnacipran 50 mg (BUP-P+MIL-50) and buprenorphine active treatment in combination with milnacipran-placebo (BUP+MIL-P). The computer-generated randomization list was prepared by the statistician prior to the start of the study. Doses were prepared by a pharmacist/technician not involved in any of the study procedures. Buprenorphine (Temgesic; RB Pharmaceuticals Limited, Slough Berkshire, UK) was administered as an intravenous solution on day 1 and day 8 in three different doses. The buprenorphine dosing schedule was based on a published population pharmacokinetic and pharmacodynamic (popPK/PD) model with the electric pain tolerance threshold as a pharmacodynamic endpoint.[11] At the end of each buprenorphine intravenous infusion, the pharmacodynamic effects of buprenorphine were expected to remain reasonably stable, which would allow the performance of the pain tests (Figure 1). First, a 30 min 0.5 μg kg^{-1} infusion, which was expected to lead to subtherapeutic plasma concentrations, followed 1.5 h later by a second 30 min 1 μg kg^{-1} infusion, which was expected to lead to minimally therapeutic plasma concentrations, finally followed 1.5 h later by a 30 min 3 μg kg^{-1} infusion, which was expected to lead to therapeutic plasma concentrations of buprenorphine. Milnacipran hydrochloride (Pierre Fabre, Castres, France) was administered orally twice daily starting from day 1 and until the morning of day 8. Intravenous metoclopramide 10 mg (Primperan; Sanofi-Aventis, Paris, France) was administered prophylactically before the second buprenorphine/placebo infusion to prevent nausea and vomiting. Additional doses of metoclopramide were administered if needed.

Each of the four study periods lasted 8 days. On the morning of day 1, subjects arrived at the clinical research unit and received the first oral dose of milnacipran or placebo. Thereafter, they received the three intravenous administrations of buprenorphine or placebo according to the different infusion schedules, separated by 1.5 h. After each infusion, the pharmacodynamic tasks were performed (evoked pain tasks and neurophysiological tests). At the end of the study day, subjects were discharged and were instructed to orally administer milnacipran or placebo twice a day at home. On day 8, subjects returned to the unit and the same procedures as on day 1 were followed. There was a 14-day wash-out interval between study periods. An overview of a study period is shown in Figure 2.

Pharmacodynamics

Pharmacodynamic measurements were performed pre-dose (twice) and 1 h after the start of each buprenorphine administration. A training session was included as part of the screening examination to reduce learning effects during the study. All measurements were performed in a quiet room with ambient illumination. Tests were performed in a fixed order (Figure 3).

Nociceptive (pain) detection and tolerance thresholds were measured using a battery of evoked pain tasks. The battery is an integrated range of tasks for measuring different modalities of nociception (electrical pain, pressure pain and cold pressor tasks). It aims to assess as objectively as possible the levels of pain induced by several noxious mechanisms in human subjects. All nociceptive tests had previously been shown to be sensitive to the effects of analgesics in healthy adults. Other pharmacodynamic tests included pupil size measurements, adaptive tracking, saccadic eye movements and body sway. These tests have previously been shown to be sensitive to the effects of several different classes of drugs.[12,13]

Pain intensity was measured continuously (beginning from when the first stimulus was applied until the predetermined end of the test) for each nociceptive task using an electronic visual analogue scale (eVAS) ranging from 0 (no pain) to 100 (most intense pain tolerable). Equipment was programmed to cease giving stimuli if pain intensity reached the maximum possible score. For each task the pain detection threshold (PDT), pain tolerance threshold (PTT) and area under the pain intensity-stimulation (-time for cold pressor) curve (AUC) were calculated.

Electrical stimulation task

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™) were placed on cleaned, scrubbed, and if required, shaved skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be <2 kW. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5; Digitimer, Cambridge, UK).

For the single stimulus, adapted from methods previously described,[14,15] (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 mA s^{-1} until the pain tolerance threshold was reached or up to a cutoff of 50 mA.

For the repeated stimulus, adapted from methods previously described,[16] each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3 to 8 seconds between the repetitions. Current intensity increased from 0 to 50 mA in steps of 0.5 mA. Pain detection threshold (PDT) was taken as the value (mA) whereby a subject indicated either: all five stimuli were painful, or the train of five stimuli started feeling non-painful but ended feeling painful (VAS > 0). The pain intensity for each stimulation was measured using the eVAS slider, until pain tolerance threshold was reached or a maximum of 50 mA was reached.

Pressure stimulation task

The method of mechanical pressure pain induction was based on methods previously described and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.[17,18] Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa s^{-1}. 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, at which point the device released pressure to the cuff.

Cold pressor task

The method of cold pressor pain was based on the methods previously described[19,20] and is the most commonly used test to induce inhibitory conditioned pain modulation (iCPM, also known as 'diffuse noxious inhibitory control').[21] Subjects placed their non-dominant hand into a water bath (minimal depth 200 mm) (Lauda, Germany) at 35 ± 0.5°C for 2 min. At 1 min 45 s, a blood pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min, the subject then moved that hand from the warm water bath, directly into a similar sized bath at 1.0 ± 0.5°C. The subjects were instructed to indicate when PDT was reached (first change in sensation from cold non-painful to painful) as well as the pain intensity, by moving the eVAS slider. When PTT 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.

Conditioned pain modulation

Conditioned pain modulation is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[21] The degree of iCPM was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the end of cold pressor task.

Pupil size

Pupil diameter was determined using a digital camera and a flash. The pupil/iris ratio was calculated as a measure of pupil size (Qpupil, Leiden University Medical Center, Leiden, the Netherlands).

Adaptive tracking

The adaptive tracker is a psychomotor task and is sensitive to impairment of eye-hand coordination. The adaptive tracking test was performed as originally described by Borland and Nicholson,[22] using customised equipment (Hobbs & Strutt, UK). A circle moves randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance over 3.5 min was used for analysis. The outcome is the average velocity of the circle as percentage of maximal velocity possible.

Saccadic eye movements

Saccadic peak velocity is one of the most sensitive parameters for sedation and was described previously.[23,24] Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements. The program for signal collection and the AD-converter was from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, MA, USA) and the sampling and analysis scripts were developed at CHDR (Leiden, the Netherlands). Disposable silver-silver chloride electrodes (Ambu BlueSensor N, Ballerup, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. The target consists of a moving dot that is displayed on a computer screen. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 s. Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were analysed.

Body sway

The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with pot string meter (Celesco, Chatsworth, CA, USA) based on the Wright ataxiameter.[25] Subjects were asked to stand still and comfortable, with their feet approximately 10 cm part and their hands in a relaxed position alongside the body and eyes closed. With a string attached to the waist, all body movements over a period of two min were integrated and expressed as mm sway.

Measurements of drug concentrations in plasma

Samples for determination of milnacipran in plasma were obtained at baseline, 1, 2, 3, 4, 5, 6, 7 and 11 h after oral administration on days 1 and 8. Samples for determination of buprenorphine and its active metabolite nor-buprenorphine were obtained 1 and 2 h after the start of each infusion.

Samples were collected in lithium heparin tubes and stored in ice. Plasma was separated within 30 min of blood collection by centrifugation at 2000 *g* for 10 min. Samples were stored at -70°C until analysis. Drug concentrations in plasma were determined using a validated Liquid Chromatography–Mass Spectrometry (LC-MS/MS) technique. The analytical range of the assay was 1.00-500 ng/mL for milnacipran and 0.100-20.0 ng/mL for buprenorphine and nor-buprenorphine.

Statistics

The sample size calculation was based on previous experiments in healthy young men. An average cold pressor AUC of 10,000 s mm was expected. We expected that the highest dose of buprenorphine would cause a decrease in the cold pressor AUC of 30%. If an increase of that difference to 37% was to

be established due to co-administration of milnacipran, then assuming an SD of 617 (based on data on file), with 80% power and a two-sided alpha = 0.05, a sample size of eight subjects was needed.

Pharmacokinetic analysis was performed using a non-compartmental model approach. For milnacipran and buprenorphine, the peak concentration (C_{max}) and the time to the peak concentration (T_{max}) was recorded as observed. In addition, the area under the plasma concentration-time curve from time zero to the time of the last sample (AUC_{0-last}) was determined for both drugs. Calculations were performed using R v2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

The pharmacodynamic data were compared, per day, with a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors, subject, subject by treatment and subject by time as random factors and the average prevalue (per treatment average of measurements before time=0 on day 1, also for the day 8 analysis) as covariate. This analysis was carried out on the four original treatments. The contrast between buprenorphine alone and buprenorphine and milnacipran 50 mg during the baseline measurements on day 8 was assessed to determine the effects of repeated milnacipran dosing.

Variables that followed a log-normal distribution were log-transformed before analysis. Transformed parameters were back-transformed after analysis.

Synergy was tested as the contrast of (buprenorphine alone) plus (milnacipran 50 mg alone) minus the overall average pre value (day 1) versus (buprenorphine and milnacipran 50 mg). The average overall prevalue of day 1 is used as there is no milnacipran-placebo and buprenorphine-placebo treatment. The values of the buprenorphine alone plus milnacipran 50 mg alone minus the overall average pre value were calculated prior to analysis. Together with the buprenorphine plus 25 mg milnacipran and the buprenorphine plus 50 mg milnacipran, the calculated synergy values were analysed in a separate repeated measure mixed model, with fixed factors of treatment, time and treatment by time, random factors of subject, subject by treatment and subject by time and the average pre-value per treatment of day 1 as covariates. 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.

RESULTS

A total of 11 subjects participated in the trial (see Figure S1 for study flowchart); subjects were aged 21-31 years (mean age 24.2 years) and had a body mass index of between 20 and 26 kg m^{-2} (mean 22.4 kg m^{-2}). Nine subjects completed the trial until the last study occasions. Two subjects dropped out from the study. One subject was withdrawn by the investigator due to side effects categorised as probably related to milnacipran (shortness of breath, palpitations, dizziness, urinary hesitation and paraesthesias). The other subject withdrew consent due to side effects (nausea and vomiting) caused by buprenorphine.

Nociceptive tests

The least squares means and the analysis results for the PTTs for the different nociceptive tests are presented in Table 1, Table 2 and figure 4. On day 1, a significant overall treatment effect was found for the PTT of the electrical stimulation tasks, the pressure stimulation task and the cold pressor tasks. On day 8, no significant overall treatment effect was found for any of the pain tests. None of the contrasts of buprenorphine plus milnacipran versus buprenorphine alone showed a significant increase of pain tolerance or detection. Buprenorphine in combination with milnacipran 50 mg significantly decreased the repeated electrical stimulation PTT and the cold pressor PDT compared with buprenorphine alone (PDT data not shown). The effects were not observed on day 8. Buprenorphine in combination with milnacipran did not lead to greater analgesic effects compared with buprenorphine alone. On day 8, buprenorphine in combination with milnacipran 50 mg and buprenorphine in combination with 25 mg milnacipran increased the cold pressor PTT compared with buprenorphine alone. The contrast between buprenorphine in combination with milnacipran 50 and 25 mg after 8 days of repeated dosing was statistically significant; however no overall treatment effect was observed for the cold pressor test on day 8 ($p = 0.0777$).

No treatment effects could be observed on ICPM on day 1 or day 8. Repeated milnacipran dosing did not affect any of the pain variables during the day 8 baseline measurements.

Synergy between treatments was assessed in a separate analysis (Table 3). No synergy was observed when buprenorphine and milnacipran were administered together, either after a single dose or after repeated dosing.

Pharmacodynamic tests of psychomotor function, body stability, eye movements and pupil size

The least squares means and the analysis results for pharmacodynamic tests for psychomotor function, body stability, eye movements and pupil size are presented in Table 4 and Figure 5. The pupil/iris ratio decreased in all treatment groups receiving buprenorphine both on day 1 and day 8, compared with milnacipran alone. On day 1, no differences were observed in pupil size between buprenorphine/milnacipran combination groups compared with buprenorphine alone. On day 8, the pupil/iris ratio was significantly larger in the buprenorphine/milnacipran combination groups compared with buprenorphine alone after the first and second buprenorphine dose. Saccadic peak velocity and adaptive tracking performance decreased after receiving the buprenorphine combinations compared with milnacipran alone. Milnacipran did not significantly potentiate the decrease due to buprenorphine in saccadic peak velocity or adaptive tracking. A significant treatment effect was observed for the body sway measurements on day 8, and no significant differences between buprenorphine combinations versus buprenorphine alone were observed. Repeated milnacipran dosing only lead to an increase in pupil/iris ratio (0.071; 95% CI 0.024-0.119; p = 0.0045; data not shown). No differences in eye movement, psychomotor function or body stability were observed during day 8 baseline measurements.

Pharmacokinetics

The sampling of buprenorphine and milnacipran was intended to corroborate adequate exposure and to detect possible pharmacokinetic interactions when co-administered. Samples taken after the first buprenorphine infusion were not analysed as it was expected that these samples would be below the lower limit of quantification. After the second and third buprenorphine infusions the measured concentrations were as expected. The mean (population) expected buprenorphine concentrations values of the pharmacokinetic model that was used to define the study is shown with the actual concentrations achieved in the study also plotted on top (Figure 6). The samples for buprenorphine near to the trough show a slight overestimation in comparison to the prediction by the model. Samples were also assayed for nor-buprenorphine but due to its low concentration in plasma, all determinations were below the lower limit of quantification (LLOQ).

Pharmacokinetic parameters for milnacipran on days 1 and 8 after administration are shown in Table 5. C_{max} and AUC_{0-last} for milnacipran increased in a dose dependent manner. AUC_{0-last} and C_{max} were approximately two-fold greater on day 8 compared with day 1.

Safety

As shown in Table 6, all subjects experienced at least one treatment emergent adverse event in each study period. The most reported adverse events were nausea, somnolence and vomiting. Vomiting occurred in all subjects receiving buprenorphine and the high dose of milnacipran. In subjects only receiving milnacipran 50 mg, only three subjects reported vomiting.

DISCUSSION

The study aimed to evaluate the possibility of potentiation or synergy of milnacipran, when co-administered with buprenorphine in evoked pain models in healthy subjects, both after single and multiple doses of milnacipran. Furthermore, the interaction of both compounds on psychomotor function, body stability, eye movements and pharmacokinetic parameters and safety was investigated.

Nociceptive tests and measurements of psychomotor function, body stability and eye movements following buprenorphine administration were consistent with the drug being a partial opioid receptor agonist. Buprenorphine showed a dose-response relationship indicative of antinociception for the variables evaluated in the electrical, cold and pressure tests. Also, a decline in the performance was seen in the adaptive tracker and saccadic eye movements after the buprenorphine administration, indicative of a mild level of sedation. Following single and repeated milnacipran (alone) administration, no changes were observed in the nociceptive tasks or other pharmacodynamic measurements. No earlier studies were performed with milnacipran using pain models. Effects of other SNRIs in human pain models have been reported before.[8] Venlafaxine increased pain tolerance thresholds on single electrical stimulation and pain summation on repetitive stimulation; however in that study no differences on the cold pressor test and on a pressure pain paradigm could be observed. These reported findings could not be replicated in our study.

Acute, single doses of 25 and 50 mg milnacipran did not potentiate the antinociceptive effects or the effects on psychomotor function, body

stability, eye movements and pupil size when given in combination with buprenorphine 0.5, 1 and 3 μg kg^{-1} and the combination did not lead to synergy. In fact, for the repeated electrical stimulation task (repeated stimulus), the combination buprenorphine and milnacipran 50 mg lead to a lower PTT compared with buprenorphine alone. For the saccadic peak velocity, the addition of milnacipran 50 mg diminished the pharmacodynamic effects (i.e. decrease in saccadic peak velocity) observed after buprenorphine alone, which may be an indication of less sedation.

On day 8, after repeated administration of milnacipran 25 or 50 mg, no potentiation or synergy was observed between buprenorphine and milnacipran on the pain variables or the other pharmacodynamic variables. A significant difference in the effect on cold pressor PTT was observed between buprenorphine alone and buprenorphine in combination with milnacipran 25 and 50 mg on day 8. However, no overall significant treatment effect was observed for the cold pressor PTT on day 8. Moreover, no adjustments for multiple testing were applied in this study, so we consider this finding not sufficiently indicative of the existence of potentiation due to the combined treatment with buprenorphine and milnacipran.

The pupil/iris ratio decreased after treatment with buprenorphine (with and without co-administration of milnacipran). However, after 8 days of treatment with milnacipran 50 mg, the pupil/iris ratio after the first and second buprenorphine infusion was significantly larger in the buprenorphine/milnacipran combination group compared with buprenorphine alone. Miosis after treatment with buprenorphine is a well-known effect of μ-opioid receptor agonists.[26] Mydriasis after treatment with an SNRI has been shown before for duloxetine[27] and for venlafaxine.[28] Here, we show both the mydriasis effect of milnacipran after 8 days of treatment and the acute miosis effect of buprenorphine. The mydriasis effect of milnacipran could only be reversed at the highest dose of buprenorphine.

In the cases that an overall treatment effect was observed for nociceptive endpoints on day 1, which were due to the effect of buprenorphine versus no buprenorphine, no overall treatment effects were observed on day 8. This may indicate that some tolerance may have occurred for the antinociceptive effects of buprenorphine or an increased (but not significant) effect of milnacipran after 8 days of treatment. In contrast, for the endpoints of psychomotor function, body stability and eye movements, when an overall treatment (buprenorphine) effect was observed on day 1, it was also observed on day 8.

Milnacipran has a modulating effect on serotonin (5-HT) and norepinephrine (NE) neurotransmitters. 5-HT and NE are involved in the modulation of endogenous analgesic mechanisms via inhibitory pain pathways in the central nervous system.[29,30] Opioids can also influence descending pain pathways.[31] Earlier, it was shown that buprenorphine is able to potentiate ICPM in a human pain model.[32] This study was not able to replicate these findings. No effects were observed in conditioned pain modulation after treatment with milnacipran, buprenorphine or the combination of the two.

In animal studies, milnacipran inhibited C-fibre-mediated nociceptive synaptic transmission in the spinal dorsal horn after the establishment of spinal long term potentiation in a neuropathic pain model.[3] Furthermore, it has been shown that milnacipran reduces thermal and mechanical allodynia in a rat model of neuropathic pain (chronic constriction injury of the sciatic nerve).[33] However, in another model of neuropathic pain in mice (central post-stroke pain model), milnacipran did not affect mechanical allodynia thresholds.[34] Co-administration of milnacipran with tramadol potentiated the antihyperalgesic effect of tramadol,[6] which used chronic constriction injury model as a model for neuropathic pain. Considering the effects of milnacipran in several hyperalgesia models in animals, a pain model which is able to measure antihyperalgesia in humans would have been a valuable addition to this study.

Only models for acute pain were used in this study. It is important to note that differences exist between these models and clinical chronic (neuropathic) pain. In a recent review Lötsch *et al.* suggested that hyperalgesia and electrical pain models might be used to predict clinical analgesia in neuropathic pain.[35] However, several pharmacological (NMDA receptor antagonists, tricyclic antidepressants, SNRIs, gabapentin) and non-pharmacological therapies (such as repetitive transcranial stimulation) that are used to treat neuropathic pain have shown contradictive results on acute pain models.[36,37] Therefore, although we were not able to show potentiation or synergy in these acute pain models, no conclusions can be drawn on a possible synergistic effect of milnacipran and buprenorphine in chronic pain conditions.

In the milnacipran pharmacokinetic analysis, C_{max} and AUC_{0-last} for milnacipran increased in a dose proportional manner. Milnacipran showed accumulation after 7 days of treatment with 25 or 50 mg twice a day and resulted in higher concentrations in all subjects on day 8 of treatment. For buprenorphine, no formal pharmacokinetic analysis was possible due to

the sparse sampling. The plasma buprenorphine concentrations were in agreement with what was expected based on the simulation prior to the study.[11] No pharmacokinetic interaction was observed between milnacipran and buprenorphine.

This was a four-way crossover study and no study period with a double placebo was included. To assess synergy between buprenorphine and milnacipran, the milnacipran only plus the buprenorphine only value minus the average pre-value over all occasions were calculated. The buprenorphine plus 25 mg milnacipran, the buprenorphine plus 50 mg milnacipran and the new values of milnacipran plus buprenorphine minus pre-values were analysed in a separate repeated measure mixed model. No synergy between these treatments could be observed, although it can be argued that the lack of a complete placebo profile made formal testing for synergy difficult.

In conclusion, buprenorphine showed dose-dependent effects consistent with its pharmacological profile; antinociception and a decrease in neurophysiological functions. Milnacipran alone did not affect any of the pain variables. The combination of both buprenorphine and milnacipran did not potentiate or show a synergistic effect on the pain models used in this study. No conclusions can be drawn on a possible synergistic effect of milnacipran and buprenorphine in clinical, chronic pain conditions.

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TABLE 1

Least squares means for pharmacodynamic outcome measures.

	LS Means (day 1)				LS Means (day 8)			
Parameter	BUP +MIL-25	BUP +MIL-50	BUP +MIL-P	BUP-P +MIL-50	BUP +MIL-25	BUP +MIL-50	BUP +MIL-P	BUP-P +MIL-50
Electrical Repeat PTT (mA)	14.11	12.33	14.18	11.23	15.03	11.28	14.11	11.34
Electrical Single PTT (mA)	26.70	22.83	24.39	20.42	27.31	23.89	27.63	19.96
Pressure PTT (kPa)	57.79	54.81	58.44	43.45	59.93	53.68	51.99	46.63
Cold Pressor PTT (s)	29.16	28.37	26.56	19.54	32.14	32.23	25.25	26.44
Conditioned Pain Modulation (mA)	1.46	0.87	1.63	0.78	2.06	1.45	0.20	0.97
Left Pupil/Iris ratio	0.328	0.304	0.321	0.484	0.377	0.355	0.308	0.554
Saccadic Peak Velocity (deg s^{-1})	418.1	430.1	391.1	463.3	430.2	416.5	426.0	479.1
Body Sway (mm)	387.5	399.3	442.4	358.5	362.5	419.3	409.2	315.0
Adaptive Tracking (%)	20.42	19.67	19.34	26.86	22.07	22.95	21.39	27.18

TABLE 2

Estimates of difference, 95% confidence intervals and P-values for main contrasts for nociceptive measurements

		Contrast (day 1)			Contrast (day 8)		
Parameter		Treatment p-value	BUP+MIL-P vs BUP+MIL-25	BUP+MIL-P vs BUP+MIL-50	Treatment p-value	BUP+MIL-P vs BUP+MIL-25	BUP+MIL-P vs BUP+MIL-50
Electrical Repeat PTT (mA)	Overall	0.0016	0.5% (-12.1%, 14.8%) P=0.9418	15.0% (1.9%, 29.7%) P=0.0258	0.2504	-6.1% (-38.4%, 43.0%) P=0.7310	25.1% (-16.0%, 86.3%) P=0.2060
	After first BUP		-0.1% P=0.9891	6.8% P=0.4035		-1.0% P=0.9586	35.1% P=0.1116
	After second BUP		6.4% P=0.4725	21.6% P=0.0190		-5.9% P=0.7456	30.5% P=0.1499
	After third BUP		-4.6% P=0.5882	17.0% P=0.0618		-11.2% P=0.5344	11.1% P=0.5384
Electrical Single PTT (mA)	Overall	0.0107	-8.6% (-22.2%, 7.3%) P=0.2357	6.8% (-8.9%, 25.3%) P=0.3712	0.2552	1.2% (-31.5%, 49.4%) P=0.9484	15.6% (-21.7%, 70.8%) P=0.4232
	After first BUP		-9.3% P=0.3158	1.0% P=0.9205		6.6% P=0.7339	13.8% P=0.4927
	After second BUP		-8.6% P=0.3793	5.7% P=0.5720		2.4% P=0.9000	10.6% P=0.5964
	After third BUP		-8.0% P=0.4645	14.3% P=0.2566		-5.2% P=0.7825	22.8% P=0.2964
Pressure PTT (kPa)	Overall	0.0057	1.1% (-16.7%, 22.7%) P=0.9065	6.6% (-11.5%, 28.4%) P=0.4831	0.1132	-13.2% (-29.9%, 7.4%) P=0.1815	-3.1% (-21.4%, 19.4%) P=0.7534
	After first BUP		-9.4% P=0.3617	-10.5% P=0.2792		-13.5% P=0.2618	2.5% P=0.8419
	After second BUP		13.9% P=0.2615	16.5% P=0.1797		0.3% P=0.9837	-4.9% P=0.7138
	After third BUP		0.1% P=0.9910	16.3% P=0.2115		-24.7% P=0.0553	-6.8% P=0.6090
Cold Pressor PTT (S)	Overall	<.0001	-8.9% (-22.2%, 6.6%) P=0.2319	-6.4% (-19.9%, 9.4%) P=0.3928	0.0777	-21.4% (-38.3%,-0.0%) P=0.0500	-21.6% (-38.3%,-0.5%) P=0.0456
	After first BUP		-4.8% P=0.6228	0.1% P=0.9930		-19.9% P=0.1167	-26.4% P=0.0319
	After second BUP		-14.1% P=0.1462	-12.4% P=0.1950		-23.9% P=0.0552	-22.7% P=0.0693
	After third BUP		-7.6% P=0.4722	-6.4% P=0.5462		-20.3% P=0.1255	-15.4% P=0.2417
Conditioned Pain Modulation (mA)	Overall	0.7541	0.16 (-2.01, 2.33) P=0.8748	0.76 (-1.51, 3.02) P=0.4875	0.4630	-1.86 (-4.42, 0.71) P=0.1387	-1.24 (-4.03, 1.54) P=0.3536
	After first BUP		-1.42 P=0.3880	-0.47 P=0.7780		-1.02 P=0.5272	-0.44 P=0.7911
	After second BUP		1.29 P=0.4917	0.94 P=0.6034		-2.18 P=0.1822	-0.88 P=0.6237
	After third BUP		0.61 P=0.7772	1.81 P=0.4312		-2.36 P=0.1994	-2.41 P=0.2165

TABLE 3

Estimates of difference, 95% confidence intervals and P-values for contrasts to assess synergy for the nociceptive measurements

	LS Means (day 1)		Contrasts (day 1)	LS Means (day 8)		Contrasts (day 8)
Parameter	BUP +MIL-50	(BUP+MIL-P)+ (BUP-P+MIL-50) -PRE	(BUP+MIL-P)+ (BUP-P+MIL-50)-PRE VS BUP+MIL-50	BUP +MIL-50	(BUP+MIL-P)+ (BUP-P+MIL-50) -PRE	(BUP+MIL-P)+ (BUP-P+MIL-50)-PRE VS BUP+MIL-50
Electrical Repeat PTT (mA)	12.08	11.07	-8.4% (-24.5%, 11.2%) P=0.3504	11.46	11.01	-3.9% (-32.9%, 37.6%) P=0.8106
Electrical Single PTT (mA)	21.42	19.49	-9.0% (-26.9%, 13.2%) P=0.3493	24.00	25.07	4.5% (-29.0%, 53.6%) P=0.8059
Cold Pressor PTT (S)	28.53	23.89	-16.3% (-32.6%, 4.0%) P=0.1020	32.84	28.45	-13.4% (-32.0%, 10.3%) P=0.2161
Pressure PTT (kPa)	53.13	51.95	-2.2% (-15.3%, 12.8%) P=0.7377	53.27	43.62	-18.1% (-36.4%, 5.4%) P=0.1121
Conditioned Pain Modulation (mA)	1.52	1.37	-0.15 (-2.33, 2.04) P=0.8446	1.58	0.50	-1.08 (-4.32, 2.17) P=0.4718

TABLE 4

Estimates of difference, 95% confidence intervals and p-values for main contrasts for the neuropsychological measurements

		Contrast (day 1)			Contrast (day 8)		
Parameter		Treatment p-value	BUP+MIL-P vs BUP+MIL-25	BUP+MIL-P vsBUP+MIL-50	Treatment p-value	BUP+MIL-P vs BUP+MIL-25	BUP+MIL-P vs BUP+MIL-50
Pupil/Iris ratio (left)	Overall	<.0001	-0.007 (-0.042, 0.028) p=0.6748	0.018 (-0.016, 0.052) p=0.2768	<.0001	-0.070 (-0.109, -0.030) p=0.0016	-0.048 (-0.086, -0.009) p=0.0182
	After first BUP		0.020 p=0.3966	0.019 p=0.4064		-0.115 p=<.0001	-0.077 p=0.0040
	After second BUP		-0.028 p=0.2455	0.002 p=0.9458		-0.065 p=0.0147	-0.047 p=0.0696
	After third BUP		-0.013 p=0.5948	0.033 p=0.2027		-0.029 p=0.2636	-0.018 p=0.4740
Saccadic Peak Velocity (deg s^{-1})	Overall	0.0005	-27.0 (-59.5, 5.4) p=0.0986	-39.0 (-71.4, -6.6) p=0.0204	0.0016	-4.2 (-34.2, 25.7) p=0.7682	9.5 (-20.7, 39.7) p=0.5157
	After first BUP		-22.0 p=0.3225	-8.7 p=0.6963		-27.8 p=0.1419	-18.3 p=0.3328
	After second BUP		-13.6 p=0.5629	-55.6 p=0.0205		24.1 p=0.2173	32.7 p=0.0988
	After third BUP		-45.4 p=0.0840	-52.8 p=0.0474		-9.0 p=0.6770	14.2 p=0.5131
Body Sway (mm)	Overall	0.3082	14.2% (-9.4%, 43.9%) p=0.2474	10.8% (-12.2%, 39.8%) p=0.3715	0.0338	12.9% (-8.2%, 38.9%) p=0.2354	-2.4% (-21.1%, 20.6%) p=0.8110
	After first BUP		12.6% p=0.4008	-9.5% p=0.4753		6.3% p=0.6500	-11.8% p=0.3602
	After second BUP		7.8% p=0.5934	34.3% p=0.0388		17.2% p=0.2441	5.7% p=0.6877
	After third BUP		22.6% p=0.1635	11.9% p=0.4560		15.4% p=0.2918	-0.3% p=0.9834
Adaptive Tracking (%)	Overall	<.0001	-1.08 (-3.62, 1.46) p=0.3883	-0.32 (-2.90, 2.25) p=0.7971	0.0014	-0.68 (-3.42, 2.05) p=0.6062	-1.56 (-4.38, 1.27) p=0.2628
	After first BUP		0.51 p=0.7674	2.80 p=0.1075		-0.50 p=0.7795	0.47 p=0.7963
	After second BUP		-0.15 p=0.9326	-2.90 p=0.0964		-0.07 p=0.9686	-1.92 p=0.2982
	After third BUP		-3.60 p=0.0527	-0.88 p=0.6432		-1.48 p=0.4280	-3.22 p=0.0829

TABLE 5

Plasma pharmacokinetic parameters for milnacipran

	Day 1			Day 8		
Parameter	BUP+MIL-25	BUP+MIL-50	BUP-P+MIL-50	BUP+MIL-25	BUP+MIL-50	BUP-P+MIL-50
C_{max} (ng ml^{-1})	46.9 ± 15.9	99.8 ± 37.2	105.2 ± 26.9	93.8 ± 11.7	188.3 ± 66.7	201.2 ± 41.6
$C_{through}$ (ng ml^{-1})	NA	NA	NA	33.2 ± 8.7	61.2 ± 24.6	58.5 ± 14.2
T_{max} (h)	3.2 ± 0.8	3.2 ± 1.0	3.2 ± 0.6	2.6 ± 0.5	3.1 ± 1.0	1.8 ± 1.0
AUC_{0-last} (ng hr ml^{-1})	318.1 ± 102.3	633.0 ±154.0	696.5 ±160.6	629.2 ± 94.1	1251.0 ± 306.7	1281.0 ± 178.6

TABLE 6

Summary of treatment emergent adverse events (AEs) by frequency [n(%)]. AEs occurring more than 3 times within one treatment are reported.

	BUP+MIL-25	BUP+MIL-50	BUP+MIL-P	BUP-P+MIL-50
Preferred term	n (%)	n (%)	n (%)	n (%)
Number of subjects with at least one AE	9 (100%)	10 (100%)	10 (100%)	11 (100%)
Nausea	8 (88.9%)	7 (70.0%)	9 (90.0%)	8 (72.7%)
Somnolence	8 (88.9%)	8 (80.0%)	8 (80.0%)	7 (63.6%)
Vomiting	6 (66.7%)	10 (100.0%)	7 (70.0%)	3 (27.3%)
Dizziness	6 (66.7%)	7 (70.0%)	6 (60.0%)	2 (18.2%)
Fatigue	6 (66.7%)	7 (70.0%)	3 (30.0%)	3 (27.3%)
Headache	4 (44.4%)	6 (60.0%)	3 (30.0%)	4 (36.4%)
Feeling hot	2 (22.2%)	1 (10.0%)	3 (30.0%)	3 (27.3%)
Hot flush	3 (33.3%)	2 (20.0%)	2 (20.0%)	2 (18.2%)
Dry mouth	2 (22.2%)	2 (20.0%)	3 (30.0%)	1 (9.1%)
Pruritus	2 (22.2%)	2 (20.0%)	3 (30.0%)	1 (9.1%)

FIGURE 1

Simulated plasma buprenorphine concentrations and electrical pain tolerance threshold (12h). Black solid line represents the mean (population) plasma concentration after three different 0.5-h buprenorphine infusions in a 70-kg subject: 0.5 (subtherapeutic), 1 (minimum therapeutic) and 3 (therapeutic) μg kg^{-1}. Dotted line represents the pain tolerance threshold.

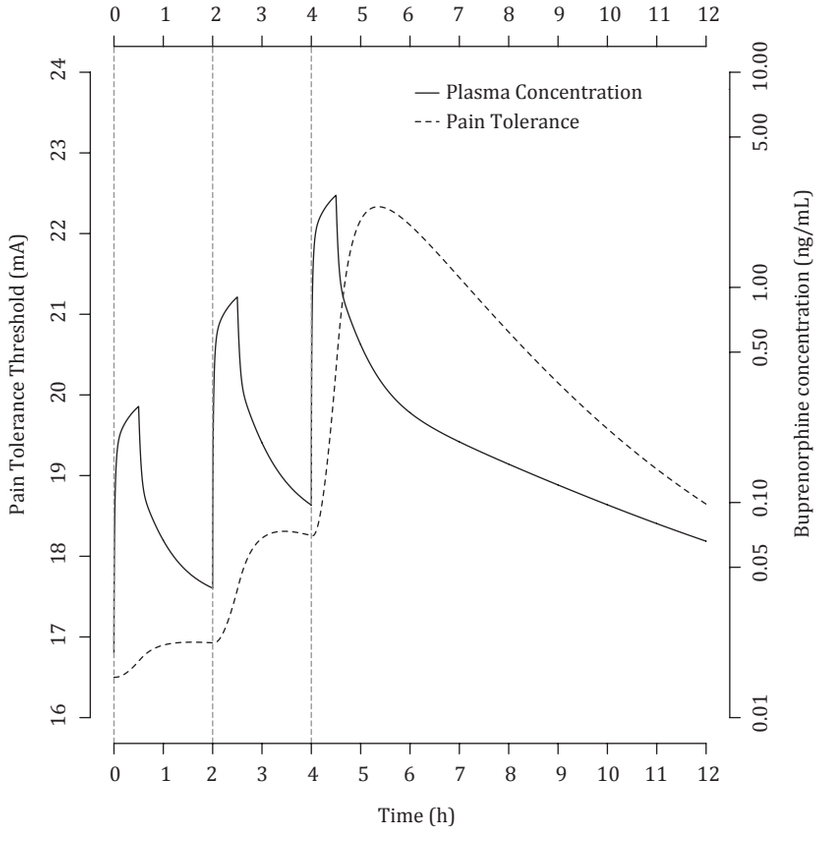
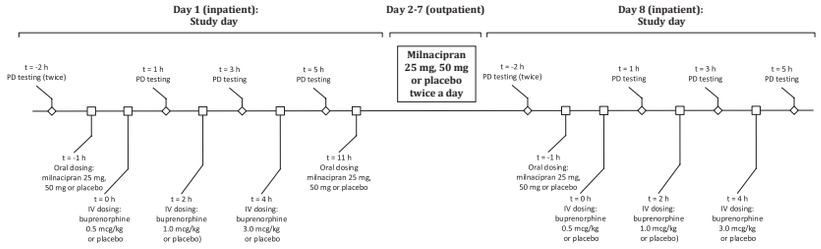


FIGURE 2

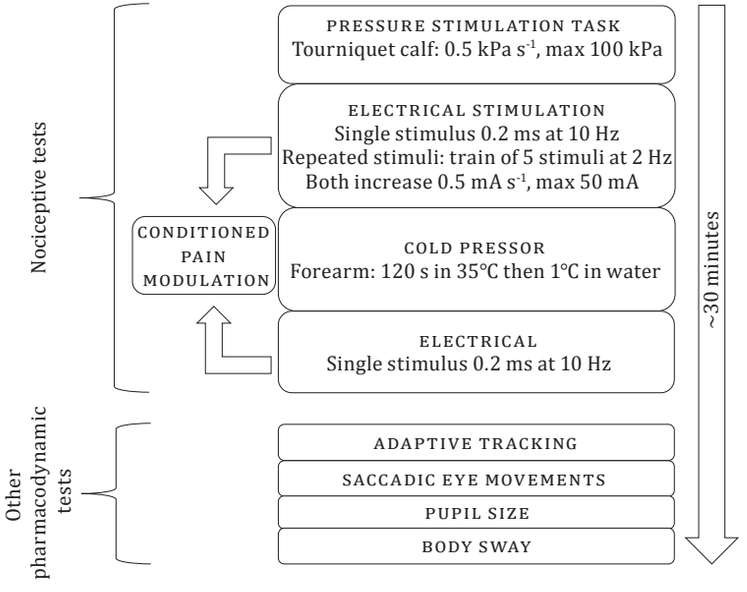
Study period overview.



PD, pharmacodynamic; t, time in hours.

FIGURE 3

Overview and sequence of pharmacodynamic tests.



kPa, kilopascal; ms, millisecond; Hz, hertz; mA, milliampere;°C, degree Celsius.

FIGURE 4

Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for electrical stimulation (repeated stimulus) [A/B], pressure pain [C/D] and the cold pressor task [E/F] after administration of milnacipran (MIL), buprenorphine 0.5 µg kg^{-1}(BUP 1), buprenorphine 1.5 µg kg^{-1} (BUP 2) and buprenorphine 3.0 µg kg^{-1}(BUP 3) on day 1 [left] and day 8 [right].

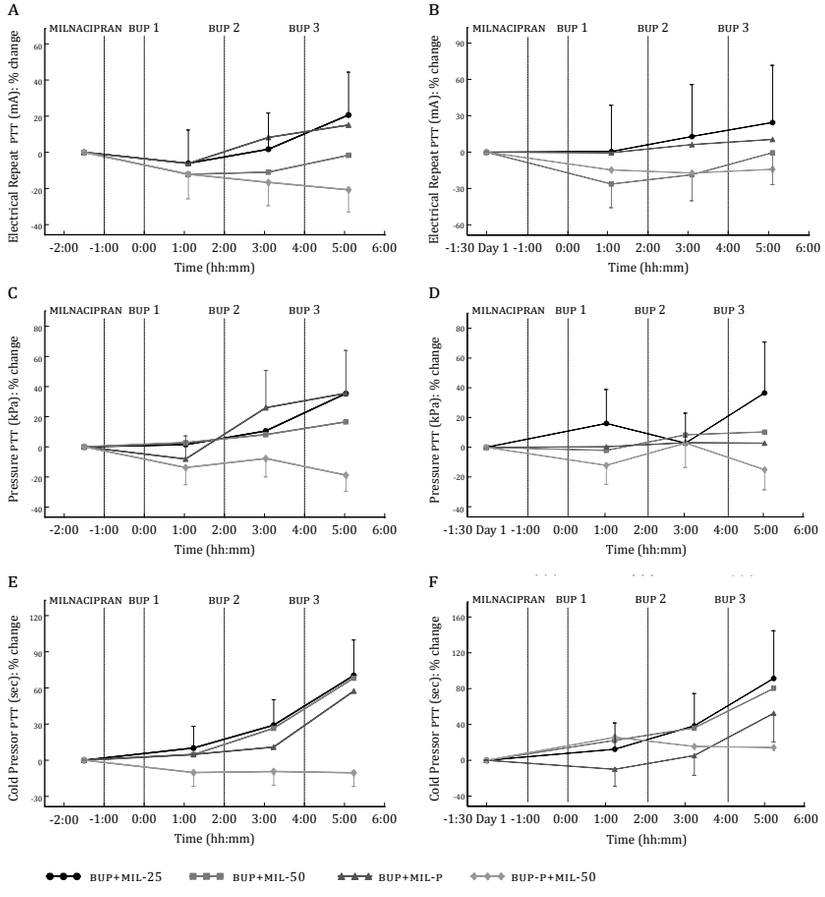


FIGURE 5

Time course of the mean change from baseline profile in least squares means for the left pupil/iris ratio [A/B] and saccadic peak velocity [C/D] after administration of milnacipran (MIL), buprenorphine 0.5 µg kg^{-1}(BUP 1), buprenorphine 1.5 µg kg^{-1} (BUP 2) and buprenorphine 3.0 µg kg^{-1}(BUP 3) on day 1 [left] and day 8 [right].

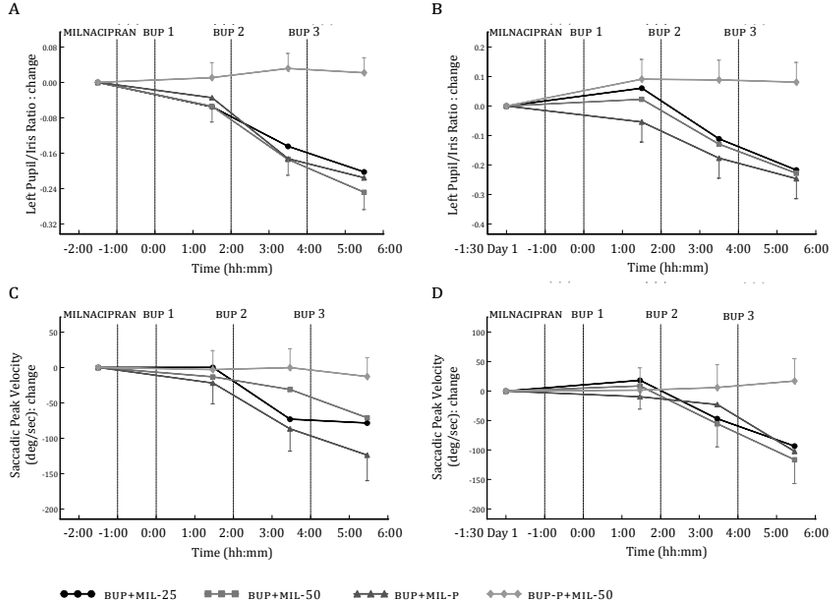


FIGURE 6

Plasma buprenorphine concentrations and popPK buprenorphine model on day 8 of the trial. The circles represent the measured concentrations and the solid line represents the mean (population) buprenorphine concentration after three different 0.5-h buprenorphine infusions in a 70-kg subject: 0.5 (sub-therapeutic), 1 (minimum therapeutic) and 3 (therapeutic) μg kg^{-1}. Vertical lines at time points 0, 120 and 240 min represent the start of the infusion for buprenorphine. The horizontal discontinuous line represents the lower limit of quantification for buprenorphine.

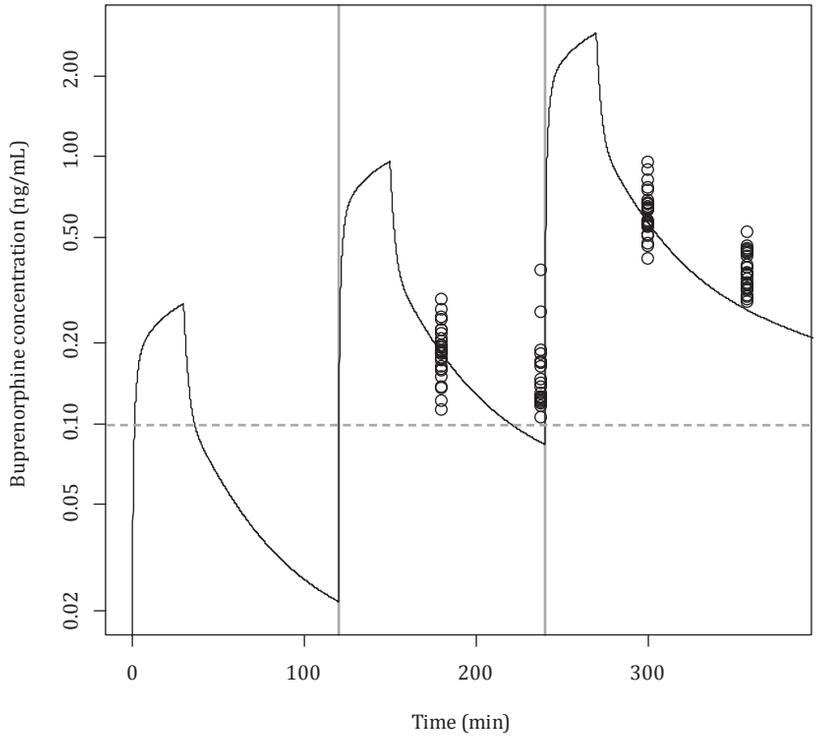
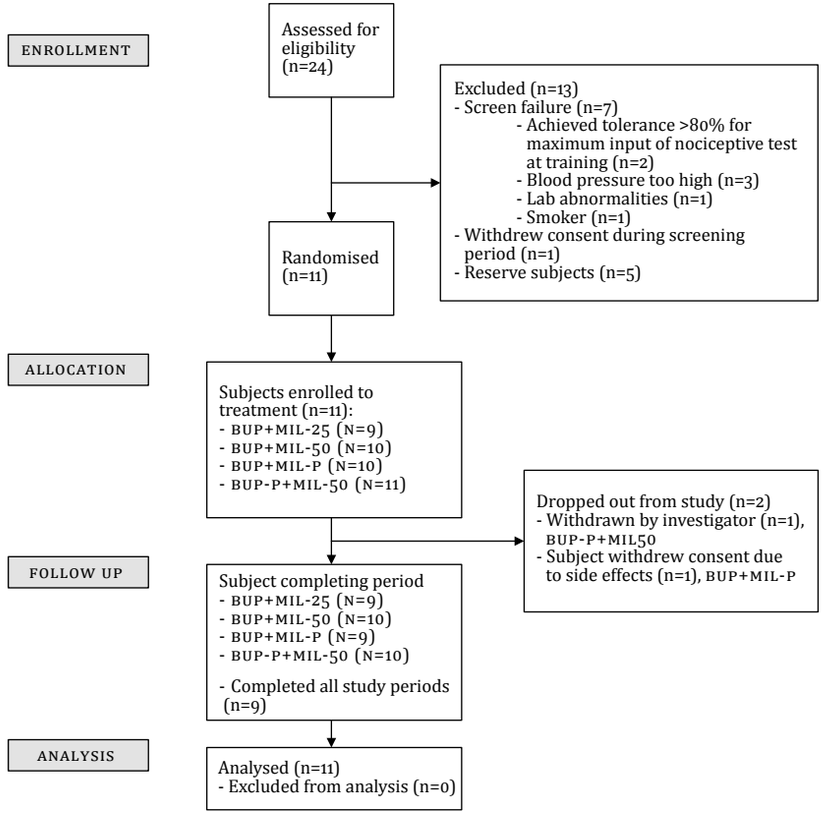


FIGURE SUPPLEMENTARY 1

CONSORT Flowchart



CHAPTER VI

The use of a battery of pain models in adolescent subjects

To be submitted

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ABSTRACT

In this two-day crossover, randomised, double-blind, placebo-controlled study, 16 adolescents (aged 16 or 17 years) received 1000 mg paracetamol or placebo on separate days. Paracetamol concentrations were measured in saliva. Plasma paracetamol concentrations were predicted using measured saliva paracetamol concentrations and pharmacokinetic data taken from published studies. Nociceptive effects of paracetamol were evaluated using a battery of pain tests (heat thermode test, electrical stimulation test, pressure stimulation test, cold pressor test and conditioned pain modulation) and analysed by mixed-model analysis of variance. A questionnaire was taken to evaluate study participation. The saliva c_{max} of paracetamol was 9.4 μg l^{-1}, t_{max} 1.5 h and the half-life was 0.9 h; predicted plasma c_{max} was 14.5 μg l^{-1}, t_{max} 0.6 h, and half-life 0.9 h. 1000mg paracetamol did not have a statistically significant effect on any of the pain parameters. 75% of the adolescents would participate again. Although no significant differences in pain detection and tolerance thresholds after paracetamol administration were observed, this study demonstrates that pain research using a comprehensive battery of evoked pain tests is feasible and acceptable to healthy adolescents. As induced pain is likely to be used increasingly in children and adolescents given the need for pediatric research on analgesics, gaining knowledge and experience in this field is an important step forward.

INTRODUCTION

Analgesics are among the most commonly used drugs in children. Paracetamol is the most frequently used on-label drug and among the most commonly used off-label drugs in this age group,[1] because of its favorable safety profile. However, there is a wide discrepancy between the consumption of this analgesic drug and knowledge of its pharmacokinetic (PK) and pharmacodynamic (PD) properties in children and adolescents. Pediatric studies investigating the effects of paracetamol and other analgesics on pain are limited, as they have mainly explored postoperative pain, for example after tonsillectomy, using pain scales.[2,3]

In adults, nociceptive pain tests are used in clinical drug development to assess efficacy of analgesics. A battery of pain models previously showed distinct profiles in pain detection and tolerance between different classes of analgesic compounds.[4] This battery can be used to benchmark new analgesics against established drugs in adults. It is already known that experimental pain thresholds and tolerance differ between children and adolescents. For instance, conditioned pain modulation, measured in an experimental setting, is lower in younger children (aged 8-11) compared to adolescents (aged 12-17 years).[5] Also, up to the age of 14, pain threshold and endurance for the cold pressor task increases.[6] Although no studies are available where a direct comparison is made between adults and children, it is unlikely that results from the adult population can be directly extrapolated to children or adolescents. Therefore, execution of a battery of pain tests in adolescents and children would be valuable in order to bridge the gap between the large consumption of analgesics and the limited information about the PK and PD in this population.

The current study was performed to investigate the antinociceptive effect profile of the commonly used analgesic, paracetamol, in healthy adolescents. Nociceptive effects of paracetamol were evaluated using a battery of evoked pain tests, which aims to assess as objectively as possible the levels of pain induced by a variety of painful stimuli, including electric stimulation, pressure, cold and heat. Some of these tests have been previously used in children and adolescents, however not for the assessment of pain relief after paracetamol, and not in an integrated manner. We, therefore, also investigated the suitability of these tests to be applied in this group of adolescent subjects. As blood sampling is less desirable in adolescents in the setting of a non-therapeutic study, and may hinder recruitment, saliva samples were collected to measure paracetamol concentrations.

METHODS

The Central Committee on Research involving Human Subjects (CCMO, The Hague, The Netherlands) approved the study protocol. The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2011-005086-20).

Study design

This was a randomised, double-blind, placebo-controlled, two-way cross-over study. All subjects received 1000 mg paracetamol or placebo on separate days. Capsules were taken with water and administration was observed for compliance. This dose was chosen as oral paracetamol has clinically proven analgesic effects with oral doses of 500 to 1000 mg in adults.[7] The washout period between study days was at least one day and paracetamol was administered at the same time of day in all subjects to avoid confounding effects from circadian variability. As PK sampling was done in saliva, paracetamol was administered as a capsule to prevent oral contamination. Capsules containing 500 mg paracetamol or placebo were prepared and provided by the pharmacy of the Leiden University Medical Center (Leiden, The Netherlands) according to GMP standards.

Subjects were not allowed to consume alcohol for 24 h and consume caffeine-containing drinks or smoke for 12 h before and during study days. Subjects were to refrain from heavy exercise from 48 h before and during study days and should have a regular day-and-night rhythm prior to study days. A standardised light breakfast was offered 1 h pre-dose and a standardised lunch was offered at 3.5 h after paracetamol administration. Water was allowed *ad libitum*. Subjects were confined to the clinical research unit for approximately 6.5 h after paracetamol administration. At the end of the study a questionnaire was taken to evaluate the way how adolescents experienced study participation.

Subjects

Healthy male and female subjects aged 16 and 17 years were included. The subjects were not allowed to smoke more than 4 cigarettes per day or use more than 14 units alcohol per week. After the informed consent was signed by the subject and parents/legal guardians, subjects were medically screened within 3 weeks prior to study participation and excluded if relevant clinical abnormalities were found. Subjects indicating nociceptive tests intolerable at screening or achieving tolerance at >70% of maximum input intensity for any nociceptive test were also excluded. Subjects were not allowed to participate in case of liver disease, renal disease, current conditions that could affect sensitivity to cold (e.g., atherosclerosis, Raynaud's disease, urticaria, hypothyroidism) or pain (e.g., paraesthesia, etc.), a previous allergic reaction to paracetamol or medical history of fainting or syncope without a known cause. Use of medications and agents known to affect paracetamol metabolism and/or effect were not allowed. Alcohol breath test and urinary drug and pregnancy tests were performed during screening and prior to each test day. Use of over-the-counter analgesic medications was not allowed within 3 days of nociceptive assessments.

Pharmacokinetics

To minimise the burden on study subjects, saliva paracetamol concentrations were measured as a surrogate marker for plasma or target site concentrations. Saliva was collected in polyester Salivette tubes (Sarstedt, Numbrecht, Germany) prior to paracetamol administration (to ensure compliance) and every 30 min (until 2 h), at 3 h and at 5 h after administration. Saliva sampling time points after administration of paracetamol were determined based on a PK-model using data available in the literature for orally and intravenously administered paracetamol in serum[8–10] (see Figure 1). Saliva concentrations were assumed to be a fraction of the serum concentrations.[11] After stimulated collection (by moving swab through mouth), swabs were centrifuged as soon as possible for 10 min (4°C, 2000G). The supernatant was frozen and stored at a maximum temperature of -20°C. At the end of the study day, samples were stored in a freezer at -80°C.

Analytical assay

Saliva paracetamol concentrations were determined using a commercially-available enzymatic assay (Cambridge Life Sciences Ltd., Ely, UK). In short, the method is based on the hydrolysis of paracetamol by aryl acylamidase yielding acetate and p-aminophenol, the latter reacting with

o-cresol in the presence of ammoniacal copper sulfate to produce a blue color. The assay is specific for the parent compound and does not detect paracetamol metabolites. The limit of quantification was 1.5 mg l^{-1} with 50 µL sample volume. Coefficients of variation for the intra-assay precision varied from 1.0% – 2.4% and for the inter-assay precision from 1.4% – 2.6%.

Pharmacodynamics

An extensive nociceptive test battery was incorporated to provide information on nociceptive detection and tolerance thresholds of an integrated range of tests measuring different modalities of nociception that could be affected by paracetamol.[12] Pharmacodynamic measurements were performed at baseline, hourly (until 3 hours) and 5 h after administration of paracetamol or placebo. Measurements were performed in a quiet room with ambient illumination with only one subject in the room per session. During screening, subjects were familiarised with the experimental procedure and given a practice session on the tests to minimise learning effects during test days. The tests were performed as described below. Pain intensity was measured continuously (beginning when the first stimulus was applied until the predetermined end of the test) for each nociceptive test. Equipment was programmed to cease giving stimuli if pain intensity reached the maximum possible score. Continuous pain intensity was measured using an electronic visual analogue scale (eVAS) ranging from 0 (no pain) to 100 (most intense pain tolerable). For the heat thermode test the heat pain detection threshold (PDT) and the pain tolerance threshold (PTT) were assessed. For the electrical stimulation test, the pressure stimulation test and the cold pressor test, PDT and PTT were calculated. In addition, for the cold pressor test, area above the pain intensity-stimulation-time curve (AAC) was calculated; for all other tests, area under the pain intensity-stimulation curve (AUC) was calculated.

HEAT THERMODE TEST

The TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel) is a computerised device that is designed to administer thermal heat or cold stimuli via a thermode to a part of the subject's body. In this study, a thermal stimulus was administered to the volar side of the dominant lower arm with an intensity that gradually increased at a constant rate. First the subject halted the stimulus when the PDT was reached, then the subject halted the stimulus when the PTT was reached. Both measurements were repeated three times.

ELECTRICAL STIMULATION TEST

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™) were placed on cleaned, scrubbed, and if required, shaved skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be less than 2 kW. The electrical stimulus was delivered by a computer-controlled constant current stimulator (DS5, Digitimer, Cambridge, UK).

For the single stimulus, a method was used which was adapted from methods previously described[13,14] (10 Hz tetanic pulse with a duration of 0.2 ms); current intensity increased from 0 mA in steps of 0.5 $mA{\cdot}s^{-1}$ (cutoff 50 mA). The pain intensity for each stimulation was measured using the eVAS slider, until pain tolerance threshold was reached or a maximum of 50 mA was reached. The delta electrical stair was used as a measure for inhibitory conditioned pain modulation (ICPM).

PRESSURE STIMULATION TEST

The method of mechanical pressure pain induction was based on methods previously described, and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.[15,16] Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 $kPa{\cdot}s^{-1}$. 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, at which point the device released pressure to the cuff.

COLD PRESSOR TEST

The method of cold pressor pain was based on the methods previously described[17,18] and is the most commonly used test to induce inhibitory conditioned pain modulation (ICPM, also known as 'diffuse noxious inhibitory control').[19] Subjects placed their non-dominant hand into a water bath (minimal depth 200 mm) at 35 ± 0.5°C for 2 min. At 1 min 45 s a blood pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min the subject then moved that hand from the warm water bath, directly into a similar sized 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.

CONDITIONED PAIN MODULATION

iCPM is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[19] The degree of iCPM was assessed by comparing the electrical pain thresholds before and within 5 min after the cold pressor test.

Statistical analysis

This was an exploratory study in adolescents. The AAC of the cold pressure test was used for the power analysis. This variable had the least intra-subject variability both within and between visits compared to other parameters of the different pain tests included in the battery of evoked pain tests (in-house data). In order to estimate the sample size for a study that has 80% power of detecting a difference in means of 5.5% in VAS% sec^{-1} of the cold AAC as observed in previous studies in adults at CHDR, a sample size of 12 was calculated (two-sided test, alpha = 0.05) for a balanced design.

Pharmacodynamics

The pharmacodynamic endpoints were analysed by mixed-model analyses of variance (using SAS PROC MIXED, SAS version 9.1.3) with subject, subject by treatment and subject by time as random effects, with treatment, test day, time and treatment by time as fixed effects, and the average baseline value as covariate. PTT and PDT variables were log transformed prior to analysis to correct for the expected log-normal distribution of the data. Analysis was performed on log-transformed data. Delta electrical stairs PTT, PDT and AUC were calculated as the difference between the electrical stair measurement before and after the cold test (after minus before). The measurement after the cold test was not used for the analysis of the electrical stairs variables, only to calculate the delta electrical stairs. Contrasts were estimated within the overall treatment effect and contrasts between treatments over 300 min were calculated. The statistical null hypothesis in this study was 'there is no difference between paracetamol and placebo' (alpha=0.05, two-sided). No adjustments for multiple comparisons were employed.

Pharmacokinetics

Pharmacokinetic analysis of saliva paracetamol concentrations was initially performed using a non-compartmental model approach. The mean peak concentration (C_{max}) and the time to the peak concentration (T_{max}) were recorded as observed. In addition, the area under the saliva concentration-time curve from time zero to the time of the last sample (AUC_{0-last}) was calculated using the trapezoidal rule. Calculations were performed using R v2.12.0 (R Foundation for Statistical Computing, Vienna, Austria).

Paracetamol plasma concentrations were expected to more closely resemble central exposure than saliva concentrations. In order to estimate central exposure of paracetamol, a PK model that correlated unobserved plasma and saliva concentrations was developed using literature data in NONMEM© v7.2 (Icon Development Solutions, Ellicott City, Maryland, United States). The basis for the PK model was a four-compartmental model originally developed by Wang et al.,[20] which was built on data only containing patients whom were rectally dosed. To allow adequate oral absorption prediction, absorption parameters (first order absorption constant, absorption lag time, and respective variabilities) for oral dosing were derived from Owens et al.[21] Finally, data from Wade et al.[22] regarding the correlation between plasma and saliva concentrations were used to extend the model based on the Wang and Owens data with an additional saliva compartment. The complete model consisted of one dosing compartment, one central plasma compartment, and three peripheral compartments (with one describing the saliva concentrations). Following estimation of saliva compartment parameters, simulations of plasma paracetamol concentrations were performed using individual parameter values.

RESULTS

Subjects

None of the screened subjects were excluded because they indicated nociceptive tests as intolerable. A total of 12 adolescent subjects (5 males, 7 females) aged 16 or 17 years (mean 16.8 years) were enrolled between December 2011 and August 2014. All adolescent subjects attended pre-university secondary education (VWO, Voortgezet Wetenschappelijk Onderwijs). None of the subjects discontinued the study. Subjects had a median weight

of 64.5 kg (range, 60-90 kg) and a mean height of 1.75 m (range, 1.62-1.90 m). Three female subjects used an oral contraceptive during the study.

Pharmacodynamic results

1000 mg paracetamol did not have a statistically significant effect on any of the pain test parameters (Table 1). A non-significant increase in PDT was observed during the heat thermode test (2.5%°C; 95% CI -0.7-5.7%; P=0.11; Figure 2) and the pressure stimulation test (17.5% kPa; 95% CI -1.2%, 39.7%; P=0.07; Figure 3) following paracetamol administration. In addition, a non-significant decrease occurred in the AUC of the pain pressure test following paracetamol administration (-477 kPa*%; 95% CI -1005.63 – 52.57; P=0.07; Figure 4).

Pharmacokinetic results

Individual concentration time plots for the saliva paracetamol concentrations are shown in figure 5. Secondary pharmacokinetic parameters for paracetamol based on predicted plasma and saliva concentrations over time by the constructed literature model taking into account individual saliva concentration data are shown in Table 2. In saliva the C_{max} was 9.4 $\mu g\ l^{-1}$ (7.4–12.2), the T_{max} 1.5 h (1-3) and the half-life was 0.9 h (0.4-1.6). The predicted plasma C_{max} was 14.5 $\mu g\ l^{-1}$ (8.5-28.7), T_{max} 0.6 h (0.3-2.1), and half-life 0.9 h (0.4-1.6). Median terminal half-life and volume of distribution as determined by the non-compartmental analysis were 2.2 h and 70.4 l respectively.

Questionnaire

The results of the evaluation questionnaire are listed in Table 3. All subjects enjoyed participating in the study. 75% of the adolescents participating in the trial would participate again. 50% of the subjects indicated the cold pressor pain test as most painful. The pain test experienced as least painful was the pressure test.

DISCUSSION

This is the first reported study in which the pharmacokinetics and pharmacodynamics of paracetamol in adolescents have been explored. An extensive nociceptive test battery was used to provide information about different modalities of nociception that could potentially be affected by paracetamol. Saliva samples were collected to measure paracetamol concentrations. Although no significant differences in pain detection and tolerance thresholds after paracetamol administration were observed, we demonstrated that pain research using a battery of nociceptive tests is feasible and acceptable in adolescents.

The prevalence of moderate to severe pain in hospitalised children remains high and adolescents especially are at risk of moderate to severe pain.[23] Children and adolescents are particularly prone to unfair exclusion from pain research.[24] Fortunately, some progress is being made in pediatric pain research. For example, recently published preliminary data illustrated the utility of pupillometry as a non-invasive method to objectively quantitate pain response/intensity in children.[25] In general, it is ethically preferable to avoid causing pain for research purposes, especially in children. However, there are some important questions in pain and analgesia research that cannot be answered without an evoked pain stimulus.[26] Pain models offer important control over the environment and provide standardisation of nociceptive stimuli, thereby enabling a more rigorous exploration of individual differences and/or the environmental factors that can affect the subjective pain experience. As the induction of pain can lack direct benefit, it is usually not deemed appropriate for use in either therapeutic or non-therapeutic research. However, as the factors that can affect the relationship between drug concentration and pain relief can differ between children and adults, findings obtained from adult studies may not necessarily translate directly to children or even adolescents. In addition to potential developmental changes in endogenous analgesic mechanisms and developmental modulation,[27,28] placebo-responses may be more robust[3] and a child's thoughts and attitudes regarding pain can change with age, thereby contributing to more intense feelings of pain during adolescence than in childhood.[29] As our current understanding of age-dependent effects and side-effects between children, adolescents and adults is based largely on data collected from animal studies, evaluating age-dependent differences in PK and PD in pediatric clinical trials will be an important step forward. Therefore, it is necessary to include adolescents and children in studies where analgesics and pain are being investigated.

Paracetamol serum and saliva concentrations were compared previously, with variable study outcomes. Rittau and colleagues compared paracetamol concentrations using venous and capillary blood and saliva

sampling.[30] They reported saliva collection as a reliable sampling method for the evaluation of paracetamol's plasma PK, as was confirmed by a study performed in patients with paracetamol concentrations in the toxic range.[31] Another study reported significant correlations between serum and saliva PK, but with a poor agreement between the two matrices.[32] In our study, in which only the paracetamol concentrations in saliva were measured, we found that these concentration data were well suitable to describe the pharmacokinetics in adolescents. Previous studies reported that, despite a difference in paracetamol metabolism between children and adults, the half-life and volume of distribution are almost identical.[33] In line with these previous findings, the median terminal half-life (2.2 h) and volume of distribution (70.4 L) that were observed in our study are in agreement with the reported kinetics of paracetamol in adults and children.[33]

1000 mg oral paracetamol did not affect the tests using electrical, thermal, pressure or cold stimuli. In adults, the cold-pressor method has been used most frequently to measure pain reduction after paracetamol.[34–36] Oral paracetamol has dose-related analgesic activity using the cold-pressor test, with no effect of doses lower than 1000 mg.[34] Using electrical and thermal stimuli, oral doses of paracetamol of 1000 mg or lower could not be discriminated from placebo.[37–39] In our study, the lack of (significant) effects on these pain stimuli may be due to the (low) dose of paracetamol administered, or the insensitivity of these tests for detecting the effects of paracetamol. The median weight in our study was relatively low (64 kg), and the weight range was relatively wide (60-90 kg); as a result, the dose per kg was relatively variable. Intravenous paracetamol exerted dose-dependent antinociceptive effects up to 2000 mg in response to selective transcutaneous electrical stimulation[40,41] and intravenous infusion of 650 mg paracetamol led to pain reduction with sustained antihyperalgesic effects.[42] Therefore, the different time course of paracetamol concentrations after intravenous administration may account for differences in paracetamol effects on electrical stimuli after intravenous versus oral administration. Paracetamol also previously showed an effect on pain detection threshold on laser-induced pain in adults.[43,44] As laser-induced pain tests has the potential to cause skin burns this test was not applied in our study. An alternative explanation for the lack of a significant antinociceptive effect in this study may be the larger intra-subject variability both within and between visits in adolescents. Furthermore, in some of the pain tests an increase in pain detection or tolerance threshold was also observed in the placebo group. It is suggested that placebo responses are higher in children and adolescents, but that drug responses are equal.[45] This could be a good explanation for lack of a significant difference between placebo and paracetamol in our study. Finally, the lack of a significant effect of paracetamol may be in part be explained by developmental differences in pain systems; for example, paracetamol may have positive effects on the serotonergic descending inhibitory pathways[46] and recent research suggests that central pain inhibitory mechanisms develop with age, as was demonstrated by a greater inhibitory conditioned pain modulation in adolescents compared to younger children.[5]

We expect that evoked pain tests will be used increasingly in children and adolescents. Anesthetics and analgesics are among the most commonly cited therapeutic subgroups with a pediatric research need on the European Medicines Agency Needs Lists.[47] As adolescents are rarely waived from pediatric development under the Pediatric Regulation (recent European legislation facilitating development of pediatric medicines), this age group will likely be involved in more studies in the near future.[48] Therefore, additional knowledge and experience should be gained in the pediatric field of evoked pain modalities. The current study demonstrates that pain research using a comprehensive battery of nociceptive tests is feasible and acceptable to healthy adolescents. It can also assist in designing future clinical studies with analgesics using nociceptive tasks in adolescents and children.

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TABLE 1

Pharmacodynamic outcome parameters for a single dose of 1000 mg paracetamol or placebo in healthy adolescents.

	Contrasts	LS Means change from baseline	
Parameter	Paracetamol Placebo	Placebo	Paracetamol
Cold AAC (s*%)	0.40 (-510, 510.9) P=0.9986	77.61	78.01
Cold PDT (s)	16.7% (-18.1%, 66.4%) P=0.3505	-4.0%	12.1%
Cold PTT (s)	9.0% (-12.9%, 36.3%) P=0.4088	-5.7%	2.7%
Electrical Stair AUC (mA*%)	92.55 (-206, 390.7) P=0.4977	-310.83	-218.28
Electrical Stair PDT (mA)	-12.7% (-28.3%, 6.3%) P=0.1544	44.3%	26.0%
Electrical Stair PTT (mA)	0.9% (-10.9%, 14.2%) P=0.8759	9.2%	10.2%
Delta Electrical Stair AUC (mA*%)	17.95 (-180, 215.4) P=0.8349	48.28	66.23
Delta Electrical Stair PDT (mA); ICPM	0.303 (-1.63, 2.234) P=0.7308	-0.439	-0.136
Delta Electrical Stair PTT (mA)	-.505 (-1.80, 0.793) P=0.3993	-0.271	-0.776
Pressure AUC (kPa*%)	-477 (-1006, 52.57) P=0.0726	-125.34	-601.87
Pressure PDT (kPa)	17.5% (-1.2%, 39.7%) P=0.0656	11.4%	30.8%
Pressure PTT (kPa)	3.4% (-11.6%, 20.9%) P=0.6460	6.2%	9.7%
Heat PDT (°C)	2.5% (-0.7%, 5.7%) P=0.1096	-1.4%	1.0%
Heat PTT (°C)	-0.1% (-2.4%, 2.3%) P=0.8946	0.3%	0.2%

TABLE 2

Secondary pharmacokinetic parameters for paracetamol based on predicted plasma and saliva concentrations over time by the constructed literature model taking into account individual saliva concentration data.

	Plasma		Saliva	
Parameter	Median	95% CI	Median	95% CI
C_{max} (mg l^{-1})	14.5	8.5-28.7	9.4	7.4-12.2
T_{max} (h)	0.6	0.3-2.1	1.5	1-3
Halflife (h)	0.9	0.4-1.6	0.9	0.4-1.6
AUC_{0-6h} (mg h l^{-1})	38.2	30.7-51.1	30.9	23.3-43.4

TABLE 3

Subject evaluation questionnaire

Question	Response (n [%])
Did you enjoy participation?	Not at all (0 [0%]) Not really (0 [0%]) Neutral (0 [0%]) Reasonably nice (9 [75%]) Nice (3 [25%])
How did you experience saliva sampling?	Very bothersome (0 [0%]) A bit bothersome (2 [16.7%]) Neutral (1 [8.3%]) Not that bothersome (3 [25%]) Not bothersome at all (6 [50%])
Which test did you experience as least bothersome?	Heat pain test (4 [33.3%]) Electrical stimulation test (1 [8.3%]) Pressure stimulation test (6 [50%]) Cold pressor test (1 [8.3%])
Which test did you experience as most bothersome?	Heat pain test (0 [0%]) Electrical stimulation test (4 [33.3%]) Pressure stimulation test (0 [0%]) Cold pressor test (8 [66.7%])
Which test did you experience as least painful?	Heat pain test (1 [9.3%]) Electrical stimulation test (2 [18.2%]) Pressure stimulation test (8 [72.7%]) Cold pressor test (0 [0%])
Which test did you experience as most painful?	Heat pain test (1 [8.3%]) Electrical stimulation test (4 [33.3%]) Pressure stimulation test (1 [8.3%]) Cold pressor test (6 [50%])
Would you participate again?	Yes (9 [75%]) No (1 [8.3%]) Not sure (2 [16.7%])

FIGURE 1

Expected plasma paracetamol concentration after a single 1000 mg oral dose based on three PK-models from literature. The solid line represents Ogungbenro (2010), the dotted line Anderson (2005), and the dashed line Wurthheim (2005). The vertical lines represent the saliva sampling times used in this study.

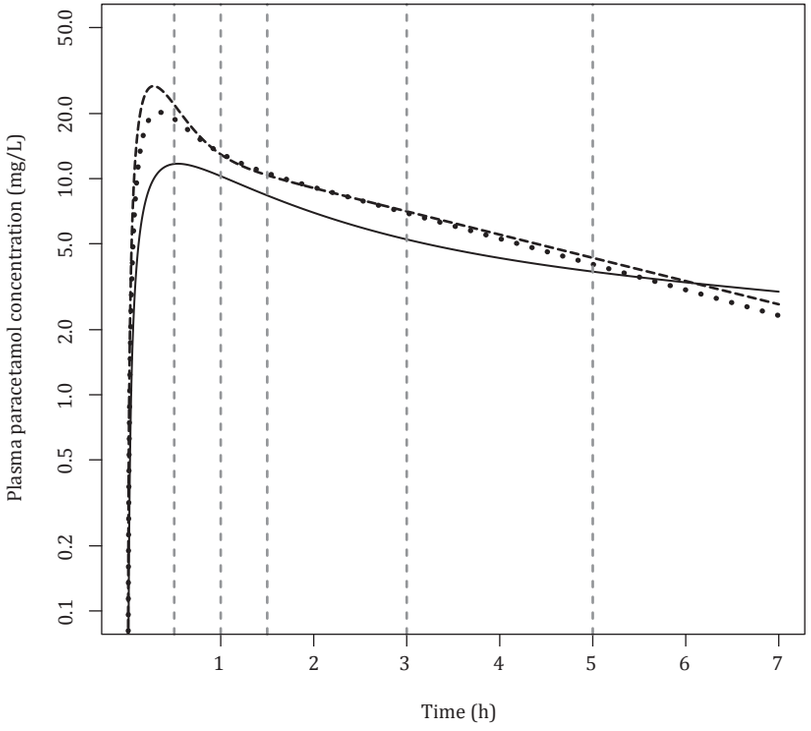


FIGURE 2

Heat pain detection threshold change in least squares mean from baseline profile with 95% CI as error bars.

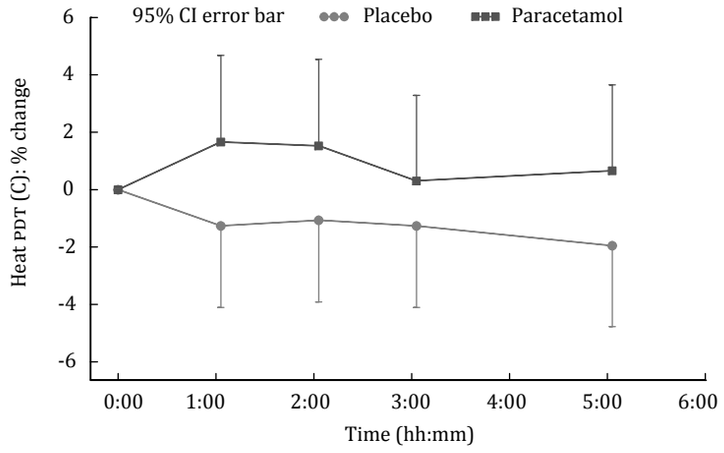


FIGURE 3

Pressure pain detection threshold change in least squares mean from baseline profile with 95% CI as error bars.

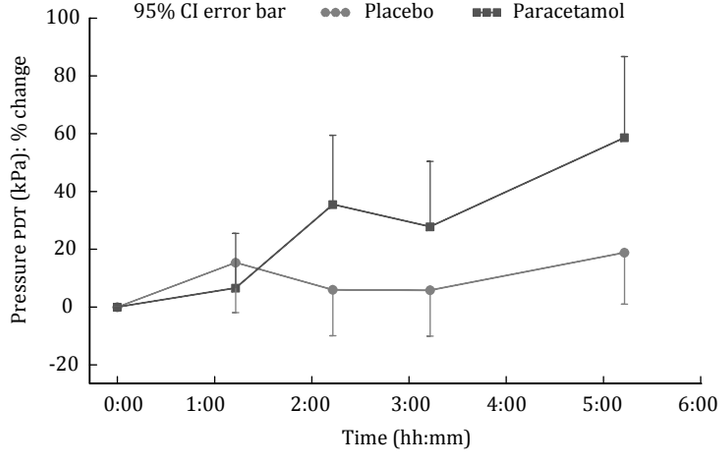


FIGURE 4

Pressure pain test AUC change in least squares mean from baseline profile with 95% CI as error bars.

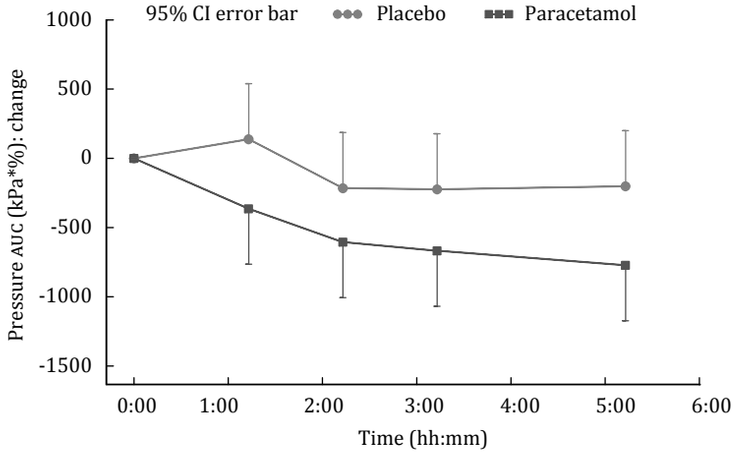
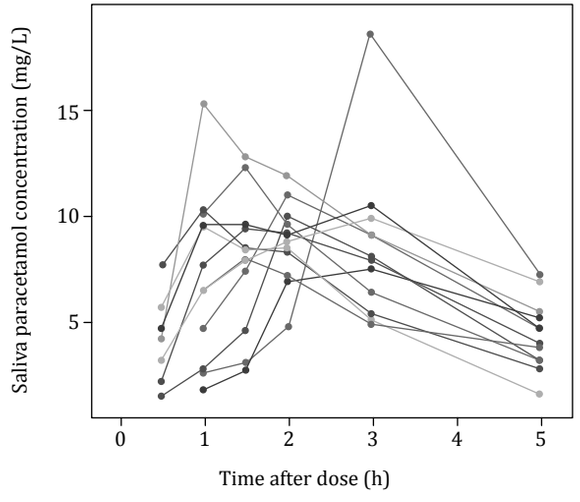


FIGURE 5

Individual plots of paracetamol concentrations in saliva



CHAPTER VII

Pharmacokinetics and pharmacodynamics of multiple doses of BG00010, a neurotrophic factor with anti-hyperalgesic effects, in patients with sciatica

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ABSTRACT

BG00010 is a protein in the glial cell line-derived neurotrophic factor (GDNF) family. It is a selective ligand for the GDNF family receptor alpha-3 (GFRα3) co-receptor that normalises cellular changes resulting from damage or disease, and potentially alleviates neuropathic pain. The main objectives of this study were to evaluate the pharmacokinetic and safety profiles and to determine the effects on pain of ascending doses of intravenous injections of BG00010 in patients with sciatica. This was a randomised, blinded, placebo-controlled multiple-dose study in subjects with sciatica. In Part I (16 patients), four IV dose levels were examined (50, 150, 400, 800 μg kg^{-1}) and in Part II (12 patients), three dose levels were examined (400, 600 and 1200 μg kg^{-1}). Safety and efficacy assessments were used as endpoints. The BG00010 concentration-time data indicated relatively low inter-patient variability and there was a dose-dependent (not dose-proportional) increase in serum exposure from 150 to 1200 μg kg^{-1}. The effective half-life was between 40 and 60 h. The most frequently occurring adverse events (AEs) reported by patients receiving BG00010 were headache (67-83%), feeling hot (50-100%) and pruritus (42-67%). Most AEs were mild; no serious AEs, or AEs leading to discontinuation occurred. Higher dose regimens of BG00010 resulted in greater pain reduction than placebo or lower dose regimens, although a clear dose-response relationship was not seen. The pharmacokinetic profile of BG00010 was characterised by low intra-patient variability. These data from a small sample suggest that BG00010 may have a benefit for patients with sciatica.

INTRODUCTION

BG00010 (neublastin, artemin) is a glial cell-derived neurotrophic factor (GDNF) family member[1] that can act as a survival factor for sensory and sympathetic neurons. BG00010 is a selective ligand for the GDNF family receptor alpha-3 (GFRα3) co-receptor. The interaction of BG00010 with GFRα3 on nociceptive sensory neurons activates downstream signalling to normalise damage- or disease-induced cellular changes and potentially alleviate neuropathic pain. GFRα3 expression is highly restricted to small dorsal root ganglion (DRG) sensory neurons, reducing the likelihood of unintended side effects.[2]

Preclinical data from surgical and chemical nerve-injury models demonstrate that BG00010 attenuates pain-related behaviours and normalises the neurochemical status of injured small DRG neurons without loss of neuronal or axonal function or integrity. Along with promoting re-entry of sensory fibres into the spinal cord and re-establishing synaptic function after crush injury, BG00010 can promote recovery of simple and complex behaviours in preclinical models.[3–5] BG00010 is being developed as a first-in-class molecule for the treatment of neuropathic pain.

Neuropathic pain results from lesions or disease affecting the peripheral or central somatosensory nervous system. Neuropathic pain is especially problematic because of its severity, chronicity and resistance to simple analgesics. Affecting 2–3% of the population, neuropathic pain is costly to the healthcare system, personally devASTating for patients,[6] and can substantially impair health-related quality of life.[7]

Sciatica is caused by spinal nerve root compression, often due to lumbar disc prolapse, and is associated with back pain radiating to the leg, occasionally accompanied by neurological deficit.[8] Sciatica is common, with reported lifetime incidence of 13–40%[9] and an annual incidence of 1–5%, peaking in the fifth decade of life.[10] Most patients with acute sciatica respond to conservative symptom management, with symptom resolution over weeks to months, although some require surgical decompression of the affected nerve root. Nevertheless, 10–40% of patients will develop a chronic pain syndrome.[11] Common pharmacotherapies for chronic neuropathic pain include tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors, calcium channel α2-δ ligands, topical lidocaine, opioid agonists and capsaicin. For sciatica, epidural steroid injections have been used for decades despite inconclusive efficacy data.[9] Treatment remains challenging, as many patients do not experience sufficient relief.[12] A

significant unmet medical need exists for a therapeutic agent with an acceptable safety profile to provide sustained neuropathic pain relief.

In a previous single-centre study (NCT00961766), BG00010 was administered to 48 patients with sciatica as single intravenous (IV; 0.3–800 μgkg^{-1}) or subcutaneous (50 μg kg^{-1}) doses.[13] The results suggested nearly linear pharmacokinetics (PK) over the tested dose range. The most frequently reported adverse events (AEs) were feeling hot, pruritus, headache and rash. In this second study of BG00010 in humans, the main objectives were to evaluate the PK, safety and pharmacodynamics of three IV injections of BG00010 given as two fixed dosing schedules.

METHODS

Patients

Eligible patients were aged 18–85 years with a diagnosis of unilateral sciatica, including pain radiating down the leg following a dermatome, suggesting L4, L5, or S1 nerve root involvement, with symptoms present for ≥3 months prior to the Screening Visit and pain rated at ≥40 mm on a 100 mm visual analogue scale (VAS) of the Dutch translation of the Short-Form McGill Pain Questionnaire (SF-MPQ)[14] at Screening and Baseline Visits.

Key exclusion criteria included: history of severe pain or signs/symptoms of peripheral neuropathy (other than that caused by sciatica) during the 3 months prior to the Screening Visit; major surgery within the 3 months prior to the Screening Visit or planned sciatica surgery within 6 months of the Screening Visit; current generalised myalgia; history of severe allergic or anaphylactic drug-related reaction; history of malignancy or clinically relevant allergy; and/or cardiac, endocrine, haematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic (not related to sciatica), dermatologic, rheumatic/joint, psychiatric, renal and/or other major disease.

Patients were allowed treatment with a selective serotonin reuptake inhibitor, a serotonin noradrenaline reuptake inhibitor, gabapentin, or a tricyclic antidepressant if doses were stable for 4 weeks prior to the Baseline Visit and pregabalin if the dose was stable for 1 week prior to the Baseline Visit. Doses of other prescription medications and/or over-the-counter products were to have been stable for 2 weeks prior to the Baseline Visit. Previous participation in a study with neurotrophic factors and participation in a study with another investigational drug or approved therapy for investigational use within 3 months prior to the Baseline Visit was not allowed.

Study design and treatment

This was a single-centre, randomised, blinded, placebo-controlled, serial-cohort, multiple-dose ascending study that examined two dose schedules (ClinicalTrials.gov NCT01405833). The study was approved by the Medical Ethics Committee (BEBO Foundation, Assen, The Netherlands) and all patients gave written informed consent. 'BG00010' is specific for the isoform of the protein used in this study (50:50 mix of 103 and 104 amino acid isoforms). The generic names 'artemin' and 'neublastin' cover all forms of the protein (104, 113, 125 and full length) and do not accurately describe BG00010. All other drug/target nomenclature is consistent with the British Journal of Pharmacology's 'Guide to Receptors and Channels'.[15] Since BG000010 is intravenously administered, less frequent dosing is preferable, and unpublished non-clinical data suggested that less frequent administration with higher doses and more frequent administration at lower doses could be explored clinically, providing the rationale for studying two dosing schedules. In Part I (Cohorts A–D), patients received BG00010 (50, 150, 400, or 800 μg kg^{-1}) or placebo once weekly for 3 weeks. In Part II (Cohorts E–G), patients received BG00010 or placebo dosed every 48 h. The starting dose in Part II was to be no more than 400 μg kg^{-1} and at least one dose level below the maximum tolerated dose (MTD) established with the once-weekly schedule.

Patients were enrolled sequentially, cohort by cohort. For each cohort, patients were randomised (three patients to BG00010 and one to placebo) to receive three IV administrations of study treatment. Following the completion of treatment for each cohort, the data safety review committee (DSRC) determined if it was appropriate to escalate to the next planned dose level. If none of the three BG00010-treated patients within a cohort experienced a treatment-related dose-limiting toxicity (DLT; defined as all BG00010-related serious AEs [SAEs] and BG00010-related AEs coded as severe by the principal investigator), dose escalation proceeded to the next cohort. If one of three BG00010-treated patients experienced a treatment-related DLT, three additional patients were to be enrolled at that dose level and escalation was to continue if there were no other DLTs among the additional BG00010-treated patients. If two or more BG00010-treated patients experienced the same or a similar treatment-related DLT, dosing was to stop and the previous dose level was to be considered the MTD. If patients experienced DLTs that were not the same or similar in nature, the cohort could be expanded or dosing stopped, as recommended by the DSRC.

Study assessments

The primary objective of the study was to evaluate the PK and safety of three IV injections of BG00010 in two fixed dosing schedules: weekly and as frequently as every 48 h (no more than three times in 1 week). Secondary objectives were to explore the potential of BG00010 to reduce pain following multiple-dose administration (as measured by a Numerical Rating Scale (NRS) and the SF-MPQ VAS) and to explore the repeated-dose immunogenicity of BG00010 (as measured by the incidence of anti-BG00010 antibodies).

In Parts I and II of the study, blood samples for PK analysis were taken: 30 min pre-dose and then 15 min and 1, 2.5, 4, 6, 9, 12, 18, 24 and 48 h following the first dose of BG00010; 30 min pre-dose and then 15 min and 4, 24 and 48 h following the second dose of BG00010; 30 min pre-dose and then 15 min and 1, 2.5, 4. 6, 9, 12, 18, 24, 48, 72 and 120 h following the third dose of BG00010. The concentration of BG00010 in serum was determined using a chemiluminescent enzyme-linked immunosorbent assay (quantification range 0.1 – 10 ng ml^{-1}) based on the binding of BG00010 to immobilised anti-BG00010 antibody (P3B3) using streptavidin conjugated with horseradish peroxidase, which upon addition of luminol substrate produced a chemiluminescent signal. At the lower limit of quantification, assay precision and bias were 12.5% and 13.6% respectively. Assay performance was fully validated in accordance with regulatory guidance and industry best practices.

Safety assessments included recording AEs and SAEs, measurements of haematology, clinical chemistry and urinalysis variables; vital signs; physical examinations; neurologic examinations; electrocardiograms (ECGs); numerical pain rating assessments; and longitudinal assessment of quantitative sensory testing (QST) in the unaffected leg (vibratory, cool thermal and heat pain). For vibratory measurements, a Rydel-Seiffer Vibratory Tuning Fork was used. The Rydel-Seiffer Vibratory tuning fork is an instrument that can determine the vibration extinction threshold. The tuning fork was set to a vibratory frequency of 64 Hz and then placed on the subject's skin. The vibration threshold was the point where the vibration was no longer perceived by the subject.[16] Measurements were performed in triplicate on the medial malleolus.

Cold and heat stimuli were applied with a thermode device (TSA-II – NeuroSensory Analyzer, Medoc) which gradually increased or decreased in temperature. The thermode was applied to the inner aspect of the calf muscle of the unaffected leg. Cold sensation, cold pain threshold, heat pain threshold and heat pain tolerance were assessed. For the QST, the change from baseline was noted if there was a change of ≥2 standard deviations (SD) of laboratory normative data from the baseline measurement.

As BG00010 is involved in nerve growth, intra-epidermal nerve fibre density (IENFD) was measured as a safety precaution. A punch biopsy of the distal part (10 cm proximal to the lateral malleolus) of the unaffected leg was performed twice (on the same leg within 1 h) to minimise patient variance.

The presence of anti-BG00010 antibodies was determined using a tiered assay approach involving a screening assay and a confirmation assay, followed by titration of positive samples. The presence of anti-BG00010 antibodies in human serum was determined using an electrochemiluminescent assay format. Samples that tested positive for binding antibodies were further evaluated in a neutralising antibody assay that measured the ability of BG00010 to bind to and activate the extracellular GFRα3 receptor. Assay performance was fully validated in accordance with regulatory guidance and industry best practices.

Plasma and serum samples were also drawn to explore potential pharmacodynamic markers: Substance P, chemokine receptor 2 (CCR2) and norepinephrine.

The efficacy assessments were an 11-point NRS assessment (general sciatic pain, back pain and leg pain) and pain as measured by the VAS of the SF-MPQ. Nociceptive testing was performed as exploratory assessment. Electrical, mechanical and cold pressor tests were performed to assess pain detection and pain tolerance thresholds.

Statistical analyses

This was an exploratory study; therefore, the sample size was not based on statistical considerations. PK parameters were calculated using noncompartmental methods and summary statistics for each PK parameter were calculated by dose. The mean concentration values for each dose group were plotted over time and dose proportionality was assessed. Calculations were performed using WinNonlin Phoenix version 6.2 (Certara, Princeton, USA).

All patients who were randomised were analysed. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 15.0. The incidences of AEs, SAEs and development of antibodies to BG00010, as well as changes in safety parameters were summarised by study dose and compared with placebo. Placebo patients were pooled together separately in Parts I and II.

The efficacy analysis population was defined as all patients who received study treatment and had pain data collected post-dose. Changes in the 11-point NRS and VAS of the SF-MPQ were summarised by study dose and compared with placebo.

RESULTS

Study population

Twenty-eight patients were randomised (see Figure S1 for study flowchart); 16 in Part I of the study to BG00010 (50, 150, 400, or 800 µg kg^{-1} once weekly) or placebo and 12 in Part II to BG00010 (400, 600, or 1200 µg kg^{-1} up to every 48 h and no more than 3 times in 1 week) or placebo. The first dose of study treatment was administered on 25 July, 2011 and the last study visit was on 20 September, 2012.

In Part I, 10 patients (63%) were women; in Part II, 9 patients (75%) were women. The mean age was 53.5 years (range 33–75 years) in Part I and 51.4 years (range 19–74 years) in Part II. The majority of patients were white (22[79%]). Concomitant medications were taken by 15/16 (94%) patients in Part I and 9/12 (75%) patients in Part II. The most frequent concomitant medication was paracetamol (9/15 [60%] patients in Part I and 3/9[33%] patients in Part II).

Pharmacokinetics

In all study cohorts, the BG00010 concentration-time data indicated relatively low inter-patient variability in the time course of BG00010 serum exposure. Serum BG00010 concentrations over time are shown in Figure 1**.** There was a dose-dependent increase in serum exposure from 150 to 1200 µg kg^{-1}, but the increase was less than dose-proportional. The log-linear BG00010 concentration *vs.* time course at all dose levels showed a distinct multiphasic disposition in which peak concentrations dropped more than tenfold in the first 2–3 h post-dose and declined more slowly thereafter. There was no trend toward increasing or decreasing clearance (CL) or steady-state volume of distribution (V_{ss}) across the body weight range tested (62.6–106.4 kg).

A summary of the PK parameters for Parts I and II of the study is given in Table 1. In Part i of the study, the area under the concentration-time curve from time zero to infinity (AUC_{inf}) increased approximately in proportion to dose from 0 to 400 µg kg^{-1} and somewhat less than dose proportionally from 400 to 800 µg kg^{-1} (Table 1). There was no difference in AUC_{inf} between the first (Dose 1) and third (Dose 3) dose within each cohort, indicating little or no BG00010 accumulation. The maximal plasma concentration (C_{max}) also increased with BG00010 dose, but reached a plateau from 400 to 800 µg kg^{-1} (Table 1). Reaching a plateau in C_{max} between these dose levels was expected due to increasing IV infusion time. CL was relatively constant over the dose range tested for Cohorts A to D. Mean V_{ss} for all cohorts increased as a function of dose. Estimation of V_{ss} at the lower dose levels (Cohorts A and B) was influenced by the limited range of concentration-time data and therefore produced underestimates of the true volume. At the higher dose levels (Cohorts C and D), mean V_{ss} fell within a tighter range (Table 1).

In Part II of the study, the increase in AUC during a dosing interval (0–48 h) at steady state (AUC_{tau}) was less than proportional to dose and AUC_{tau} for Dose 3 was higher than that for Dose 1 in all cohorts, indicating some degree of accumulation (Table 1). C_{max} for Cohorts E–G increased with dose, but formed a similar plateau as observed in Cohorts A–D. There was little or no BG00010 accumulation with a 168-h dose interval in Cohorts A to D and some accumulation (29–55%) with a 48-h dose interval in Cohorts E–G, indicating an effective half-life ($t_{½(eff)}$) for BG00010 between 30 and 60 h.

Safety

All patients experienced AEs. The most frequently reported AEs reported by patients receiving BG00010 were headache (10 BG00010-treated patients[83%] vs 3 placebo-treated patients[75%] in Part I and 6[67%] *vs.* 1 [33%] in Part II), feeling hot (6[50%] *vs.* 0 in Part i and 9[100%] *vs.* 0 in Part II), generalised pruritus (8 [67%] *vs.* 1[25%] in Part i and 3[33%] *vs.* 0 in Part II) and pruritus (5 [42%] *vs.* 2[50%] in Part i and 6 [67%] *vs.* 0 in Part II). The majority of AEs were mild; no severe AEs, SAEs or AEs leading to discontinuation were observed. There was no indication for increased reporting of AEs with increasing BG00010 dose or increasing frequency of BG00010 dosing.

An overview of pruritus-related AEs is shown in Figure 2. All AEs pertaining to pruritus were considered related to study treatment and resolved by the end of the follow-up period. Pruritus that lasted >28 days was reported in five patients: this was mild in severity and no modification of study treatment was required in any of the five patients. One patient reported a generalised rash during the study. The patient was in the BG00010 50 µg

kg^{-1} group and the AE was mild in severity and lasted 5 days. One patient in the BG00010 150 μg kg^{-1} group had a temperature-related AE of moderate severity (feeling hot) that lasted 3 days. The same event occurred after the second and third dose, but were of mild severity. Five patients in Part i experienced mild temperature-related AEs that lasted between 1-5 days. Nine patients in Part II experienced mild temperature-related AEs with duration of 1-2 days. No clear trends in response to multiple doses of BG00010 were observed in blood levels of Substance P or CCR2 in Parts I or II of the study.

In Parts I and II, the most frequent concomitant medication was paracetamol. Use of concomitant medications as permitted by the protocol was not expected to affect study results.

No clinically significant changes were found in vital signs, ECG, IENFD, QST or safety laboratory tests. Although some patients, at some points, had QST values outside the normal range, none of these changes were determined to be a reason for concern. Observed changes were considered within the expected variability of QST assessments (data not shown). The change in IENFD from baseline is shown in Figure 3. No trends in IENFD data were observed and no clear dose effect was documented in Parts I or II of the study. There was no correlation between changes in IENFD and clinical findings and the changes that were seen were considered not clinically significant.

The only case of anti-BG00010 antibodies detected in the study was in a patient receiving placebo. This was a false positive result on Day 20; results were negative on Day 43. No neutralising antibodies were detected.

Pharmacodynamics

Change from baseline in NRS over time is shown in Figure 4. On the NRS in Part i, there was little differentiation between placebo-treated patients and patients treated with BG00010 50 or 150 μg kg^{-1} in mean change in general sciatica pain from baseline. There was more differentiation for patients treated with BG00010 400 or 800 μg kg^{-1}, observed particularly after Dose2. After Dose 2, patients receiving BG00010 ≥400 μg kg^{-1} reported greater reductions in general sciatica pain and these reductions were maintained after Dose 3. However, overall there was substantial variability in the NRS data. In Part II of the study, mean changes from baseline among placebo-treated patients remained within a lower range relative to that observed for placebo treatment in Part I and the treatment response was far more evident with the Part II dosing regimen. Results for leg pain and back pain, as measured by the NRS, were similar to those seen for general pain.

In Part I, BG00010 800 μg kg^{-1} resulted in the greatest mean (SD) decrease from baseline in VAS score (-34 [14] mm at Week 6). The greatest mean decrease from baseline for placebo-treated patients was seen at Week 10 (-20.67 [31.63] mm). For the other doses tested in Part I, no pain reduction was seen compared with placebo-treated patients. In Part II of the study, the greatest mean decrease from baseline was seen 56 days after Dose 3 in the 600 μg kg^{-1} dose group (mean[SD] of -33.33 [33.38] mm *vs.* -3.67 [20.03] mm with placebo). A trend in pain reduction from baseline as measured by the VAS of the SF-MPQ was seen in the BG00010 600 and 1200 μg kg^{-1} dose groups compared with placebo. No notable trends were seen in the nociceptive testing data (Table 2a-f; Figure 5).

DISCUSSION

Overall, BG00010 dosed weekly or every 48 h demonstrated dose-dependent PK in this ascending-dose study in patients with sciatica. There were no DLTs, the MTD was not identified and BG00010 administration appeared to lead to a reduction in back pain and leg pain in some patients.

When administered as multiple doses by IV infusion at 48- or 168-h dose intervals, serum BG00010 exposure increased in a dose-dependent, but less than dose-proportional, manner with relatively low variability within dose cohorts. At all dose levels, the log-linear BG00010 concentration-time course showed a distinct multiphasic disposition in which peak concentrations dropped more than tenfold in the first 2–3 h after administration and then declined more slowly over the following days with a $t_{½}$ that generally fell between 40 and 60 h. There was no accumulation of BG00010 when dosed at a 168-h interval, but there was some accumulation when dosed every 48 h (accumulation ratio: 29–55%), suggesting a $t_{½\ (eff)}$ for BG00010 of approximately 30-60 h. The PK results in the current study are consistent with those observed in the previous single ascending-dose study with BG00010.[13]

All patients experienced at least one AE during the study and AEs were more frequently reported in patients receiving BG00010 versus placebo. There was no clear increase in the incidence of the most frequent AEs for the higher- versus lower-dose cohorts or in the cohorts where BG00010 was administered three times during 1 week versus once weekly for 3 weeks. One of the most frequently reported AEs in this study was mild or moderate

pruritus, starting approximately 1–3 days following administration of Dose 1. The exact mechanism of the pruritus is not known, but pruritus was also seen in a single ascending-dose study where it was more common with higher BG00010 doses (≥100 μg kg^{-1}).[13] In our study, with weekly dosing, patients who received the highest BG00010 dose (800 μg kg^{-1}) experienced pruritus AEs of longer duration than patients in the other treatment groups. However, in Part II of the study, when BG00010 was dosed every 48 h, there was no clear indication of pruritus AEs having a longer duration with increasing BG00010 dose. The single case of anti-BG00010 antibodies being detected in a patient receiving placebo was a false positive result, not unexpected as the assay cut point incorporates a 5% false positive rate.

The effects of BG00010 on neuropathic pain behaviour have been studied extensively in rats. Three rat models of neuropathic pain have been examined: spinal nerve ligation,[3] chronic constriction injury of the sciatic nerve[17] and distal root crush.[18] In these models, BG00010 substantially attenuated neuropathic pain behaviour. Exposure to BG00010 at single doses of 400 or 800 μg kg^{-1} in humans was comparable to exposure in rats at doses that have shown efficacy in these nonclinical pain models. In this study, based on the NRS and VAS of the SF-MPQ, the Part II multiple-dose BG00010 regimen resulted in greater pain reduction versus placebo and the lower-dose regimens, although a clear dose-response relationship was not seen. These limited data suggest that BG00010 may benefit patients with sciatica and that further development of this compound for the treatment of neuropathic pain is warranted.

No formal sample size calculation was performed for the efficacy endpoints; therefore, owing to the small sample size of each cohort, no final conclusions can be made about the efficacy of BG00010 at this point. Nociceptive testing was included as an exploratory measure. With only three subjects per dose group, large variability was observed in baseline values of the different nociceptive tasks. No effects of BG00010 on evoked pain were observed. Likely the group size was too small to detect clear effects on evoked pain.

A recent review[19] concluded that available evidence does not clearly show favourable effects with nonsteroidal anti-inflammatory drugs, corticosteroids, antidepressants, or opioid analgesics for the treatment of sciatica. There has, however, been significant progress over the last 20 years in the understanding of the biology of pain sensory neurons and the discovery that neurotrophic factors play an important role in neuropathic pain has provided several new therapeutic targets. Although sciatica probably has mixed neuropathic and nociceptive/inflammatory components, neurotrophic factors such as the GDNF family of ligands may address certain aspects of the underlying causes of neuropathic pain.

In conclusion, PK results were consistent with those observed in previous research. There were no DLTs and a MTD was not identified. These data from a small sample suggest that further evaluation of BG00010 in patients with sciatica is warranted. Additionally, a phase II study in patients with radiculopathy has recently been completed.

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TABLE 1

Summary of the PK parameters for Parts I and II of the study

	Part I (weekly dosing)				Part II (dosing every 48 h)		
	BG00010 50 µg kg^{-1} (n = 3)	BG00010 150 µg kg^{-1} (n = 3)	BG00010 400 µg kg^{-1} (n = 3)	BG00010 800 µg kg^{-1} (n = 3)	BG00010 400 µg kg^{-1} (n = 3)	BG00010 600 µg kg^{-1} (n = 3)	BG00010 1200 µg kg^{-1} (n = 3)
Injection 1							
$AUC_{(inf)}$ h ng ml^{-1}	45.0 (9.1)	156.7 (36.7)	399.0 (37.3)	652.7 (243.2)			
$AUC_{(tau)}$* h ng ml^{-1}	40.7 (12.7)	142.3 (28.4)	347.0 (27.5)	606.0 (247.7)	180.7 (5.0)	294.7 (51.5)	313.7 (113.6)
Clearance (l h^{-1} kg^{-1})	1.1 (0.2)	1.0 (0.2)	1.0 (0.1)	1.3 (0.4)			
C_{max} (ng ml^{-1})	33.2 (2.6)	76.4 (17.3)	156.0 (32.9)	149.3 (29.5)	100.9 (9.7)	174.7 (52.3)	84.0 (18.1)
$t_{½}$[b] (h) †	24.1 (9.6)	54.9 (8.5)	63.8 (16.5)	49.9 (4.0)			
V_{ss} (L kg^{-1})	18.3 (7.4)	44.5 (2.5)	62.3 (11.0)	71.4 (27.6)			
Injection 2							
C_{max} (ng ml^{-1})	28.5 (3.6)	84.1 (12.4)	118.9 (35.9)	135.0 (32.9)	118.3 (25.8)	154.7 (22.0)	135.0 (43.6)
Injection 3							
$AUC_{(inf)}$ h ng ml^{-1}	52.1 (22.6)	159.7 (20.5)	435.7 (121.2)	516.3 (70.5)			
AUC (0-168) h ng ml^{-1}	47.6 (20.5)	149.7 (16.5)	354.7 (52.5)	471.3 (73.0)	232.3 (8.1)	386.0 (61.1)	487.7 (167.9)
Clearance (l h^{-1} kg^{-1})	1.1 (0.4)	0.9 (0.1)	1.0 (0.2)	1.6 (0.2)			
C_{max} (ng ml^{-1})	38.8 (15.2)	65.2 (7.8)	140.3 (47.9)	106.8 (23.2)	96.9 (24.4)	175.0 (47.9)	121.3 (30.4)
$t_{½}$[c] (h) ‡	41.5 (27.1)	48.4 (12.7)	86.6 (53.4)	53.8 (14.2)	66.1 (15.5)	62.5 (15.8)	99.0 (37.5)
V_{ss} (L kg^{-1})	25.3 (8.5)	40.5 (9.2)	74.8 (32.1)	91.2 (23.5)			

All values are mean (±SD). AUC, area under the concentration_time curve; AUC_{tau}, AUC during a dosing interval at steady state; $AUC_{(inf)}$, AUC from time zero to infinity; C_{max}, maximal plasma concentration; $t_{½}$, half-life; V_{ss}, steady-state volume of distribution. *AUC_{tau}: Tau =0-168 for Part I; 0-48 for Part II; † For Dose 1, Part I, mean terminal $t_{½}$ values are based on concentration data to 168 h post-dose; ‡ For Dose 3, Part I, mean terminal $t_{½}$ values are based on concentration data to 120 h post-dose.

TABLE 2A

Nociceptive testing data, Part I cold pressor test, pain tolerance threshold in seconds, mean (± Standard deviation)

	Part I (weekly dosing)				
	Placebo (n = 4)	**BG00010 50 µg kg^{-1} (n = 3)**	**BG00010 150 µg kg^{-1} (n = 3)**	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 800 µg kg^{-1} (n = 3)**
Baseline	15.5 (11.8)	20 (4.7)	18.2 (9.4)	50.2 (60.5)	16.6 (4.1)
Day 1-4 h post-dose	10.5 (6.8)	17.2 (4.4)	15.7 (5.8)	48.9 (61.7)	14.9 (3)
Day 2	12 (7)	15.9 (4.4)	15 (4.6)	48.2 (62.1)	15.1 (5.1)
Day 3	18.5 (13.7)	18.5 (4.6)	17.9 (5.2)	47.6 (62.7)	19.6 (11.4)
Day 8-60 min pre-dose	13.1 (10.2)	18.5 (8.2)	17.1 (4.9)	49.7 (61.2)	19.2 (2.9)
Day 8-4 h post-dose	12.4 (8.5)	17.5 (9.4)	17.5 (4.9)	49.5 (61.4)	14.9 (4.6)
Day 9	9.3 (4.7)	16 (6.2)	18.6 (2)	47.7 (62.8)	15.4 (8.2)
Day 10	13.1 (7.9)	15.3 (5)	18.9 (4.1)	49.8 (60.8)	19.5 (10.8)
Day 15-60 min pre-dose	13.3 (2.9)	14.7 (2.5)	20.5 (6.3)	49.9 (61)	14.7 (7.1)
Day 15-4 h post-dose	12.8 (2.6)	15.5 (5.4)	18.9 (6.7)	48.8 (61.7)	11.7 (2.5)
Day 16	14.1 (2.3)	15.1 (2.8)	17.4 (5.1)	47.8 (62.6)	12.5 (5.9)
Day 17	19.5 (9)	16.9 (6.3)	19.5 (4)	47.6 (62.8)	13.4 (7.3)
Day 29	21.6 (10.8)	10.8 (4.8)	22 (6.8)	48.2 (62.3)	15.9 (5.8)
Day 43	12.1 (6.8)	16.9 (3.9)	20.5 (7.3)	48.2 (62.4)	15.3 (5.5)
Day 71	24.7 (9.2)	13.9 (2.9)	16.4 (6.7)	47.4 (63)	15.8 (3.7)

TABLE 2B

Nociceptive testing data, Part I, electrical pain test, pain tolerance threshold in mA, mean (± Standard deviation)

	Part I (weekly dosing)				
	Placebo (n = 4)	**BG00010 50 µg kg^{-1} (n = 3)**	**BG00010 150 µg kg^{-1} (n = 3)**	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 800 µg kg^{-1} (n = 3)**
Baseline	36.2 (16.9)	24 (10.5)	31.9 (7.9)	28.3 (19.1)	23.2 (7.5)
Day 1-4 h post-dose	28.2 (17.2)	23.8 (12.1)	28.1 (4.2)	29.3 (18.9)	23.5 (12.1)
Day 2	30.9 (16.6)	18.1 (9.2)	28.3 (3.7)	27.5 (19.8)	17.7 (10.9)
Day 3	34 (17.1)	20.1 (10.1)	28.6 (5.3)	28.4 (18.8)	30.3 (15.4)
Day 8-60 min pre-dose	36 (16.1)	23.7 (12.8)	30.4 (2.5)	31.6 (17.4)	25.8 (17)
Day 8-4 h post-dose	32.1 (14.8)	24.6 (18.9)	33.2 (2.3)	31.4 (17.2)	26.3 (21.3)
Day 9	39.7 (17.4)	25.3 (16.9)	34.1 (2.1)	31.6 (17)	24.6 (22.2)
Day 10	36 (19.4)	24.5 (21.4)	34.9 (3.8)	32.1 (18.1)	32.3 (15.5)
Day 15-60 min pre-dose	42.2 (1.2)	17 (8.7)	36.3 (4.4)	32.5 (15.8)	26.9 (19.5)
Day 15-4 h post-dose	30.4 (7.9)	18.2 (8.1)	31.6 (0.8)	33.4 (14.9)	23.6 (19.3)
Day 16	35.9 (5.8)	20.1 (11.5)	33.3 (4)	32.2 (15.7)	24.1 (22.7)
Day 17	41.1 (12.4)	20.8 (10.9)	36.7 (8)	32.4 (15.5)	27.4 (19.6)
Day 29	23.8 (0)	17.1 (8.5)	36 (11.7)	35.8 (17.2)	24.6 (20.8)
Day 43	33.2 (17.9)	25.7 (10)	34.5 (12.2)	28.2 (18.9)	23.2 (18.7)
Day 71	35.6 (19.1)	17 (3.7)	33.8 (5.8)	27.5 (19.6)	25.7 (21.3)

TABLE 2C

Nociceptive testing data, Part I, pressure pain test, pain tolerance threshold in kPa, mean (± Standard deviation)

	Part I (weekly dosing)				
	Placebo (n = 4)	**BG00010 50 µg kg^{-1} (n = 3)**	**BG00010 150 µg kg^{-1} (n = 3)**	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 800 µg kg^{-1} (n = 3)**
Baseline	43.3 (15.6)	44.1 (23.4)	45.6 (19.3)	46.5 (17.1)	51.2 (31.1)
Day 1-4 h post-dose	55.5 (31)	41.1 (19.7)	40.7 (8.5)	44.1 (19)	55.3 (29.9)
Day 2	55.9 (30.6)	42.7 (18.2)	38.3 (11.7)	42.5 (19)	51.6 (16.1)
Day 3	53.6 (31.9)	44.5 (22.1)	41.7 (12.9)	37.3 (16.4)	50.3 (28.6)
Day 8-60 min pre-dose	51.4 (24)	44.1 (16.8)	45.1 (8.7)	40.6 (18)	34.7 (15.2)
Day 8-4 hour post-dose	51 (26.1)	35.8 (20.4)	40.1 (14)	39.7 (13.7)	55.3 (35.2)
Day 9	54.5 (33.5)	38.7 (20.3)	40.7 (10.9)	44.5 (17)	45.1 (29.2)
Day 10	38.8 (15.2)	44.5 (14.4)	45.4 (13.9)	42.4 (14.2)	57.6 (32.9)
Day 15-60 min pre-dose	47.8 (11.7)	31.8 (5.3)	42.2 (9.2)	37.9 (11.9)	49.4 (34.9)
Day 15-4h post-dose	46.3 (7.6)	37.3 (9.5)	45.3 (18.2)	37.6 (13.5)	46.8 (30.4)
Day 16	44 (8.4)	37 (12.8)	39.7 (10.4)	37.4 (13.1)	46.9 (31.1)
Day 17	54.1 (12.9)	38.1 (17.4)	40.5 (12.9)	45.6 (17.8)	51.3 (34.9)
Day 29	58.8 (18)	39 (11)	48.2 (19.3)	46.1 (17.3)	46.1 (31.1)
Day 43	39.9 (13.9)	32.8 (9)	47.2 (16.2)	47.5 (22.2)	38.9 (28.4)
Day 71	39.3 (2.5)	32.4 (11.3)	47.5 (16.3)	45.4 (22.8)	27.6 (14.2)

TABLE 2D

Nociceptive testing data, Part II, cold pressor test, pain tolerance threshold in seconds, mean (± Standard deviation)

	Part II (dosing every 48 h)			
	Placebo (n = 3)	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 600 µg kg^{-1} (n = 3)**	**BG00010 1200 µg kg^{-1} (n = 3)**
Baseline	22 (9)	35.8 (46.1)	15.1 (5.3)	8.8 (1.3)
Day 1-4 h post-dose	20.4 (4)	23 (16)	19.2 (10.8)	8.6 (1.1)
Day 2	16.6 (4.1)	17.1 (11.9)	15.7 (7)	6.7 (0.5)
Day 3-4 h post-dose	18.2 (9.4)	15.4 (10.2)	15.4 (9.2)	7.9 (0.8)
Day 4	19.2 (2.1)	20.3 (14.4)	19.2 (12.9)	7 (1.3)
Day 5-4 h post-dose	37.2 (39.8)	20.9 (15)	21.6 (15.8)	8 (2.2)
Day 6	35.6 (37.9)	18.4 (13.3)	24.3 (17.6)	8.8 (3.2)
Day 7	44.2 (39.3)	21.4 (14.9)	19.1 (14.8)	11.5 (2.1)
Day 19	37.5 (38.4)	24.8 (25.1)	17.5 (11.5)	12.8 (0.4)
Day 33	18.9 (7.7)	25 (19.5)	23 (16.5)	11.6 (4.3)
Day 61	18.8 (8.3)	17.3 (12)	17.8 (12.7)	16.4 (11.8)

TABLE 2E

Nociceptive testing data, Part II, electrical pain test, pain tolerance threshold in mA, mean (± Standard deviation)

	Part II (dosing every 48 h)			
	Placebo (n = 3)	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 600 µg kg^{-1} (n = 3)**	**BG00010 1200 µg kg^{-1} (n = 3)**
Baseline	32.7 (17.6)	26.3 (16.5)	32.8 (15.3)	15.2 (7.5)
Day 1-4 h post-dose	31.5 (10)	26.3 (12.4)	29.1 (7.8)	15.9 (6.5)
Day 2	26.1 (5.2)	25.4 (12.1)	26.6 (12.3)	13.6 (5.5)
Day 3-4 h post-dose	30.7 (4)	24 (8.9)	25.1 (6)	17.4 (1.6)
Day 4	28.1 (2.9)	24.1 (11.5)	26.7 (12.5)	18.9 (9.2)
Day 5-4 h post-dose	32.7 (10.3)	21.9 (10.4)	30.8 (13.8)	15.6 (8.4)
Day 6	31.5 (6)	25.3 (9.2)	31.7 (9.1)	13.3 (3.3)
Day 7	38.3 (9.8)	26.2 (13.8)	31.2 (13.1)	16 (5.3)
Day 19	37.5 (10.9)	39.5 (12.4)	49.9 (0)	20.2 (12.7)
Day 33	41.3 (10)	38.9 (5.8)	37.8 (7.3)	18.7 (12.1)
Day 61	34.5 (18)	33.9 (10.8)	25 (9.6)	16.7 (7.2)

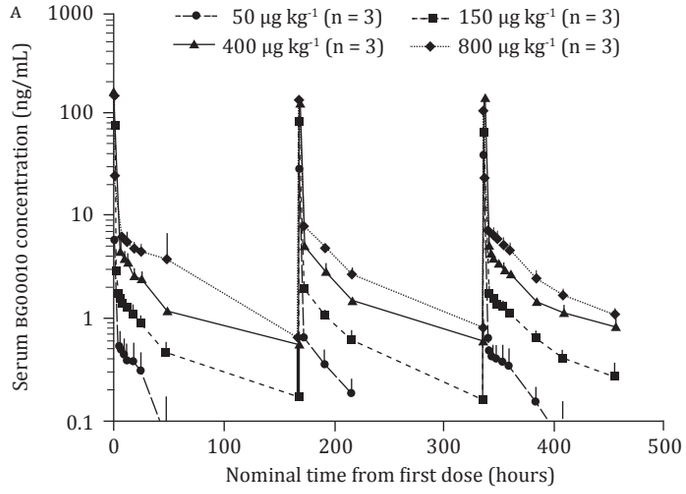
TABLE 2F

Nociceptive testing data, Part II, pressure pain test, pain tolerance threshold in kPa, mean (± Standard deviation)

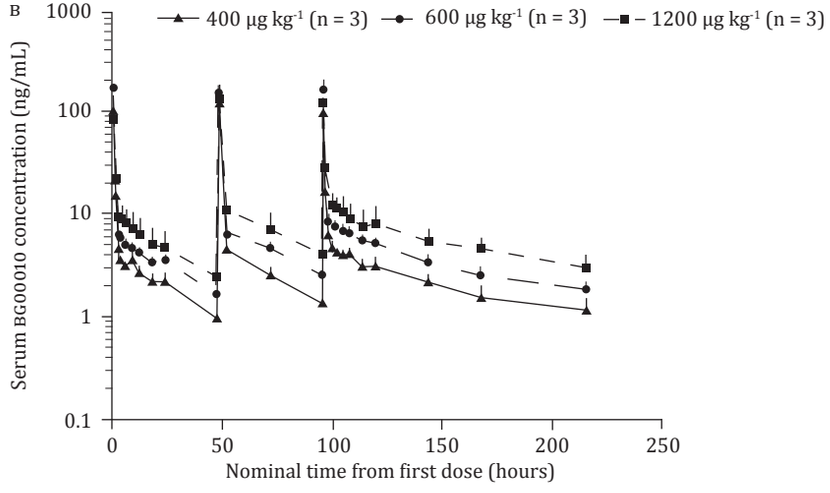
	Part II (dosing every 48 h)			
	Placebo (n = 3)	**BG00010 400 µg kg^{-1} (n = 3)**	**BG00010 600 µg kg^{-1} (n = 3)**	**BG00010 1200 µg kg^{-1} (n = 3)**
Baseline	64.9 (26.6)	36.6 (12.4)	35.9 (18.3)	27.2 (8.1)
Day 1-4 h post-dose	62.5 (30.9)	33.5 (11.5)	35 (9.7)	25.9 (4.9)
Day 2	56.8 (30.8)	31.3 (5.1)	23.2 (5.1)	29.5 (11.1)
Day 3-4 h post-dose	46 (7.5)	27.9 (3.3)	31.7 (13.2)	24.9 (4.2)
Day 4	48.6 (4.7)	28.8 (6.7)	23.2 (4.9)	24.9 (4.4)
Day 5-4 h post-dose	42.1 (1.8)	29.1 (2.5)	26.8 (8)	22.7 (5)
Day 6	58.1 (30.7)	27.9 (8.6)	28.4 (6.3)	23 (5.6)
Day 7	65.3 (21.4)	30.9 (7.5)	30.4 (7.5)	33.7 (12.8)
Day 19	66.7 (30.5)	35.8 (13.1)	37.1 (6.7)	33.4 (0.6)
Day 33	58 (21.3)	30.5 (13.4)	34.6 (10)	37 (13.3)
Day 61	58.5 (50.5)	23.3 (6.7)	34.5 (7.5)	23.2 (3.4)

FIGURE 1

Mean (+ SD) serum BG00010 concentration *vs.* time by cohort in Part I (A) and Part II (B).



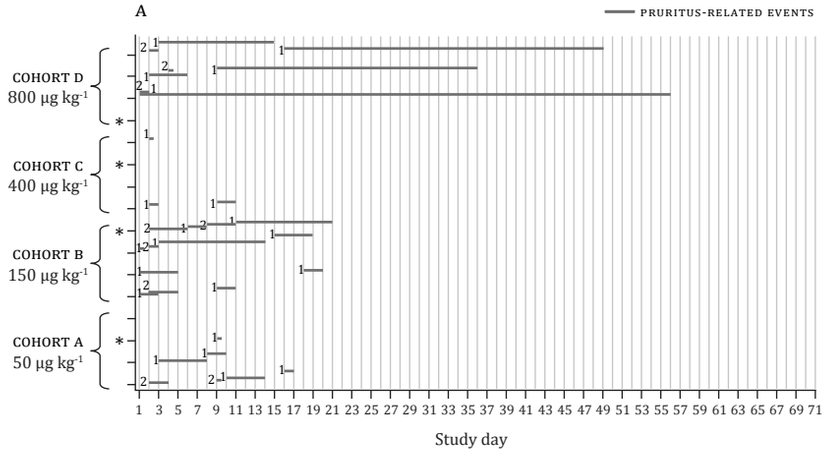
Dosing: once weekly for 3 weeks



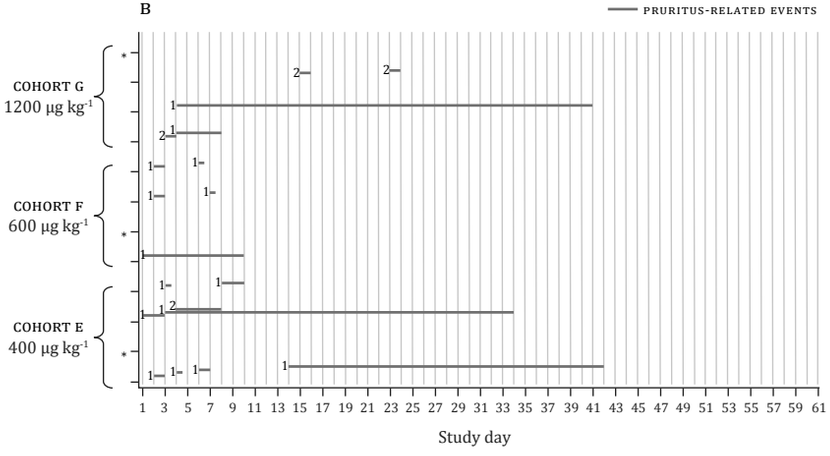
Dosing: up to 3 times weekly for 1 week

FIGURE 2

Adverse-event overview in Part I (A) and Part II (B) for pruritus events. *Patient received placebo. Severity: 1=mild; 2=moderate.



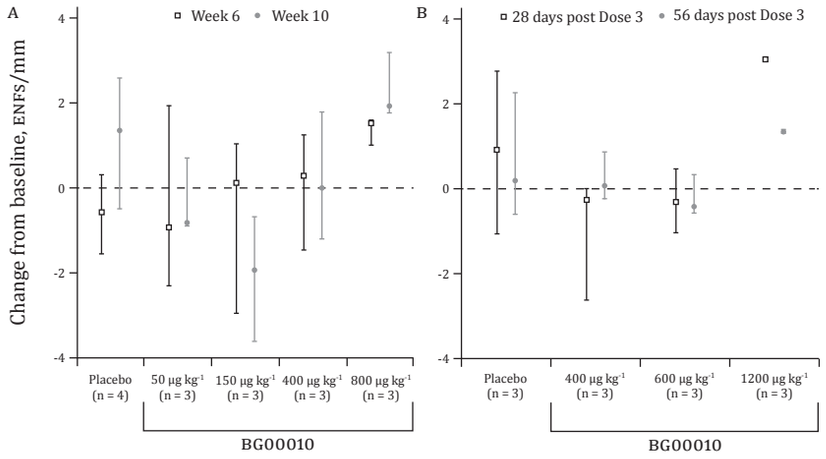
Dosing: once weekly for 3 weeks



Dosing: up to 3 times weekly for 1 week

FIGURE 3

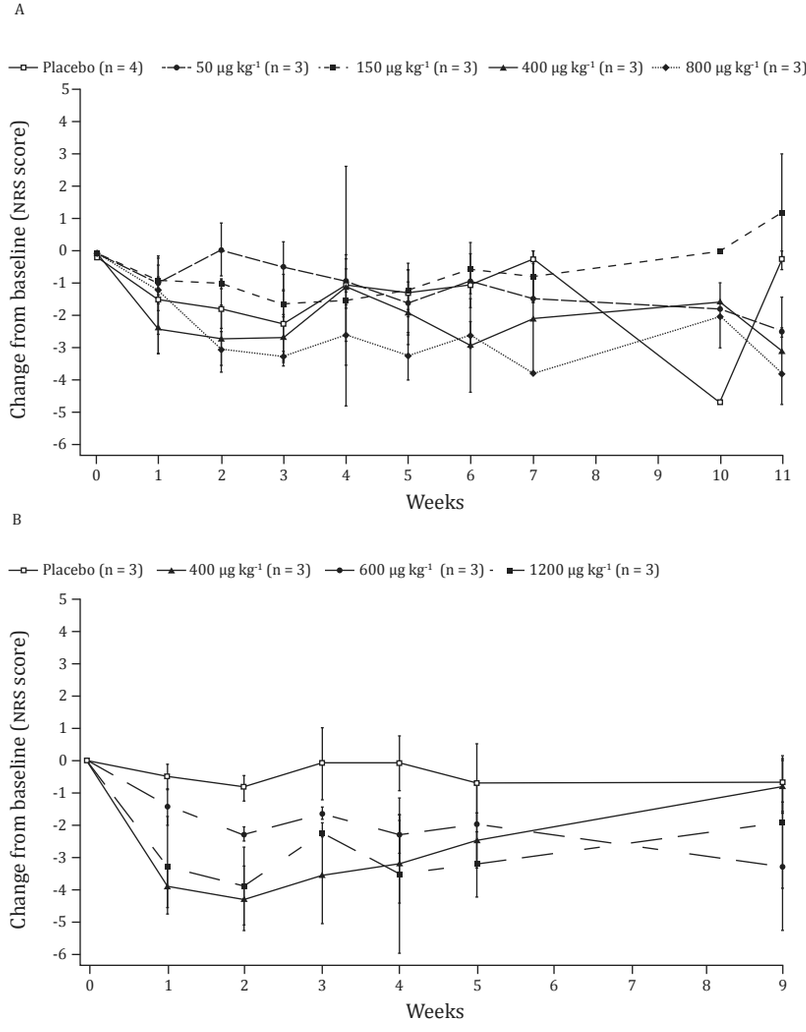
Median (± range) change in IENFD from baseline by treatment and visit in Part I (A) and Part II (B).



ENF, epidermal nerve fiber; IENFD, intra-epidermal nerve fiber density.

FIGURE 4

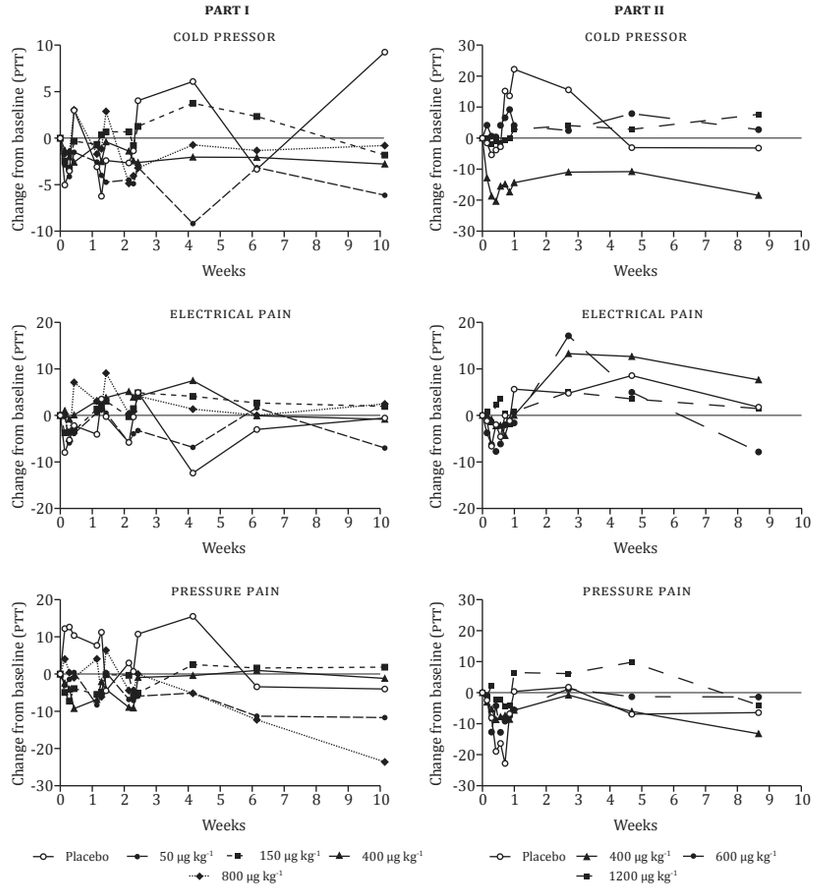
Mean (± SE) NRS pain general assessment change from baseline for Part I (A) and Part II (B).



NRS, Numerical Rating Scale.

FIGURE 5

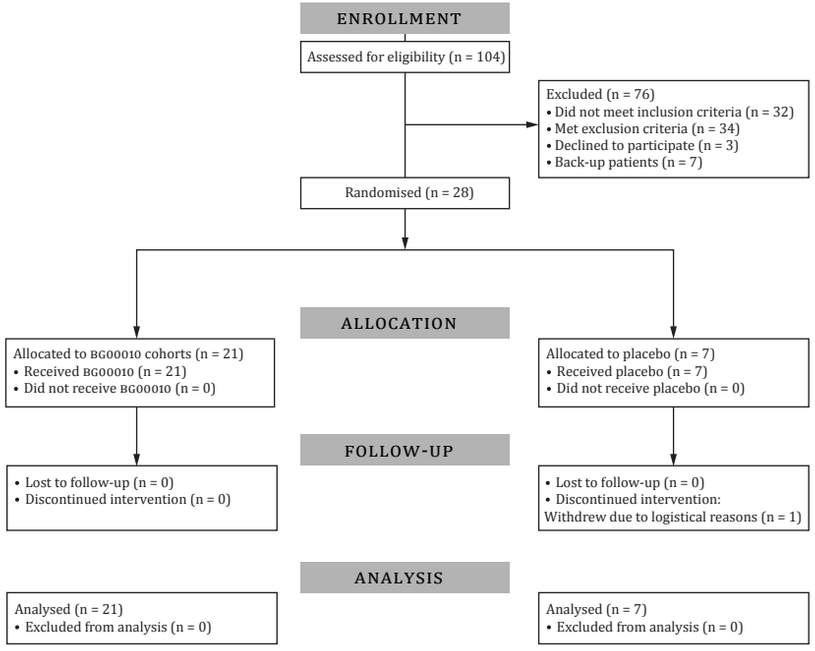
Nociceptive data.



PTT, pain tolerance threshold

SUPPLEMENTARY FIGURE 1

Consort-flowchart.



CHAPTER VIII

Use of human evoked pain models in chronic pain patients and comparison to healthy subjects

To be submitted

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ABSTRACT

Tests that can measure nociceptive thresholds are widely used in healthy subjects in early drug development to predict therapeutic potential of analgesics. Early phase trials can be performed in the target patient population instead of in healthy subjects. We compared baseline measurements from seven studies in order to identify possible differences in nociceptive thresholds between healthy subjects and patients with a chronic pain state. Data from 7 studies in healthy subjects and different pain patient populations in which a battery of human pain models was used were included to perform an exploratory analysis on mean baseline values of all subjects. Differences between healthy subjects and patients with sciatica, diabetes (DM), painful diabetic neuropathy (PDN) and chronic idiopathic axonal polyneuropathy (CIAP) were analysed. Furthermore, we investigated if age and body mass index were covariates in the analysis. The test battery consisted of a sequence of tests eliciting cutaneous electrical, mechanical (pneumatic-) and thermal (cold pressor)-pain. Pain detection thresholds (PDT) for electrical pain were lower in PDN and CIAP patients; pain tolerance thresholds (PTT) for cold pressor pain and pressure pain were lower in DM and CIAP patients compared to healthy subjects. Intra-subject variability of pain thresholds tended to be higher in patients than in healthy controls. Significant differences in pain thresholds exist between healthy subjects and patients, which may influence the extent to which results of analgesic effects using evoked pain tests in healthy subjects can be extrapolated to patients with chronic pain conditions.

INTRODUCTION

Tests that can measure nociceptive thresholds are widely used in healthy subjects in early drug development to predict the therapeutic potential of analgesics. The advantage of the use of human evoked pain models is that they can be performed in controlled circumstances. Although pain is a subjective experience, which is difficult to objectify, several evoked pain tests correlate well with clinical pain syndromes.[1] By using evoked pain tests in humans in an early stage of the clinical development of a new drug, the possible analgesic potency can be investigated.[2–5]

Early phase clinical trials with new analgesics can be performed both in healthy subjects and in a target patient population. When performing early phase clinical drug studies in a target patient population, traditional safety and pharmacokinetic endpoints can be investigated together with exploratory endpoints on the possible desired analgesic effects of a new compound. In addition to subjective pain scales such as visual analogue scales (VAS) and numeric rating scales (NRS) on the current pain condition, more objective methods can be used to assess other analgesic properties of a compound. If no positive evidence for the efficacy of a drug in the chosen target patient population can be found, the use of one or more human pain models can give further insights in the possible effect of a compound for the treatment of pain with another aetiology.[4]

Evoked pain tests have previously been performed in pain populations to investigate the effects of chronic pain conditions on the experience of pain.[6] Patients with chronic pain have a tendency to respond differently to painful stimuli. Changes in pain tolerance levels, pain modulation and augmented brain responses have been found in several chronic pain populations, such as sciatica,[7,8] diabetic painful neuropathy,[9] chronic whiplash associated disorders,[10] rheumatoid arthritis,[11] vulvodynia[12] and fibromyalgia.[13] Quantative sensory testing (QST) is regularly used to determine sensory profiles of patients with pain syndromes.[7,14] The aim of the current analysis was to identify possible differences between healthy subjects and patients with a chronic pain state in their response to a battery of pain models and to investigate potential confounding factors. Possible differences may be important in the design of early phase clinical drug studies in which multi-modal pain testing is considered. Comparing and contrasting the way healthy subjects and patients respond to pain testing, can help to bridge results obtained in healthy subjects to patient populations. Patients with sciatica, diabetes mellitus (without polyneuropathy) (DM), painful

diabetic polyneuropathy (PDN) and chronic idiopathic axonal neuropathy (CIAP) were included in this analysis. Findings from this analysis can be used in decisions to perform a study in a patient population or to use nociceptive tasks in healthy subjects.

METHODS

Data collection

Data from 7 studies performed at CHDR in which a battery of human pain models was used were included to perform an exploratory analysis on the baseline values of all subjects. Each subject had two baseline measurements on each test. Differences between groups (healthy vs. different patient populations) were analysed. Furthermore, we investigated if sex, age and body mass index were covariates in the analysis. All the studies were approved by the local ethics committee and were conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Subjects

Main study and subject characteristics are listed in Table 1. All subjects gave written informed consent. All subjects underwent a full medical screening, at least including medical history taking, a physical examination and vital signs. Healthy subjects were excluded if they had any clinically significant findings as determined by the medical screening. Patients with sciatica included male and female patients, aged 18-85 years with a diagnosis of unilateral sciatica, including pain radiating down the leg following a dermatome, suggesting L4, L5, S1 nerve root involvement, with symptoms present for > 3 months prior to screening visit and pain rated at >40 mm on a 100 mm visual analogue scale (VAS). Patients with diabetes mellitus but without neuropathy included subjects with DM according to the World Health Organization criteria,[15] with a stable glycaemic control regimen and without neuropathy. Patients with painful diabetic neuropathy included subjects with diabetes and polyneuropathy according to AAN criteria.[16] Pain had to be attributable to a symmetrical stocking distribution neuropathy in the lower limbs and average daily pain scores were ≥4 on 0-10 numeric rating scale (NRS). Chronic idiopathic axonal neuropathy patients had to be diagnosed with CIAP as defined by Visser and colleagues.[17] Patients were included if average daily pain scores were ≥4 on 0-10 NRS scale.

Subjects that were regular user of any illicit drugs or had an history of drug abuse, a positive drug screen at screening, consumption of more than 8 units of (methyl)xanthines per day or smoking more than 5 cigarettes/day were excluded. Use of xanthine-containing products and alcohol was not allowed from one day prior to admission to the clinical research unit and during the stay at the research unit. Healthy subjects were not allowed to take analgesics within 3 days of the nociceptive assessments. If patients used medication, they had to be stable on their medication 2 weeks prior to the first study visit.

In studies 1, 2, 3 and 6, subjects were excluded from study participation if they indicated the nociceptive tests intolerable during a training session or achieved >70% (studies 1 and 2) or >80% (studies 3 and 6) of maximum input intensity for any of the nociceptive tasks. For studies 4, 5 and 7 subjects were excluded from this analysis if they achieved >80% of maximum input intensity for any of the nociceptive tasks during the first session.

Evoked pain tests

Nociceptive (pain) detection and tolerance thresholds were measured using a battery of evoked pain tests. The test battery is an integrated range of tasks for measuring different modalities of nociception. It aims to assess as objectively as possible the levels of pain induced by several noxious mechanisms in human subjects. At baseline the evoked pain tests were performed twice. A training session was included in all studies to reduce learning effects during the study. The battery of pain tests was performed multiple times during the day. Exact timing of the tests was different in the individual studies. All tests had previously been shown to be sensitive to the effects of analgesics in healthy adults.[4] All measurements were performed in a quiet room with ambient illumination. Per session, there was only one subject in the room, to avoid any distraction or other external influence.

Pain intensity was measured continuously (beginning from when the first stimulus was applied until the predetermined end of the test) for each nociceptive 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 pain intensity reaches the maximum possible score. For each test the pain detection threshold (PDT) and pain tolerance threshold (PTT) were calculated.

ELECTRICAL STIMULATION TASK

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™) were placed on cleaned, scrubbed, and if required, shaved skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be less than 2 kW. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5, Digitimer, Cambridge, UK).

For the single stimulus, adapted from methods previously described,[18,19] each single stimulus (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 mAs-1 (cutoff 50 mA).

For the repeated stimulus, adapted from methods previously described,[20] each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated 5 times with a frequency of 2 Hz at the same current intensity with a random interval of 3 to 8 seconds between the repetitions. Current intensity increased from 0 mA in steps of 0.5 mA (cutoff 50 mA). Pain detection threshold was taken as the value (mA) whereby a subject indicated either: all 5 stimuli were painful, or the train of 5 stimuli started feeling non-painful but ended feeling painful (VAS > 0). The pain intensity for each stimulation was measured using the eVAS slider, until pain tolerance threshold was reached or a maximum of 50 mA was reached.

PRESSURE STIMULATION TASK

The method of mechanical pressure pain induction was based on methods previously described, and was shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors.[21,22] Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 $kPas^{-1}$. 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, at which point the device released pressure to the cuff.

COLD PRESSOR TASK

The method of cold pressor pain was based on the methods previously described[23,24] and is the most commonly used test to induce inhibitory conditioned pain modulation (CPM, also known as 'diffuse noxious inhibitory control').[25] Subjects placed their non-dominant hand into a water bath (minimal depth 200 mm) at 35 ± 0.5°C for 2 min. At 1 min 45 s a blood pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min the subject then moved that hand from the warm water bath, directly into a similar sized 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.

CONDITIONED PAIN MODULATION

Conditioned pain modulation is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system.[25] The degree of inhibitory conditioned pain modulation (CPM) was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the cold pressor task.

Statistics

All baseline measurements and pre-dose measurements were included to estimate the average values, intra-subject and inter-subject coefficient of variation (CV). All PDT and PTT variables followed a log-normal distribution and were therefore log-transformed before analysis. Transformed parameters were back-transformed after analysis.

To estimate the difference between group and between sex, parameters were analysed with a mixed model with group (healthy, sciatica, DM, PDN, CIAP), sex (male, female) and measurement (1, 2) as fixed factor and with subject as random factor. In order to assess the confounding effects of gender by group, age and BMI, a mixed model analysis with fixed factor group, sex and measurement and covariate age and BMI respectively were performed. Contrasts between groups are reported along with 95% confidence intervals. To assess the confounding effects of age and BMI p-values were calculated. A p-value below <0.05 was considered as a significant covariate. No correction for multiple testing was applied. All calculations were performed using SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

In total 171 subjects were included in the analysis. 108 healthy subjects were included (62 male, 46 female), 23 patients with unilateral sciatica (7/16), 15 patients with diabetes mellitus without polyneuropathy (9/6), 11 patients

with painful diabetic neuropathy (6/5) and 14 patients with CIAP (10/4). 5 patients with sciatica from study 4, 18 healthy subjects from study 5, 12 subjects (4 healthy, 1 with DM, 5 with PDN and 2 with CIAP) from study 7 were excluded from the analysis because they achieved >80% of maximum input intensity for any of the nociceptive tasks during the first session.

Means, intra-subject CVs and inter-subject CVs for the outcome variables for the different pain tasks for healthy subjects and different patient populations are presented in Table 2. The intra-subject CVs were larger for the PDT variables than the PTT variables in all populations. Inter-subject CVs were largest for the PDT variables in all populations, except for the cold pressor test in sciatica subjects. Variability was largest for the CPM variables, in both groups the largest intra- and inter subject CVs were observed for this test. Overall, intra-subject variability tended to be higher in patient populations compared to healthy subjects. Inter-subject variability was not consistently different in healthy subjects versus pain populations.

Least squares means for groups and p-values for the effects of sex, age and BMI are presented in Table 3 and Figure 1**.** No differences in cold PDT were observed. Cold PTTs were lower in DM (contrast versus healthy -34.2%, (95%CI -54.7%, -4.5%), P<0.05) and in CIAP patients (-36.3%, (-58.5%, -2.4%), P<0.05) compared to healthy volunteers. Compared to healthy volunteers, electrical (repeated) PDT was lower in CIAP patients (-41.3%, (-63.3%, -6.1%), P<0.05). No differences were observed in tolerance thresholds. For the electrical single stimulus task PDT was lower in PDN patients compared to healthy volunteers (-40.7%, (-60.9%, -9.9%), P<0.05). No statistically significant differences were observed between healthy subjects and patients for CPM. Compared to healthy subjects, DM (-26.9%, (-41.4%, -8.9%), P<0.01), PDN (-26.6%, (-42.5%, -6.3%), P<0.05) and CIAP patients (-23.8%, (-40.1%, -3.2%), P<0.05) had lower tolerance thresholds for the pressure pain test. Sex was a significant covariate for the PDT of both electrical pain tests. Female subjects reported lower PDTs for both tests (electrical repeat, -43.8%, (-59.8%, -21.3%), P<0.001; electrical single, -34.9%, (-51.4%, -12.9%), P<0.01). Age was not a significant covariate in any of the tests. BMI was a significant covariate for the PDT of both electrical tests and the pressure pain test, but not for the PTTs of these tests.

DISCUSSION

In the present study results from 7 studies were pooled in order to compare differences in pain detection and pain tolerance thresholds in different patient populations and in healthy subjects. The aim of the current analysis was investigate the differences between healthy subjects and patients with chronic pain in their response to a battery of pain models. The analysis showed that the pain detection threshold for electrical pain was lower in patients with PDN (single stimulus) and CIAP (repeated stimulus) compared to healthy subjects. Also the pain tolerance threshold for cold pressor pain and pressure pain was lower in patients with DM and CIAP compared to healthy subjects. No differences were observed between healthy subjects and sciatica subjects. CPM did not show significant differences between the different populations. Intra-subject variability tended to be higher within the patient cohorts. Inter-subject variability for the different nociceptive tests was not consistently different in patients compared to healthy subjects. In general pain detection threshold outcomes were more variable than pain tolerance thresholds.

Several studies have previously investigated pain sensitivity in chronic pain patients. These mostly involved one chronic pain condition with one pain testing modality. This is the first time that pain sensitivity in four different chronic pain conditions were compared using a multi-modal battery of pain models. Pain and sensitivity in patients with chronic lower back pain was previously investigated. Patients with sciatica have an impaired sensitivity for warm and cold sensory thresholds measured in the affected dermatomes.[26] Another study previously performed evaluated the effects of intradermal capsaicin administration in both the affected and the unaffected leg in sciatica patients and an increased hyperalgesia response was observed in both legs in sciatica patients compared to healthy subjects.[8] Another study used QST to create a complete sensory profile of CLBP patients. Widespread changes in somatosensory sensitivity were found in the affected body parts but also at sites distinct from the region of pain.[7] In contrast to the hyperalgesic response observed previously, in our study no changes in PDT or PTT compared to healthy subjects for any of the pain tests were observed. We only performed measurements in sciatica subjects in unaffected body parts, but since others also found impaired sensitivity in unaffected extremities, this does not explain the differences between our and other studies.

In our study we found no differences in pain detection and pain tolerance thresholds for electrical pain in patients with diabetes without neuropathy. In patients with PDN, a lower PDT for electrical pain compared to healthy subjects was observed. For the other pain modalities cold PTT and pressure PTT were lower in patients with diabetes compared to healthy subjects. In

PDN subjects a lower PDT and PTT for pressure pain compared to healthy subjects were observed. In a study previously performed, patients with diabetes had higher thresholds and tolerance for electrical pain both in the lower and upper extremity compared to healthy subjects. Patients with diabetes and neuropathy had higher thresholds than patients with diabetes without neuropathy.[9] In another study, glucose infusions in healthy subjects resulted in decreased pain detection and tolerance thresholds. In the same study patients with diabetes were hyperalgesic compared to healthy subjects.[27] In contrast, Luft and colleagues found no differences in cold pressor pain intensity between healthy and diabetic subjects.[28] Results from literature are contradictory as some studies show a hyperalgesic and other a hypoalgesic response in diabetic subjects with or without neuropathy. However, it is certain that these patients have alterations in their somatosensory nervous system and hence changes in their pain response.[29] Therefore, reliable measurements of pain detection and tolerance thresholds in this patient category in studies where analgesic compounds are tested might be challenging.

We found a lower PTT for the cold pressor task and the pressure pain task in CIAP patients, indicative of increased sensitivity to pain in these patients. No prior studies are available in which pain detection and tolerance thresholds are determined in CIAP patients.

Differences were observed between pain thresholds in healthy subjects compared to patients with diabetes, PDN and CIAP. No differences were observed between healthy subjects and subjects with sciatica. The electrical pain paradigm, the pressure pain paradigm were performed on the lower extremities, the cold pressor test on the upper extremity. In diabetes, PDN and CIAP nerve fibers of all extremities can be injured;[30,31] in sciatica only the nerve root of the affected leg is injured. In the sciatica patients measurements were performed in the unaffected leg. So in all patient populations except for the sciatica patients, pain measurements were performed in a possibly compromised part of the nervous system. Performing these measurements in less affected parts of the nervous system would possibly have yielded other outcomes.

Recent studies suggest a role of an impaired inhibitory conditioned pain modulation in patients with chronic pain states.[10,32] Here, no differences in CPM values were observed between healthy subjects and patients. Of all pain measurements, CPM had the largest intra- and inter-subject variability. We compared the electrical pain detection and tolerance thresholds before and within 5 min after the cold pressor task to quantify CPM. The study by Deanen and colleagues measured CPM by comparing pain intensity (and not pain detection and tolerance) during (and not after) the conditioning stimulus.[10] The study by Olesen measured CPM by performing somatic pressure stimulation direct, 2 and 5 min after immersion of the hand of a cold water bath. Maximum CPM response was observed directly after the cold pressor test. The highly variable noted for the CPM in our study is the most likely reason why we were not able to detect differences in CPM between the different patient groups.

Sex was a significant covariate in electrical pain (PDT) and pressure pain (PTT). In all cases where differences between male and female subjects were observed, female subjects had lower detection and tolerance thresholds compared to male subjects. A systematic review about sex differences in experimental pain perception mentions that there is strong evidence that female subjects tolerate less thermal and pressure pain compared to male subjects and the female and male subjects have comparable thresholds for cold and ischemic pain.[33] Body mass index was a significant covariate for the pain detection thresholds for both electrical pain paradigms and for pressure pain. Higher BMI was associated with higher PDTs. A recent review observed a tendency towards higher pain threshold in obese subjects compared to non-obese subjects.[34] Although obese subjects were excluded from our studies, a trend towards higher PDTs with increasing BMI was observed.

The QST battery is used as tool to determine somatosensory profiles in different pain disorders. Several studies showed distinct somatosensory profiles in different neuropathic pain disorders.[35,36] We only observed differences between healthy subjects and patients in a small number of outcome measurements. Subjects who achieved more than 80% of the maximum input intensity for any of the nociceptive tasks were excluded from participation or excluded from the analysis. Therefore, the presented values might be an underestimation of the actual differences in pain threshold and tolerance within the groups. This, in combination with the high variability in baseline outcome measurements, makes the battery of human pain models not a useful tool to quantify somatosensory profiles of pain syndromes. The battery is more suitable as a screening tool to predict analgesic and pharmacological potential of existing and new compounds. Due to the variability of the measurements and confounding effects of sex, setting up studies that are placebo-controlled with a crossover design are preferable. Furthermore, outcomes should always be corrected for individual baseline values.

In this study we aimed to make a comparison between healthy subjects and different patient populations. Results from seven studies were used to do this exploratory analysis. Study designs were different and were not specifically designed to look at the endpoints described in this analysis. The patient population differed from the healthy subject population in baseline characteristics such as weight, age and concomitant medication use. Healthy subjects were not allowed to use concomitant medication, patients were allowed to use medication if the use was stable 2 weeks prior to the first study visit. This could have influenced the outcomes in the patient populations.

If patients respond to the pain tests in a similar way as healthy subjects do, these tests can be implemented in pharmacological studies in patient populations. Subsequently, if a correlation exists between outcomes in pain tests and clinical efficacy after a pharmacological intervention, results from pain tests can be used to predict clinical efficacy. Results of pain tests performed in healthy subjects can then be genuinely extrapolated to clinical efficacy in patient populations. However, significant differences were observed between healthy subjects and patient populations for several of the pain tests. Intra-subjects variability tended to be higher in the patient populations. These differences may influence the extent to which results of analgesic effects using evoked pain tests in healthy subjects can be extrapolated to patients with chronic pain conditions. Pharmacological validation studies in patients using this battery of pain models could be performed in order to further explore how this battery of pain models responds in patients. As pointed out previously, a possible point of improvement to future studies would be to perform pain tests in parts of the body unaffected by the pain condition.

This is the first time that a study is reported in which pain sensitivity in four different chronic pain conditions are compared using a multi-modal battery of pain models. It can be concluded that patients with a chronic pain state respond differently to pain tests than healthy subjects. Future studies are needed to investigate if a correlation exists between outcomes in pain tests and clinical efficacy after a pharmacological intervention in chronic pain patients.

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TABLE 1

Overview of original studies

Study	Subjects	Main inclusion criteria	N (male/female)	Age (years)*	BMI (kg m^{-2})*	Reference
1	Healthy	Healthy, aged 18-65 years, BMI 18-30 kg m^{-2}, no clinical relevant abnormalities at screening.	20 (10/10)	22.5 (19-39)	22.0 (18.0-25.9)	Not available
2	Healthy	Healthy, aged 18-65 years, BMI 18-30 kg m^{-2}, no clinical relevant abnormalities at screening.	10 (5/5)	20.5 (18-23)	22.4 (20.1-28.4)	Not available
3	Healthy	Healthy, aged 18-65 years, BMI 18-30 kg m^{-2}, no clinical relevant abnormalities at screening.	12 (6/6)	22 (19-25)	22.3 (20.6-29.6)	Not available
4	Sciatica patients	Aged 18-85 years, unilateral sciatica, pain radiating L4, L5 or S1. For at least 3 months prior to screening. Must rate their pain at ≥ 40 mm on the 100 mm VAS of the SF-MPQ.	23 (7/16)	55 (18-75)	27.4 (22.5-32.9)	[37]
5	Healthy	Healthy, aged 18-65 years, BMI 18-30 kg m^{-2}, no clinical relevant abnormalities at screening.	15 (14/1)	25 (22-43)	24.8 (18.3-29.5)	[38]
6	Healthy	Healthy, aged 18-45 years, BMI 18-30 kg m^{-2}, no clinical relevant abnormalities at screening.	39 (21/18)	22 (18-30)	21.7 (18.5-25.5)	[4]
7	Healthy and patients with diabetes mellitus, painful diabetic neuropathy and chronic idiopathic axonal polyneuropathy.	-Healthy, aged 18-80 years, BMI 18-32 kg m^{-2}, no clinical relevant abnormalities at screening.	12 (6/6)	63 (27-78)	24.2 (20.1-27.0)	Not available
		-DM, aged 18-80 years, BMI 18-32 kg m^{-2}, according the WHO criteria with stable glycaemic control regimen[15].	15 (9/6)	58 (19-74)	26.5 (21.4-30.7)	
		-PDN, aged 18-80 years, BMI 18-32 kg m^{-2}, according to AAN criteria[16], average daily pain score ≥ 4 on 0-10 NRS scale.	11 (6/5)	64 (30-75)	28.7 (20.7-32.0)	
		-CIAP, aged 18-80 years, BMI 18-32 kg m^{-2}, diagnosis as defined by criteria in Visser et al.[17], average daily pain score ≥ 4 on 0-10 NRS scale.	14 (10/4)	67 (49-73)	25.9 (19.5-32.1)	

DM, diabetes mellitus; PDN, painful diabetic neuropathy; CIAP, chronic idiopathic axonal polyneuropathy. *values are presented as median (range)

TABLE 2

Mean values and the intra- and inter-subject variability for the different pain tasks

	Healthy			Sciatica			DM			PDN			CIAP		
Parameter	**Mean**	**Intra-subject CV (%)/SD***	**Inter-subject CV (%)/SD***	**Mean**	**Intra-subject CV (%)/SD***	**Inter-subject CV (%)/SD***	**Mean**	**Intra-subject CV (%)/SD***	**Inter-subject CV (%)/SD***	**Mean**	**Intra-subject CV (%)/SD***	**Inter-subject CV (%)/SD***	**Mean**	**Intra-subject CV (%)/SD***	**Inter-subject CV (%)/SD***
Cold PDT (s)	7.9	32.3	91.7	5.4	34.0	69.3	6.8	44.4	66.3	5.1	21.8	56.3	9.1	35.7	126.2
Cold PTT (s)	29.0	13.5	84.3	22.7	12.8	82.4	15.8	19.4	34.9	14.9	12.1	36.0	13.2	17.9	43.3
Electrical (repeated) PDT (mA)	3.5	19.7	77.5	4.4	12.2	86.9	3.3	41.6	75.6	2.6	42.2	71.1	4.6	54.7	119.3
Electrical (repeated) PTT (mA)	11.4	10.7	54.8	10.1	7.9	47.9	10.4	17.5	38.5	12.6	39.2	56.0	12.3	12.7	58.7
Electrical (single) PDT (mA)	8.2	24.1	55.6	10.2	24.4	67.2	9.7	34.7	83.5	6.2	52.7	86.7	10.4	33.1	98.2
Electrical (single) PTT (mA)	22.8	8.9	43.3	22.2	11.2	42.2	22.5	18.7	37.0	20.0	35.1	33.6	20.3	15.9	33.3
CPM: Delta Electrical (single) PDT (mA)	0.9	2.3	2.2	0.3	2.5	2.8	0.0	3.0	3.1	0.9	3.8	3.7	1.8	2.3	2.1
CPM: Delta Electrical (single) PTT (mA)	1.2	1.7	2.2	2.4	3.3	4.0	1.7	2.3	3.0	1.8	3.4	3.3	2.2	2.8	3.4
Pressure PDT (kPa)	17.6	23.7	66.8	17.2	20.4	86.3	12.0	30.7	75.1	11.1	27.4	99.2	14.5	35.3	80.5
Pressure PTT (kPa)	48.7	10.7	41.2	39.6	9.2	44.2	34.3	25.0	26.8	34.7	21.4	36.8	39.3	11.7	46.9

CV, coefficient of variance; SD, standard deviation; AUC, area under the curve; PDT, pain detection threshold; PTT, pain tolerance threshold; VAS, visual analogue scale and CPM, Conditioned Pain Modulation, sec, seconds; kPa, kilopascal; mA, milliampere. *For CPM parameters the SD is reported, for the other parameters the CV.

TABLE 3

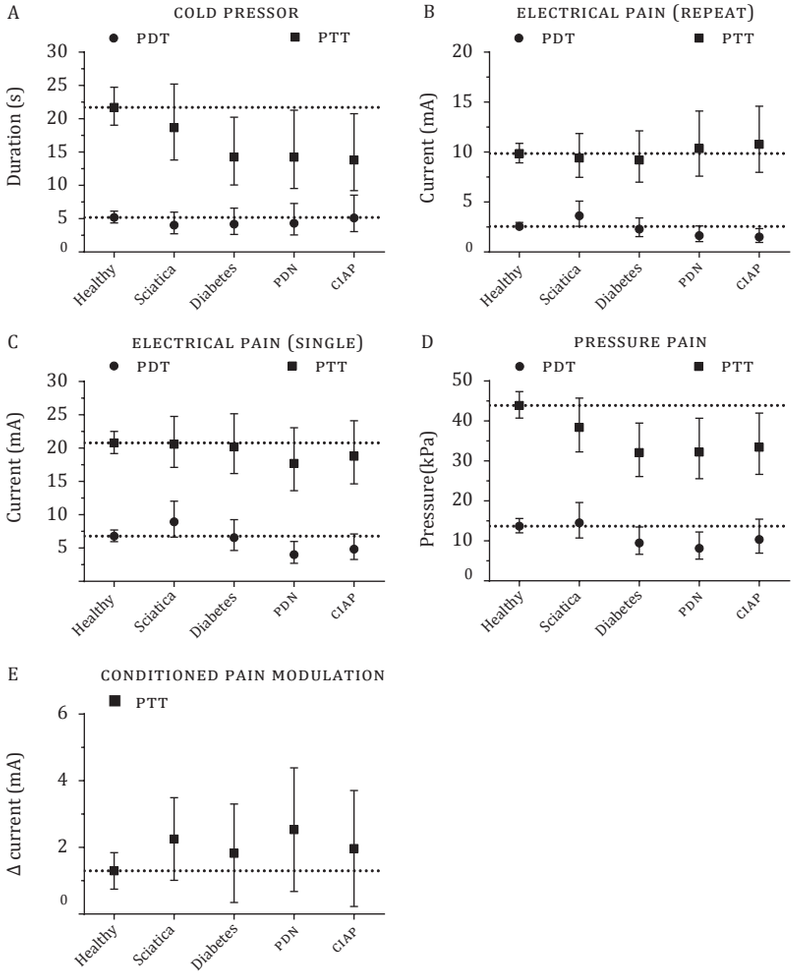
Least squares means of healthy subjects versus sciatica, DM, PDN and CIAP patients. P-values are presented for the confounding effects of sex, group by sex, age and BMI.

	Least squares means					Contrast				Covariates			
Parameter	**Healthy**	**Sciatica**	**DM**	**PDN**	**CIAP**	**Healthy vs Sciatica**	**Healthy vs DM**	**Healthy vs PDN**	**Healthy vs CIAP**	**Sex**	**Group * Sex**	**Age**	**BMI**
Cold PDT (s)	5.18	4.04	4.18	4.31	5.12	-21.9% (-49.1%, 19.6%) P=0.25	-19.2% (-50.3%, 31.4%) P=0.39	-16.7% (-52.0%, 44.5%) P=0.51	-1.1% (-42.3%, 69.6%) P=0.97	0.3384	0.7136	0.1675	0.2659
Cold PTT (s)	21.71	18.66	14.27	14.25	13.82	-14.0% (-38.1%, 19.4%) P=0.3645	-34.2% (-54.7%, -4.5%) P=0.0280	-34.4% (-57.0%, 0.2%) P=0.0509	-36.3% (-58.5%, -2.4%) P=0.0386	0.5491	0.3061	0.2105	0.3386
Electrical (repeated) PDT (mA)	2.56	3.62	2.29	1.65	1.50	41.6% (-2.5%, 105.5%) P=0.0671	-10.5% (-41.4%, 36.9%) P=0.6082	-35.5% (-60.1%, 4.3%) P=0.0736	-41.3% (-63.3%, -6.1%) P=0.0266	0.0009	0.0396	0.2559	0.0065
Electrical (repeated) PTT (mA)	9.85	9.41	9.20	10.35	10.78	-4.5% (-25.7%, 22.9%) P=0.7207	-6.6% (-30.3%, 25.2%) P=0.6450	5.1% (-24.1%, 45.4%) P=0.7641	9.4% (-20.4%, 50.4%) P=0.5773	0.1847	0.6381	0.3010	0.3603
Electrical (single) PDT (mA)	6.78	8.93	6.56	4.02	4.83	31.7% (-4.7%, 82.0%) P=0.0953	-3.3% (-33.1%, 39.8%) P=0.8574	-40.7% (-60.9%, -9.9%) P=0.0146	-28.8% (-52.7%, 7.2%) P=0.1030	0.0042	0.0697	0.6407	0.0047
Electrical (single) PTT (mA)	20.78	20.59	20.19	17.72	18.79	-0.9% (-19.0%, 21.2%) P=0.9257	-2.8% (-23.1%, 22.8%) P=0.8084	-14.8% (-35.3%, 12.3%) P=0.2537	-9.6% (-30.4%, 17.5%) P=0.4492	0.0809	0.8659	0.382	0.0834
CPM: Delta Electrical (single) PDT (mA)	0.8209	-0.0638	0.1667	1.0880	1.3342	-0.8848 (-2.1136, 0.344) P=0.1569	-0.6543 (-2.0533, 0.7447) P=0.3569	0.2671 (-1.345, 1.8791) P=0.7439	0.5133 (-1.1161, 2.1427) P=0.5348	0.0985	0.3123	0.4532	0.9571
CPM: Delta Electrical (single) PTT (mA)	1.2951	2.2529	1.8240	2.5326	1.9680	-0.8848 (-2.1136, 0.344) P=0.1569	-0.6543 (-2.0533, 0.7447) P=0.3569	0.2671 (-1.345, 1.8791) P=0.7439	0.5133 (-1.1161, 2.1427) P=0.5348	0.3328	0.6985	0.3657	0.9065
Pressure PDT (kPa)	13.69	14.51	9.48	8.14	10.34	6.0% (-23.7%, 47.2%) P=0.7276	-30.7% (-52.4%, 0.8%) P=0.0550	-40.5% (-61.2%, -8.9%) P=0.0173	-24.4% (-50.4%, 15.1%) P=0.1901	0.0824	0.8179	0.4449	0.0234
Pressure PTT (kPa)	43.91	38.41	32.10	32.24	33.44	-12.5% (-27.6%, 5.7%) P=0.1653	-26.9% (-41.4%, -8.9%) P=0.0056	-26.6% (-42.5%, -6.3%) P=0.0135	-23.8% (-40.1%, -3.2%) P=0.0261	0.0014	0.7648	0.9656	0.3952

LSM, least squares mean; SD, standard deviation; MIN, minimum; MAX, maximum; CV, coefficient of variance; AUC, area under the curve; PDT, pain detection threshold; PTT, pain tolerance threshold; VAS, visual analogue scale; CPM, Conditioned Pain Modulation; sec, seconds; kPa, kilopascal; mA, milliampere.

FIGURE 1

Least squares means for PDT and PTT for the different pain tasks in different populations. A represents the cold pressor task; B, electrical stimulation repeated stimulus; C, electrical stimulation single stimulus; D, pressure stimulation task and E, conditioned pain modulation (only PTT).



PDT, pain detection threshold; PTT, pain tolerance threshold; s, seconds; kPa, kilopascal; mA, milliampere; PDN, painful diabetic neuropathy; CIAP, chronic idiopathic axonal polyneuropathy.

SUMMARY AND GENERAL DISCUSSION

Drug development scientists are on a search for suitable biomarkers that can assist in predicting the therapeutic potential of analgesic medication and, therefore, it's efficacy in the target population. This is particularly appropriate for human pain where models can assist to bridge the preclinical and clinical findings. These models can provide valuable information about the mechanism of action of existing and new drugs. However, a single human pain model cannot be used exclusively to screen the pharmacological mechanism of a compound as it inherently only tests a single mechanism.

In this thesis the performance of a battery of pain models (PainCart) was investigated. Three main topics were investigated. (1) The validation of the PainCart was described in which the effects of different classes of analgesics on this battery of pain models were explored. (2) The PainCart was used in different chronic pain populations. (3) The performance of the battery during the development of new analgesic compounds was studied.

The PainCart can potentially act as biomarker to assess the effect of analgesics on pain in early phase drug studies. A biomarker has to meet the following four criteria;[1]

- There must be a consistent response of the biomarker across studies (preferably from different research groups) and drugs from the same mechanistic class.
- The biomarker must respond clearly to therapeutic (not supratherapeutic) doses.
- There must be a clear dose- or concentration-response relationship.
- There must a plausible relationship between the biomarker, pharmacology of the drug class, and disease pathophysiology.

In this chapter of the thesis the main outcomes are summarised. The biomarker criteria in relation to the PainCart are discussed point by point and limitations and future perspectives are discussed.

Main outcomes

Chapter 2 describes the methodology of the different PainCart measurements. In **Chapter 3** the ability of the battery of pain models to detect analgesic properties of commonly used analgesics in healthy subjects is investigated. The study consisted of two parts. Subjects were administered fentanyl, phenytoin, (S)-ketamine and placebo (part I), or imipramine, pregabalin, ibuprofen and placebo (Part II). In this chapter it was shown that the battery of pain models is able to detect changes in pain detection and pain tolerance thresholds after administration of different classes of analgesic compounds in healthy male and female subjects. Compounds with different mechanisms of action demonstrated a distinct response pattern on the different pain models.

In **Chapter 4**, the analgesic effects of a novel compound for treatment of acute pain, Xen2174, were assessed with the PainCart. Xen2174 is a small peptide, derived from the venom of a marine cone snail. This peptide binds to the norepinephrine transporter, which results in inhibition of norepinephrine uptake. The study was performed to assess the pharmacodynamics and the pharmacokinetics in plasma and cerebrospinal fluid (CSF) of Xen2174 in healthy subjects. In a randomised, blinded, placebo-controlled study, 25 subjects were administered Xen2174 or placebo. CSF was sampled for 32 hours using an intrathecal catheter. Pharmacodynamic assessments were performed using the PainCart. This study showed that the highest dose of Xen2174 administered intrathecally was able to influence pain thresholds in several pain models. The pain models showed an increase in pain tolerance thresholds for the electrical pain models and the pressure pain model following administration of the highest dose of Xen2174 tested, although statistical significance was not reached. At the highest dose level tested in this study, concentrations of Xen2174 in cerebrospinal fluid exceeded the required exposure limit based on nonclinical safety margins, making it unlikely that the compound can be used in practice for the treatment of acute pain. However, in this study it was shown that intrathecal drug administration in combination with performing a battery of evoked pain tasks in humans is feasible, even with concurrent CSF sampling.

Chapter 5 describes a study which investigated the synergistic effects of milnacipran and buprenorphine in healthy subjects using the PainCart. Buprenorphine is known to be a potent opioid agonist. Animal studies suggest that milnacipran co-administered with opioids may potentiate the analgesic effect of μ-opioid receptor agonists. A randomised double-blinded, placebo-controlled, 4-way cross-over, multiple dose clinical trial to investigate the analgesic effects of buprenorphine in combination with milnacipran in healthy subjects was performed. Buprenorphine showed a dose dependent analgesic response, but no potentiation or synergy on a battery of evoked pain tasks could be observed after co-administration of both milnacipran and buprenorphine.

The studies presented in the previous chapters were all performed in healthy adult subjects. **Chapter 6** described the use of the PainCart in a

population of healthy adolescents. In this two-day crossover, randomised, double-blind, placebo-controlled study, 16 adolescents (aged 16 or 17 years) received paracetamol or placebo on separate study days. Paracetamol concentrations were measured in saliva. Plasma paracetamol concentrations were predicted using measured saliva concentrations and pharmacokinetic data taken from published studies. Paracetamol did not have a statistically significant effect on any of the pain parameters. Nonetheless, a majority of the adolescents would participate again in the study. In this chapter it was demonstrated that pain research using the PainCart with concurrent saliva sampling is feasible and acceptable in a population of healthy adolescents.

Chapter 7 describes a study in which a novel neurotrophic factor, BG00010, was administered in patients with sciatica. The main objectives of this study were to evaluate the pharmacokinetic and safety profiles and to determine the effects on pain of ascending doses of intravenous injections of BG00010 in patients with sciatica. In this randomised, blinded, placebo-controlled multiple-dose study, different dose levels of BG00010 were examined. Safety and efficacy assessments were used as endpoints. Efficacy assessment of pain were performed by using a numerical rating scale of the sciatic pain. PainCart measurements were included in this study as exploratory measurements. In this study no notable trends were observed within the PainCart measurements. The main conclusion of this chapter is that BG00010 may have a benefit for patients with sciatica. Although no effects of BG00010 on the PainCart were observed, it was shown that performing a battery of pain models at the same time as the more traditional endpoints in a clinical trial in patients with sciatica was feasible.

In **Chapter 8**, an analysis was performed in which data from 7 PainCart studies were analysed. Healthy subjects and patients with sciatica, diabetes mellitus (DM), painful diabetic neuropathy (PDN) and chronic idiopathic axonal polyneuropathy (CIAP) were included in this analysis. Significant differences in pain thresholds existed between healthy subjects and patients. These observed differences may influence the extent to which results of analgesic effects using evoked pain tests in healthy subjects can be extrapolated to patients with chronic pain conditions.

The PainCart as biomarker for analgesic efficacy

As discussed previously, in order to assess if a measurement or a combination of measurements can be used as biomarker, it has to fulfil four criteria.

THERE MUST BE A CONSISTENT RESPONSE OF THE BIOMARKER ACROSS STUDIES (PREFERABLY FROM DIFFERENT RESEARCH GROUPS) AND DRUGS FROM THE SAME MECHANISTIC CLASS

The PainCart is not the first multimodal battery of pain models that is described in the literature. Other groups also describe a multimodal approach to investigate effect of analgesics. Staahl *et al.* assessed the reproducibility of a pain test battery that consisted of pain models evoking pain in skin, muscle and viscera.[2] This battery of test was later used to compare the analgesic potency of both morphine and oxycodone. All pain models used in this study were sensitive to the effects of strong opioids.[3]

Olsesen *et al.* used a multi-modal, multi-tissue approach to explore which models were able to assess morphine analgesia in a group of healthy subjects.[4] They concluded that models that provide deep tonic stimulation including C fibre activation (muscle pressure, bone pressure, cold pressor and visceral pressure) were more sensitive to morphine analgesia than models that stimulate superficial structures with phasic impulses. The findings from literature are in agreement with our observations. The strong opioids that were used in **Chapter 3 and Chapter 5** increased pain tolerance in a broad range of pain models (Table 1). The effects on evoked pain tests of the other compounds described in this thesis are also shown in Table 1. If this table is compared with Table 1 from **Chapter 1**, most drug/pain model combinations show comparable results. The agreement of the results observed in this thesis with the findings found in literature suggests that the individual models from this battery show consistent responses across studies. However, in order to draw any definite conclusions about the reproducibility of the PainCart between studies similar or equal compounds should be tested repeatedly.

THE BIOMARKER MUST RESPOND CLEARLY TO THERAPEUTIC (NOT SUPRATHERAPEUTIC) DOSES

The following doses were administered in the studies described in **Chapter 3, 5, and 6**; fentanyl 3 μg kg^{-1} in 30 min, buprenorphine 0.5-3 μg kg^{-1}, ibuprofen 600 mg, imipramine 100 mg, phenytoin 300 mg, pregabalin 300 mg, (S)-ketamine 10 mg and paracetamol 1000 mg. For ibuprofen, imipramine and pregabalin these doses are on the upper limit of normal, but not supratherapeutic. For the other compounds, dosages administered are within the normal range prescribed. For all compounds, except paracetamol, a statistically significant effect on one of the pain models

was observed. Therefore, it can be concluded that the PainCart responds clearly to therapeutic doses. Furthermore, in **Chapter 5** three ascending doses of buprenorphine were administered; a subtherapeutic dose of 0.5 μg kg^{-1}, a dose of 1 μg kg^{-1} dose which was expected to lead to minimally therapeutic plasma concentrations and a 3 μg kg^{-1} infusion, which was expected to lead to therapeutic plasma concentrations of buprenorphine. The subtherapeutic did not lead to pronounced effects on the PainCart while the minimally therapeutic and the therapeutic doses did (Figure 1).

THERE MUST BE A CLEAR DOSE- OR CONCENTRATION-RESPONSE RELATIONSHIP

In **Chapter 4** three ascending doses of Xen2174 were administered. After administration of 0.5 mg Xen2174 and 1 mg Xen2174, no changes in pain detection and pain tolerance were observed. After administration of 2.5 mg Xen2174 an increase in PTT was observed for the electrical pain and the pressure pain tests. As discussed, in **Chapter 5** three ascending doses of buprenorphine were administered. Similar observations as in **Chapter 4** were made in **Chapter 5**. A clear dose response and concentration-response was observed for the different doses administered in this study (Figure 1).

THERE MUST A PLAUSIBLE RELATIONSHIP BETWEEN THE BIOMARKER, PHARMACOLOGY OF THE DRUG CLASS, AND DISEASE PATHOPHYSIOLOGY

The individual models of the PainCart induce pain via different mechanisms. As described in Chapter 1, electrical stimulation directly stimulates sensory nerve endings.[5] The pressure stimulation test assesses nociception generated from the muscle.[6] Cold pain induced by the cold pressor test mainly activates C-fibers.[7] The pain response from the thermal stimulation initially activates Aδ fibers, followed by C-fiber activation. After induction of UVB inflammation, cytokines are produced which lead to sensitisation of cutaneous nociceptors.[8] The drugs administered in the studies all have their own specific effects on these pain pathways. For instance, opioid receptors are widely distributed in the brain, the spinal cord and the periphery. Opioids have direct central effects, where they modulate descending pathways which exert a strong inhibitory effect on pain transmission in the dorsal horn. They also inhibit pain transmission by directly acting on the dorsal horn and by inhibiting excitation from peripheral nociceptors.[9] Nonsteroidal anti-inflammatory drugs inhibit cyclo-oxygenase enzyme (COX) 1, 2 and 3. In this way they inhibit conversion of arachidonic acid into prostaglandins and consequently inhibit the inflammatory response by elicited by cytokines.[9] The extensive distribution of opioid receptors throughout the body might explain the broad overall effect of fentanyl and buprenorphine on the PainCart. Ibuprofen only had a significant effect on the heat PDT of UVB irradiated skin, this can also be explained by the mechanism of action of NSAIDs. Therefore, it can be concluded that there is a plausible relationship between the pharmacology of the drugs administered and their effect exerted on the PainCart. Based on the four criteria previously mentioned, the collection of pain models combined in the PainCart can act as biomarker to assess the pharmacodynamic responses of analgesic drugs. As mentioned previously, a single human pain model cannot be used exclusively to screen the pharmacological mechanism of a compound as it inherently only tests a single mechanism. Therefore, it is the combination of pain models that makes the PainCart useful as a biomarker.

The use of the PainCart in clinical drug development

We concluded that the PainCart can act as biomarker for the assessment of the pharmacodynamic effects of analgesic drug. But if an effect is observed in human pain models in healthy subjects, does this also predict clinical efficacy? If effects observed on evoked pain models are predictive for clinical efficacy is discussed in **Chapter 1**. Lotsch *et al.* concluded that a small set of pain models seemed predictive for efficacy in the clinic.[10] In this study nine experimental pain models are mentioned that predict analgesia in drug development. Of these nine models, three models are included in the PainCart battery, namely UVB + contact heat, blunt pressure and electrical pain. This suggests that pharmacodynamic effects observed on the PainCart might predict clinical efficacy. However, the review from Lotsch and colleagues only provided an indirect link from healthy subjects to chronic pain patients. For this reason the study described in **Chapter 8** was performed. The aim was to identify possible differences between healthy subjects and patients with a chronic pain state in their response to the PainCart. If patients respond to the pain models in a similar way as healthy subjects, extrapolation of results obtained in healthy subjects to patient populations may be better justified. The results from this study showed that there are differences in pain perception between healthy subjects and chronic pain patients. This is in agreement with previous research which showed that chronic pain patients and chronic opioid users have a tendency to respond differently to painful stimuli.[11–14] Therefore, although the PainCart is perfectly suitable as a tool to assess effects of analgesics in

healthy volunteers, in order to draw conclusions about the extrapolation of PainCart results from healthy subjects to pain patients, further research in this specific area needs to be performed. This future research is challenged by the fact that the 'pain patient' does not exist. In patients with chronic (neuropathic) pain, different sensory profiles exist. These profiles possibly match with different neurobiological mechanism of pain.[15] Selecting and clustering the patients in groups is necessary to obtain meaningful results.

Limitations of the PainCart

As previously discussed in **Chapter 3**, the large number of pain models that were used in this study, yielded an even greater number of outcome variables. No correction for multiple testing was applied in any of the studies as this were early phase clinical studies designed to explore the pharmacodynamics of the analgesic compounds. This multi-modal test battery should be considered as a screening tool for analgesic properties of compounds in development for the treatment of pain, and not as a way to definitely prove effects on a specific evoked pain model with statistical significance. When the analgesic effect of a new drug on a certain pain mechanism has already been established, predefining a primary outcome measure would prevent the need to correct for multiple testing.

The magnitude of a placebo response can be large in pain research, especially in neuropathic pain.[16] However, large placebo responses are also reported in healthy subjects. Olofsen *et al.* reported that placebo analgesia contributed to 20% of the total analgesic effect after administration of alfentanil in healthy subjects,[17] although this study reported that the placebo response was additive to the alfentanil effect. It is of utmost importance to use cross-over study designs when pain models are used to reduce variability and correct for potential placebo effects.

In this thesis no pharmacokinetic-pharmacodynamic (PK/PD) modelling and simulation techniques were used. A review of Martini *et al.* shows the usefulness of PK/PD modelling of analgesics to assess not only their efficacy, but also to estimate potency, identify sources of variability and predict time course of drug effects.[18] In **Chapter 5** PK/PD data was used to estimate the timing and dosages of the buprenorphine infusions. Other studies described in this thesis report the PK and PD results somewhat separately. For instance, in **Chapter 3 and 4** besides the PainCart measurements, concentrations of the different compounds used were also measured. Only a non-compartmental analysis was performed. However, these data could be very well used to do more advanced analyses, such as defining responders and non-responders and predict a more specific time course of the drug effects.
As discussed in **Chapter 8** significant differences were observed between healthy subjects and patient populations for several of the pain tests. These differences may influence the extent to which results of analgesic effects using evoked pain tests in healthy subjects can be extrapolated to patients with chronic pain conditions. Pharmacological validation studies in patients using this battery of pain models could be performed in order to further explore how this battery of pain models responds in patients. This way, clinical efficacy can be directly correlated to the effects on the PainCart measurements. However, the larger variability that was observed in patients compared to healthy volunteers should be taken into account when designing these studies.

Future of the PainCart

In all studies in this thesis, the battery of pain models included two paradigms for electrical stimulation, the pressure stimulation test, the cold pressor test and a paradigm for conditioned pain modulation. **Chapter 3** also included the UVB model and the thermal grill test. However, choices have to be made about what pain models should be included in the battery. An example is the thermal grill test. In **Chapter 3**, no significant decrease on thermal grill maximum unpleasantness or maximum pain ratings could be observed in this study, while other research groups report significant outcomes after the use of this test.[19] Due to the apparent necessity to tailor this method to each individual subject, it is difficult to standardise this method and incorporate it into our battery of pain models. Also, the thermal grill test does not fit the first two biomarker criteria as mentioned earlier in this chapter (consistent response of the biomarker across studies preferably from different research groups/the biomarker must respond clearly to therapeutic (not supratherapeutic) doses). Therefore, the thermal grill does not prove to be an added value to the PainCart setup.

Another example is the CPM paradigm that is used in the PainCart. It is the test with the highest inter- and intra-subject variability in their outcome parameters (**Chapter 8**). To quantify CPM, the electrical pain detection and tolerance thresholds were compared before and within 5 minutes after the cold pressor task. A study by Deanen and colleagues measured CPM by comparing pain intensity (and not pain detection and tolerance) during (and not after) the conditioning stimulus.[20] A study by Olesen measured CPM

by performing somatic pressure stimulation directly, 2 and 5 minutes after immersion of the hand of a cold water bath. Maximum CPM response was observed directly after the cold pressor test. It appears that CPM measured in our way leads to more variable CPM. CPM is an important measurement of the pain perception in healthy subjects and chronic pain patients, however if a measurement of CPM is considered as a valuable addition to the PainCart, another method of measuring CPM should be selected.

Two methods for electrical stimulation were used. A single stimulus method in which intensity of a current gradually increases and the repeated stimulus method in which each single stimulus pulse is repeated 5 times with a frequency of 2 Hz at the same current intensity and the repeated stimulus intensity increases gradually. This repeated application of a stimulus over time induces an integrated and more painful response, known as temporal summation. It is suggested that temporal summation might act as a biomarker of drug effects on neuropathic pain.[21] However, a statistically significant treatment effect for the electrical repeated stimulus is only observed after administration of buprenorphine (Table 1). And in this specific case buprenorphine also increased the single stimulus PTT. It could be argued that discarding one of the electrical tests could be an improvement for the PainCart.

EXPANSION OF MODELS INCLUDED IN THE PAINCART

Currently the battery focuses on nociceptive and inflammatory pain models. There are pain models that are not included in the PainCart, which could be of value for this battery.

For instance, induction of chemical pain by intranasal gaseous CO2 stimuli and punctate heat induced by laser heat stimuli. Both methods have the advantage that they are using short-lasting stimuli and that evoked potentials can be used as possible read out. Both methods have been shown to be predictive of clinical analgesia.[10]

Stimulation of the viscera is also not a part of the PainCart. Visceral pain can be evoked by applying distension to hollow organs, such as the oesophagus, small intestines or rectum. Other evoked pain methods inducing visceral pain include electrical, thermal or chemical stimulation of a part of the gastrointestinal tract.[7] Distension techniques have been used to evaluate the effects of several NSAIDs and opioids.[22] If the PainCart is considered as a screening tool for potential analgesic effects of compounds, then addition of a methodology of visceral stimulation would give a wider range of possible analgesic findings. However, major disadvantages of visceral stimulation include that they are highly invasive (which limits tolerability by the subjects), technically difficult to perform (for instance insertion of a gastroesophageal probe), more difficult to include in a study setup where measurements are performed throughout the day and the pain is more difficult to measure to the diffuse nature of the pain.

Another model that the PainCart is lacking, is a model for neuropathic pain. Numerous models exist that evoke neuropathic pain in animals. For instance central neuropathic pain is evoked by injecting excitotoxic agents (e.g. picrotoxin or kainate) into the somatosensory cortex and peripheral neuropathic pain is evoked by ligating or transecting peripheral nerves.[23]

For obvious reasons these models are not performed in human subjects. Models that are frequently used to induce symptoms that are also observed in neuropathic pain include models that evoke pain via intradermal capsaicin, UVB irradiation or a freeze lesion.[24] These models evoke several symptoms that are typically observed in neuropathic pain such as allodynia and/or hyperalgesia, but do not lead to chronic neuropathic pain as such is normally caused by a lesion or disease of the somatosensory system.[25] The UVB model that is incorporated in the PainCart causes hyperalgesia and is a good model for inflammatory pain. Hyperalgesia is a symptom observed in neuropathic pain, however, none of the compounds of which you would expect an effect on neuropathic pain (imipramine, pregabalin or phenytoin) increased the pain detection threshold of this test. It may be that neuropathic pain is too complex to be imitated by a human pain model. A possible strategy to predict efficacy of analgesic drugs against neuropathic in pain models is to back-translate results from the clinic to studies in healthy volunteers as suggested by Lotsch and Oertel.[10,26] For instance, if pregabalin is effective against neuropathic pain; and pregabalin affects the cold pressor test; then this test might be a predictor for a drug's effect on neuropathic pain. Besides, neuropathic pain is not a single entity, it is a group of syndromes that is caused by a lesion or disease of the nervous system. Neuropathic pain can manifest itself in many ways; such as peripheral sensitisation, central sensitisation and deafferentation.[27] A single model for neuropathic pain as an entity is unnecessary, if all these manifestations can be mimicked in a model separately. For instance, dorsal horn sensitisation can be induced by intradermal injection of capsaicin.[28]

READOUTS OF THE PAINCART

The psychophysical readout of the PainCart is a combination of a response-dependent and a stimulus-dependent methods (see also **Chapter 1**). In

the response-dependent method the subjects rates the intensity of a given stimulus, with the PainCart this is done continuously with the eVAS slider. In the stimulus-dependent threshold, the stimulus increases until a certain threshold (pain detection threshold, pain tolerance threshold) is reached; this is also done in the PainCart measurements. In the studies performed, the primary outcomes were pain detection threshold, pain tolerance threshold and area under the pain-intensity curve. These readouts have in common that they provide a subjective rating of the pain. More objective readouts include electrophysiological readouts such as somatosensory evoked potentials, electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). Although they have a larger variation in outcome measurements and are technically more difficult to perform in a large group of subjects, a more objective outcome measurement would be valuable addition to the PainCart.[29–31]

Overall conclusion

While the PainCart has demonstrated that it is robust, reliable and sensitive to a wide range of analgesics, one should realise that the PainCart is not a static entity, it is a dynamic process in which we continuously have to search for opportunities to improve the overall outcome of this methodology.

As discussed previously targets of novel analgesic drugs can fall into three main classes: (1) incremental improvement on an existing drug mechanism, (2) novel selective mechanism arising from better understanding of the mechanism of an existing analgesic drug and (3) completely novel mechanism arising from basic biological studies or from human pathophysiological or genomic studies.[32] In all these classes the PainCart can assist the development of new analgesics. In PainCart studies where improvement on an existing drug mechanism is tested, the original compound should be used as a positive control. If novel mechanisms are tested; the PainCart can be used to screen for analgesic properties of a drug and benchmark the compound with existing analgesics.

Possible new drug targets include anti-nerve growth factor (NGF) antibodies, tropomyosin receptor kinase (Trk) A inhibitors, cannabinoid agonists, selective sodium channels blockers, transient receptor potential vanilloid 1 (TRPV1) receptor antagonists.[33–36] Based on the expected pharmacokinetic and pharmacodynamic properties of these new targets, trials can be set up with a tailored study design using the PainCart. For instance, anti-NGF antibodies mediate anti-inflammatory responses. The UVB model would be an excellent model to assess pharmacodynamic properties of this class of drugs in healthy subjects.

In 1998 the European Medicines Agency issued a guideline named "Note for guidance on general considerations for clinical trials". In the introduction they state "The essence of rational drug development is to ask important questions and answer them with appropriate studies".[1,37] In this thesis, the battery of pain models was able to answer some of these important questions in early phase, analgesic drug development. Although there is still room for improvement of this current methodology, at this stage the PainCart can be used to benchmark analgesic properties of new drugs against established analgesics in early phase clinical studies.

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TABLE 1

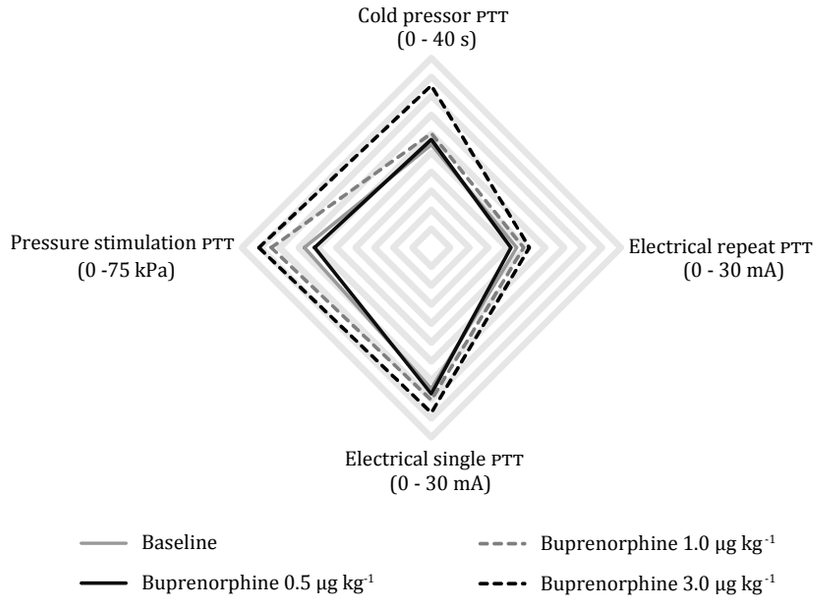
Overview of analgesics described in this thesis and their effect on different pain models.

Drug class	Drug	Cold pressor	Electrical repeat	Electrical single	CPM	Pressure pain	Thermal skin pain	Thermal skin UVB	Thermal grill
Strong opioid	Fentanyl	PTT ↑ (P<0.05)	PTT ↑ (P<0.10)	PTT ↑ (P<0.10)	NS	PTT ↑ (P<0.10)	PDT ↑ (P<0.05)	PDT ↑ (P<0.05)	Unpleasentness ↓ (P<0.10)
	Buprenorphine	PDT ↑ (P<0.05) PTT ↑ (P<0.05)	PTT ↑ (P<0.05)	PTT ↑ (P<0.05)	NS	PTT ↑ (P<0.05)	NP	NP	NP
NSAID	Ibuprofen	NS	PTT ↑ (P<0.10)	NS	NS	NS	NS	PDT ↑ (P<0.05)	Intensity ↑ (P<0.10)
TCA	Imipramine	PTT ↑ (P<0.10)	NS	NS	PDT ↑ (P<0.05)	PTT ↑ (P<0.10)	NS	NS	NS
Sodium channel blocker	Phenytoin	NS	NS	PDT ↑ (P<0.05) PTT ↑ (P<0.05)	NS	PDT ↑ (P<0.10)	NS	NS	Intensity ↑ (P<0.10)
A2δ ligand	Pregabalin	PDT ↑ (P<0.05) PTT ↑ (P<0.05)	PTT ↑ (P<0.10)	PTT ↑ (P<0.05)	PDT ↑ (P<0.05) PTT ↑ (P<0.10)	PTT ↑ (P<0.05)	PTT ↑ (P<0.05)	NS	NS
NMDA antagonist	(S)-ketamine	NS	PTT ↑ (P<0.10)	PTT ↑ (P<0.05)	NS	NS	PDT ↑ (P<0.05)	NS	NS
Other	Paracetamol	NS	NS	NS	NS	PDT ↑ (P<0.10)	NS	NP	NP
	Xen2174	NS	PTT ↑ (P<0.10)	PDT ↑ (P<0.10)	NS	PTT ↑ (P<0.05)	NP	NP	NP

CPM, conditioned pain modulation; UVB, ultraviolet B; PTT, pain tolerance threshold; PDT, pain detection threshold; NS, not significant; NP, not performed; NSAID, nonsteroidal anti-inflammatory drug; TCA, tricyclic antidepressant and NMDA, N-methyl-D-aspartate.

FIGURE 1

Least squares means of different pain tasks at baseline and after administration of three ascending doses of buprenorphine.



PTT, pain tolerance thresholds; s, seconds; kPa, kilopascal; mA, milliampere.

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Acute pijn, te definiëren als pijn die minder dan 12 weken aanhoudt, is onderdeel van het normale leven en wordt regelmatig ervaren door bijna iedereen. Daarnaast wordt geschat dat ongeveer 19-31% van de Westerse bevolking lijdt aan chronische pijn; pijn die meer dan 12 weken aanhoudt. Chronische pijn komt vaker voor bij het toenemen van de leeftijd en meer bij vrouwen dan bij mannen. Veel gebruikte medicijnen tegen pijn zijn paracetamol, niet-steroïde anti-inflammatoire medicijnen (zoals ibuprofen en diclofenac) en opioïden (zoals morfine en tramadol). Ook worden antidepressiva en anti-epileptica gebruikt bij de behandeling tegen pijn. Door bijwerkingen en soms ook beperkte effectiviteit, blijft een continue zoektocht gaande naar nieuwe pijnstillers.

Men kan nieuwe pijnstillers ontwikkelen door bestaande aangrijpmechanismen te verbeteren of op basis van nieuwe aangrijpmechanismen die vanuit fundamenteel geneesmiddelenonderzoek of vanuit menselijk pathofysiologisch onderzoek ontdekt worden. Ook al zijn er in de afgelopen jaren diverse nieuwe pijnstillers op de markt zijn gekomen, zijn er toch veel nieuwe geneesmiddelen tegen pijn die gedurende de ontwikkelingsfase sneuvelen. Met name als dat laat in de klinische fase plaatsvindt, zal dat gepaard gaan met zeer hoge kosten. Een methode die bij zou kunnen dragen aan een meer succesvolle ontwikkeling van geneesmiddelen in het algemeen is het gebruik van biomarkers. Een biomarker is een 'kenmerk dat objectief gemeten en geëvalueerd kan worden als een indicator voor een normaal biologisch proces, een pathogeen proces of een farmacologische respons op een therapeutische interventie.' Biomarkers die zijn bedoeld om farmacologische activiteit te meten, kunnen worden gebruikt om te bepalen of een middel aan een specifiek moleculair aangrijpingspunt bindt en wat de concentraties en doseringen zijn waarbij farmacologische activiteit optreedt. Het gebruik van biomarkers in vroege-fase-geneesmiddelonderzoek heeft als voordeel dat in een vroege fase kan worden bepaald of een potentieel geneesmiddel zijn beoogde effect heeft. Op die manier kan het falen van een geneesmiddel in een latere fase door gebrek aan effectiviteit worden voorkomen. Daarnaast zorgt het aantonen van afwezigheid van effectiviteit in een vroege fase, voor de mogelijkheid vroegtijdig te kunnen stoppen met de ontwikkeling van een middel, met belangrijke kostenbesparingen tot gevolg.

Pijnmodellen in mensen

Een voorbeeld van het gebruik van biomarkers in geneesmiddelonderzoek is het gebruik van pijnmodellen in mensen. Klinische pijn in patiënten wordt beïnvloed door veel factoren, waaronder emotionele, psychologische en cognitieve. Door pijnmodellen te gebruiken in onder andere gezonde proefpersonen kunnen deze factoren worden gecontroleerd. Een pijnmodel bestaat uit twee onderdelen; een externe pijnlijke stimulus wordt toegediend en deze stimulus wordt gemeten. De stimulus kan onder andere een mechanische, thermische, elektrische of chemische prikkel zijn. De prikkel kan op verschillende weefseltypes zoals huid, organen en spieren worden uitgevoerd. Het meten van de pijn kan met behulp van psychofysische-, elektrofysiologische- en beeldvormingstechnieken worden gedaan. De psychofysische uitkomsten bestaan uit twee componenten; een stimulus-afhankelijke bepaling en een respons-afhankelijke bepaling. Bij de stimulus-afhankelijke bepaling neemt de intensiteit van de stimulus toe totdat een bepaalde drempel wordt bereikt (bijvoorbeeld een pijn detectiedrempel of een pijn tolerantiedrempel). Bij de respons-afhankelijke bepaling wordt gevraagd de intensiteit van een bepaalde prikkel te beoordelen (bijvoorbeeld met een visuele analoge schaal of een numerieke schaal). Een voorbeeld van een elektrofysiologische meetmethode is elektro-encefalografie (EEG). Beeldvormingstechnieken kunnen bestaan uit metingen met behulp van een fMRI (functional magnetic resonance imaging) en een PET (positron emission tomography) scan. De grootste voordelen van het gebruik van pijnmodellen ten opzichte van klinische pijn zijn dat de intensiteit, duur en modaliteit van de stimulus zijn te controleren, dat de reactie op een pijnprikkel kwantitatief kan worden bepaald en dat deze reactie kan worden vervolgd in de tijd. Een van de belangrijkste nadelen van pijnmodellen in mensen is dat de pijnlijke stimuli van kortdurende aard zijn en klinische pijn, die vaak langer duurt, niet goed nabootsen. Daarnaast zal een bepaald pijnmodel hooguit voldoende zijn om één specifieke vorm van pijn na te bootsten. Door meerdere pijntesten te combineren kunnen meerdere vormen van pijn worden onderzocht. Om die reden is dan ook het onderzoek, dat beschreven is in dit proefschrift, uitgevoerd. Hierbij is onderzocht of een multimodale benadering, waarbij meerdere receptoren en pijnbanen gestimuleerd worden, beter in staat is de potentiële pijnstillende effecten van middelen op te sporen.

De PainCart

De PainCart, de pijntest batterij beschreven in dit proefschrift, is een batterij van testen die verschillende pijnmodellen combineert. De verschillende pijnmodellen induceren pijn via verschillende stimuli en in verschillende weefsels. De pijnmodellen die onderdeel uitmaken van de PainCart zijn uitgebreid gebruikt in eerder onderzoek. Een uniek aspect van de PainCart is dat het verschillende pijnmodellen combineert waardoor op een gecontroleerde manier meerdere pijntesten in een relatief korte tijd uitgevoerd kunnen worden. De specifieke pijnmodellen die onderdeel uitmaken van de PainCart zijn gekozen op basis van de mogelijkheden om pijnprikkels via verschillende modaliteiten (elektrisch, mechanisch en thermisch), in verschillende structuren (oppervlakkige en diepe structuren) en met verschillende duur (kort, lang) toe te dienen. De PainCart bestaat uit modellen voor nociceptieve en inflammatoire pijn. De batterij bevat de volgende modellen; elektrische stimulatie test, druk stimulatie test, koud water test, hitte stimulatie test (met en zonder ultraviolet (UV) inductie) en methoden om de verwerking en modulatie van pijn door het zenuwstelsel te meten (geconditioneerde pijn modulatie en temporele summatie).

Bij de elektrische stimulatietest worden twee elektroden bevestigd op het scheenbeen. Door middel van een computer aangestuurde stimulator wordt elektrische stroom door de elektroden geleid. De elektrische stroom neemt langzaam toe in intensiteit en dit veroorzaakt pijn. De proefpersonen wordt gevraagd om aan te geven wanneer de prikkel pijnlijk begint aan te voelen (de pijn detectiegrens) en om aan te geven wanneer zij de prikkel niet meer kunnen verdragen (de pijn tolerantiegrens). Bij het bereiken van de pijn tolerantiegrens stopt de elektrische stroom direct.

Bij de druk stimulatie test wordt een tourniquet om de kuitspier bevestigd. Via een luchtpomp wordt het tourniquet langzaam opgeblazen. Dit veroorzaakt pijn, met name door stimulatie van pijnvezels in de spier. Hierbij wordt eveneens aan de proefpersonen gevraagd om de pijn detectie en de pijn tolerantiegrens aan te geven.

De methode van de koud water test bestaat uit een bad met koud water van ongeveer 1°C en een warm waterbad met een temperatuur van ongeveer 35°C. Proefpersonen plaatsen hun hand eerst in het bad met warm water en na een bepaalde periode plaatsen zij hun hand in het koude water. De pijn detectiegrens is de tijd waarna de proefpersonen de koude sensatie als pijnlijk ervaren. De pijn tolerantiegrens, het moment waarop de proefpersonen het koude water niet meer tolereren. Hierna wordt de hand uit het water verwijderd. De koud water test wordt ook gebruikt om geconditioneerde pijn modulatie te induceren. Geconditioneerde pijn modulatie is het principe waarbij een pijnlijke prikkel op een bepaald lichaamsdeel ervoor zorgt dat pijnlijke prikkels naar andere lichaamsdelen verminderd doorkomen. Deze geconditioneerde pijnmodulatie is een natuurlijke respons van het lichaam om de pijnwaarneming bij te sturen. Dit proces wordt aangestuurd door het centraal zenuwstelstel en maakt onderdeel uit van de endogene pijn aansturing van het lichaam.

Bij de hitte stimulatie test wordt pijn geïnduceerd door een thermode op de rug te bevestigen. De temperatuur van de thermode loopt langzaam op. Aan de proefpersonen wordt gevraagd om aan te geven wanneer de hitteprikkel als pijnlijk wordt ervaren. De UV test is een toevoeging aan de hitte stimulatie test. Hierbij wordt een stuk huid van de rug van 3 x 3 cm bestraald met UV licht. Dit zorgt voor een verbranding van de huid (zonnebrand). Deze verbranding veroorzaakt hyperalgesie en een verlaging van de detectie grens voor warmte pijn. Het UV model wordt gebruikt als model voor inflammatoire pijn.

Dit proefschrift beschrijft de validatie van deze batterij van pijnmodellen, het gebruik van de batterij in verschillende groepen en het gebruik van de batterij bij de ontwikkeling van nieuwe geneesmiddelen. De belangrijkste vraag van dit proefschrift is of de batterij gebruikt kan worden als biomarker voor klinische effectiviteit in de ontwikkeling van nieuwe pijnstillers.

Validatie van de PainCart

De methodologie van de afzonderlijke testen van de PainCart wordt uitgebreid beschreven in **hoofdstuk 2**. **Hoofdstuk 3** beschrijft een studie waarin de validatie van de pijntest batterij wordt uitgevoerd. Deze studie bestaat uit twee delen. In het eerste deel heeft een groep van 16 gezonde proefpersonen op vier verschillende studiedagen een eenmalige intraveneuze dosering van fentanyl, fenytoïne, (S)-ketamine en placebo gekregen. In deel 2 kregen 16 andere gezonde proefpersonen een orale dosering imipramine, pregabaline, ibuprofen en placebo. In dit hoofdstuk wordt aangetoond dat de pijntest batterij in staat is verschillen in pijn detectie en pijn tolerantie na toediening van verschillende klassen pijnstillers te detecteren. Wij ontdekten dat middelen met verschillende werkingsmechanismen een uniek uitkomstenprofiel vertonen op de verschillende pijntesten.

Het effect van nieuwe en bestaande pijnstillers op de PainCart

De PainCart kan worden gebruikt om de pijnstillende effecten van zowel bestaande als nieuwe geneesmiddelen te onderzoeken. Een van de onderzochte nieuwe stoffen is Xen2174. Xen2174 is een klein eiwit, dat is afgeleid van het gif van een zeeslak die leeft in de Great Barrier Reef in Australië. Dit eiwit bindt aan de norepinefrine transporter en zorgt voor remming van de heropname van norepinefrine in het zenuwstelsel. Dit versterkt de dempende werking die de hersenen uitoefenen op de pijnprikkels die vanuit het lichaam naar de hersenen lopen. De studie, die wordt beschreven in **hoofdstuk 4**, is uitgevoerd om het farmacokinetische en farmacodynamische profiel van Xen2174 te onderzoeken. Hiervoor hebben 25 proefpersonen, in een gerandomiseerde, dubbelblinde studie, een intrathecale toediening van een dosering Xen2174 of placebo gekregen. Vervolgens is in de 32 uur na toediening liquor (ruggenmergvloeistof) afgenomen door middel van een intrathecale catheter. De farmacodynamische metingen zijn gedaan door middel van de PainCart. Deze studie heeft laten zien dat de hoge dosis van Xen2174 de pijndrempels van een aantal pijntesten gunstig kan beïnvloeden. Tijdens dezelfde studie bleek echter ook dat de concentraties van het middel in het liquor hoger waren dan de vooraf vastgestelde veiligheidslimiet. Het is dan ook onwaarschijnlijk dat dit middel verder ontwikkeld zal worden voor de behandeling van pijn. Wel toont deze studie aan dat het uitvoeren van pijntesten en het doen van liquor afnames na toediening van een geneesmiddel via een ruggenprik goed mogelijk is.

Van sommige pijnstillers is bekend dat ze elkaars werking versterken indien ze in combinatie met elkaar worden gebruikt. Zo is in dierstudies aangetoond dat wanneer milnacipran in combinatie met buprenorfine wordt toegediend, zij elkaars werking versterken. Buprenorfine is een potente opioïd receptor agonist. Milnacipran is een serotonine norepinefrine heropname remmer. In **hoofdstuk 5** wordt een gerandomiseerd, dubbelblind, cross-over onderzoek beschreven waarin het effect van milnacipran in combinatie met buprenorfine wordt onderzocht in een groep gezonde mannen. De effecten van beide middelen, apart en in combinatie, op pijn werden onderzocht met behulp van de PainCart en op een aantal functies van het zenuwstelsel (zoals stabiliteit, oogbewegingen, pupilgrootte en aandacht) met behulp van de NeuroCart testbatterij. Na toediening van buprenorfine werd een dosis-afhankelijke toename in pijnstilling waargenomen. Na toediening van alleen milnacipran werd een toename van de pupilgrootte geconstateerd, maar was er geen effect op pijndrempels of op de andere testen. Uit dit hoofdstuk kan worden geconcludeerd dat buprenorfine en milnacipran tezamen in deze studieopzet geen versterkend effect op pijn hebben.

Paracetamol is een van de meest gebruikte pijnstillers ter wereld. Niet alleen in de volwassen populatie, maar ook bij jongeren. In **hoofdstuk 6** wordt een onderzoek beschreven waarin paracetamol werd toegediend aan een groep adolescenten (16- en 17-jarigen). Hierbij werd gekeken naar de effecten van paracetamol op de pijntest batterij en werden de concentraties van paracetamol in het speeksel gemeten. Aan de adolescenten werd aan het eind van de studie gevraagd hoe zij het onderzoek hadden ervaren. De resultaten van het onderzoek toonden aan dat paracetamol in vergelijking met placebo bij deze populatie geen statistisch significant effect had op de PainCart metingen. Wel kan worden geconcludeerd dat het uitvoeren van pijntesten in een groep minderjarigen goed uitvoerbaar is. Het merendeel van de adolescenten zou opnieuw deelnemen aan een dergelijk onderzoek.

Het gebruik van de PainCart in patiënten

De studies beschreven in de vorige hoofdstukken werden uitgevoerd in gezonde proefpersonen. **Hoofdstuk 7** beschrijft een onderzoek waarin de PainCart werd gebruikt in een groep patiënten met lage rugpijn, uitstralend naar een van beide benen. In dit onderzoek werd BG00010 onderzocht. BG00010 is een neurotrofe stof; een eiwit dat zorgt voor een herstel en groei van zenuwcellen. Dit middel wordt ontwikkeld als mogelijke therapie voor neuropathische pijn. Het onderzoek beschreven in dit hoofdstuk was het tweede onderzoek waarin dit middel aan mensen werd gegeven. Het vaststellen van de farmacokinetiek in het bloed en van het effect van het middel op pijn waren de belangrijkste onderzoeksdoelen. De studie was gerandomiseerd, dubbelblind, en placebo-gecontroleerd. In het onderzoek werd aan de patiënten in totaal 3 doseringen van BG00010 toegediend. In totaal namen 28 patiënten aan het onderzoek deel. Bij hogere doseringen van het middel werd een afname in pijnklachten waargenomen, maar er was geen duidelijk dosis-gerelateerd effect. De bijwerkingen die door de patiënten het meest gerapporteerd werden, waren jeuk, hoofdpijnklachten en een warm gevoel na toediening. Er werd geen duidelijk effect gevonden van het middel op de testen van de PainCart. Op basis van dit onderzoek kan worden geconcludeerd dat BG00010 in de toekomst mogelijk een therapie zou kunnen zijn voor de behandeling van neuropathische pijn. In deze

studie kon geen relatie tussen de effecten van BG00010 op klinische pijn en de effecten van dit middel op de PainCart worden aangetoond.
Behalve in het onderzoek beschreven in hoofdstuk 7, werd de PainCart vaker gebruikt om de pijn drempel en pijn tolerantie te onderzoeken in patiënten. Naast patiënten met lage rugpijnklachten uitstralend naar het been, werd de batterij gebruikt in patiënten met diabetes mellitus, met pijnlijke diabetische neuropathie (PDN) en met chronische idiopathische axonale neuropathie (CIAP). **Hoofdstuk 8** beschrijft een analyse waarin de pijn detectie drempel en pijn tolerantie drempel in deze patiënten groepen werd vergeleken met gezonde proefpersonen. Voor deze analyse werden de resultaten van zeven PainCart gebruikt. De pijn detectie grenzen voor elektrische pijn bleken lager in patiënten met PDN en CIAP. De pijn tolerantie gemeten met de koud water test en de drukstimulatie test waren lager in patiënten met diabetes en CIAP in vergelijking met gezonde proefpersonen. Deze analyse laat zien dat er verschillen bestaan in de PainCart metingen tussen gezonde proefpersonen en patiënten. Uitkomsten gevonden in gezonde proefpersonen kunnen dus niet zo maar geëxtrapoleerd worden naar patiënten met chronische pijn.

De PainCart als biomarker

Om een bepaalde meting als biomarker te kunnen gebruiken moet deze aan een aantal voorwaarden voldoen;

» Er moet een consistente respons zijn van de biomarker tussen verschillende onderzoeken en tussen geneesmiddelen met hetzelfde werkingsmechanisme.
» De biomarker moet een duidelijke respons hebben op therapeutische doseringen van een middel (en niet op subtherapeutische doseringen).
» Er moet sprake zijn van een dosis-respons of een concentratie-respons relatie.
» Er moet een logische relatie zijn tussen de biomarker, de farmacologie van het onderzoeksmiddel en de pathofysiologie van de ziekte.

Aangezien de PainCart voldoet aan al deze vier criteria, kan geconcludeerd worden dat de PainCart als biomarker gebruikt kan worden om de farmacodynamische respons van pijnstillers te onderzoeken. Daarbij is het belangrijk dat één enkel pijnmodel niet gebruikt kan worden om het effect van een pijnstiller te onderzoeken. Het is de combinatie van pijntesten die de PainCart bruikbaar maakt als biomarker.

Vervolgens dient de volgende vraag zich op; indien van een bepaald middel een pijnstillend effect wordt aangetoond met behulp van de PainCart, beïnvloedt dit middel dan ook de spontaan optredende pijn bij patiënten? Er bestaan diverse overzichtsartikelen die deze vraag hebben onderzocht. Het lijkt zo te zijn dat een aantal pijntesten goed voorspellend zijn voor klinische effectiviteit. Een aantal van deze modellen maakt deel uit van de PainCart. Dit zijn de hitte pijntest in combinatie met de UV inflammatie, drukkende pijntest en de elektrische stimulatie test. Het eerder gepubliceerde artikel suggereert dat de PainCart in sommige gevallen klinische effectiviteit kan voorspellen. Het is echter belangrijk te beseffen dat deze overzichtsartikelen alleen indirect bewijs tonen voor deze relatie. Voor direct bewijs zou men nieuw vergelijkend onderzoek in patiënten moeten uitvoeren. Daarnaast kan de voorspellende waarde van de PainCart worden bewezen als middelen, waarbij met de PainCart een pijnstillend effect is aangetoond, in latere klinische studies in patiënten ook effectief tegen pijn blijken.

Toekomst van de PainCart

Een groot aantal verschillende pijnmodellen wordt gebruikt in de studies die in dit proefschrift worden beschreven. Het ene model is gevoeliger voor het aantonen van pijnstilling dan het andere model. In **hoofdstuk 3** wordt bijvoorbeeld de 'thermal grill' pijntest gebruikt, maar werden geen duidelijke effecten van de toegediende pijnstillers op deze test gevonden. In de literatuur blijkt dat veel verschillende onderzoeksgroepen net iets andere meetmethoden voor deze test gebruikten. Daarnaast wordt deze test per proefpersoon net iets anders ingesteld. Om deze redenen concludeerden wij dat dit pijnmodel minder geschikt is als model binnen de PainCart.

De PainCart bevat een methode voor het onderzoeken van geconditioneerde pijn modulatie. Ondanks dat deze meting in alle hoofdstukken wordt uitgevoerd, wordt in bijna geen van de studies een effect aangetoond met deze meting. Een van de redenen is de hoge variabiliteit van de meting binnen individuele proefpersonen en tussen de proefpersonen. Andere onderzoeksgroepen vinden wel regelmatig verschil in geconditioneerde pijn modulatie, maar zij gebruiken vaak een net andere meetmethode dan beschreven in dit proefschrift. De huidige methode van de PainCart om geconditioneerde pijn modulatie te meten zal dan ook kritisch moeten worden geëvalueerd en eventueel worden vervangen door een andere methode. De PainCart bestaat voornamelijk uit nociceptieve en inflammatoire pijn-

modellen. De weefsels die geprikkeld worden zijn huid en spieren. Hierin zou de PainCart kunnen worden uitgebreid. Er bestaan modellen die pijnprikkels kunnen toedienen aan de interne organen (bijvoorbeeld slokdarm en endeldarm). Toevoeging van een orgaanmodel zou een bredere uitkomst opleveren wanneer de mogelijke pijnstillende effecten van een middel op pijn aan de ingewanden worden onderzocht. Een nadeel van dergelijke modellen is echter de invasiviteit van de meting en de ongemakkelijkheid voor de proefpersoon.

Daarnaast ontbreekt in de PainCart een model voor neuropathische pijn. Een lastig aspect hiervan is dat neuropathische pijn een complexe aandoening is, die een grote groep aan verschillende syndromen bevat. Deze verschillende syndromen hebben als gemeenschappelijke overeenkomst dat er schade of ziekte is aan een onderdeel van het sensibele zenuwstelsel. Uiteindelijk is het met pijnmodellen alleen mogelijk om symptomen van neuropathische pijn na te bootsten. Zo kan bijvoorbeeld met intradermale injectie van capsaïcine, de pitte stof in rode pepers, centrale sensitisatie worden nagebootst.

Bovenstaande voorbeelden geven aan dat continu kritisch moet worden gekeken naar de afzonderlijke testen van de PainCart. Voegt een bepaalde meting nog wel iets toe? Is er misschien een andere meetmethode beschikbaar? De PainCart is geen statische methode. Het is een dynamisch model waarin continu gezocht wordt naar verbeteringen van de methodologie.

Conclusie

Eerder is al benoemd dat potentieel nieuwe pijnstillers kunnen worden ontwikkeld door bestaande aangrijpmechanismen te verbeteren of door nieuwe aangrijpmechanismen te ontdekken vanuit fundamenteel (geneesmiddelen)onderzoek. In dit proces kan de PainCart zijn nut bewijzen. Als een studie wordt uitgevoerd met een nieuwe stof, waarbij een bestaand aangrijpingsmechanisme is verbeterd, kan het reeds bestaande geneesmiddel met een vergelijkbaar werkingsmechanisme worden gebruikt als positieve controle. Indien een nieuw mechanisme wordt onderzocht, kan de PainCart worden gebruikt als screeningsmiddel van de pijnstillende activiteit en kunnen de uitkomsten gebruikt worden om de plaats te bepalen van dit nieuwe middel ten opzichte van reeds bestaande pijnstillers.

In 1998 bracht de Europese Geneesmiddelen Autoriteit een richtlijn uit met de titel ‘een richtlijn voor algemene overwegingen voor klinische trials’. In de inleiding hiervan staat: ‘de essentie van rationeel geneesmiddelenonderzoek is om belangrijke vragen te stellen en deze te beantwoorden met geschikte studies’. In dit proefschrift, is de batterij van pijnmodellen in staat om enkele van deze belangrijke vragen te beantwoorden in vroege-fase-geneesmiddelenonderzoek. Ook al bestaat nog ruimte om de methodologie verder te ontwikkelen, de PainCart kan in dit stadium gebruikt worden om de pijnstillende effecten van nieuwe middelen te onderzoeken. De gevonden resultaten kunnen worden vergeleken met de effecten die reeds bestaande pijnstillers eerder vertoonden op de PainCart.

CURRICULUM VITAE

Petrus (Pieter) Okkerse was born on 7 June 1985 in Rijnsburg, the Netherlands. After completing secondary school at Visser 't Hooft Lyceum in Leiden in 2003, he studied medicine at Leiden University. In 2007 he temporarily halted his study to become chairman of the board of the medical student association (M.F.L.S.). In 2009 he received his doctoral degree and in 2010 he received his qualification as Medical Doctor (*cum laude*). He started working as research physician at the Centre for Human Drug Research in the field of neurology and pain in 2011. During this period the research described in this thesis was performed under supervision of G.J. Groeneveld and prof. A.C. Cohen. In 2015 he started his residency in anesthesiology at the LUMC under supervision of prof. L.P.H.J. Aarts. In 2016 he completed his registration as clinical pharmacologist. Pieter lives together with his partner Carlijn.

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