INTEGRATED ASSESSMENT OF NEUROCOGNITIVE, NEUROPHYSIOLOGICAL AND PAIN PROCESSING IN EARLY CLINICAL DRUG DEVELOPMENT

Guido van Amerongen

Opgedragen aan mijn schoonmoeder:
Elisabeth Engelina Maria (Els) de Greef
1953 - 2020

INTEGRATED ASSESSMENT OF NEUROCOGNITIVE, NEUROPHYSIOLOGICAL AND PAIN PROCESSING IN EARLY CLINICAL DRUG DEVELOPMENT

Proefschrift ter verkrijging van de graad van
Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 20 oktober 2020
klokke 15:00 uur

Door Guido van Amerongen
geboren te Leidschendam in 1986

Promotores
Prof. dr. J.M.A van Gerven
Prof. dr. A.F. Cohen

Co-promotor
Dr. G.J. Groeneveld

Leden promotiecommissie
Prof. Dr. H.J. Guchelaar
Dr. G.E. Jacobs
Prof. Dr. M.A.H. Steegers (Amsterdam University Medical Center)
Em. Prof. dr. M. de Visser (Amsterdam University Medical Center)

DESIGN
Caroline de Lint, Voorburg (caro@delint.nl)

The publication of this thesis was financially supported by the foundation Centre for Human Drug Research in Leiden, the Netherlands.

CHAPTER 1

INTRODUCTION

In spite of the advances made since the description of willow bark to induce pain relief by Hippocrates in ancient Greece, many millions worldwide today still suffer from untreated pain. (Verhaak et al., 1998; Dureja et al., 2014; Breivik et al., 2006) Chronic pain has a negative influence on an individual's wellbeing and may be associated with psychiatric disorders including mood disorders and anxiety disorders (Tsang et al., 2008). (Racine, 2018) The unmet medical need pushes clinical drug development for the treatment of pain into two directions. The first direction is the development of new compounds, which can be further subdivided into refinements of existing and first-in-class compounds. This direction is quite rare – especially completely novel concepts – illustrating the many challenges that are faced in the development of a novel pain therapy. The second direction is to repurpose already marketed pharmacological agents. Some eminent examples are the antihypertensive clonidine, the anticonvulsant pregabalin, and the tricyclic antidepressant amitriptyline. These two potential avenues in analgesic drug development illustrate the spectrum of potential pharmacological targets to treat pain: from highly specific sodium channels exclusive for the nociceptive system, to largely serendipitous effects of serotonin and norepinephrine re-uptake blockage and Gamma-Aminobutyric acid (GABA) modulation. This extensive assortment of potential targets throughout the central and peripheral nervous system provides many opportunities, but is also a peril in finding new effective and safe treatments against chronic pain.

Nociception versus pain

Pain serves an evolutionary purpose as the body's warning system for any potential physical threat. Since the threat of injury may require anticipatory or immediate conscious actions, the system is inherently connected to affect and cognition. This results in different dimensions of pain to be distinguished. The pain cascade is initiated within the *nociceptive* system.

In 1664, René Descartes laid the foundation for a mechanical approach to a specialised pain system in humans, which was further developed in the nineteenth century as a distinct sensory system responding to high intensity sensory input. In the twentieth century understanding of the pain system deepened with the introduction of functional neurophysiological and neuroimaging techniques including evoked potentials, fMRI and PET.

This physiological nociceptive mechanism is defined by the transduction of a noxious signal at the peripheral sensory nerve terminal, generating an action potential after the activation threshold is reached. This signal is subsequently transmitted via nociceptors, of which there are two main subtypes. Myelinated Aδ fibres transmit signals fast and respond to weaker stimuli. The unmyelinated C fibres respond to stimuli of stronger intensity and provide slower longer lasting signalling.

The nociceptive signal is transmitted onto the dorsal horn of the spinal cord where the peripheral nerve afferent synapses into a dorsal horn neuron. From here the signal can be transmitted higher up to the central nervous system. At this level, many different

neurotransmitters have been identified and implicated to play a role in modulation of the action potential; these include excitatory (glutamate) and inhibitory (GABA, glycine) neurotransmitters, neuro-active peptides (e.g. Substance P, Calcitonin gene-related peptide [CGRP], nociceptin), opioid peptides (enkephalins and dynorphins), and various other biogenic amines (serotonin, dopamine, norepinephrine, acetylcholine, ATP, nitric oxide, etc).

Depending on which type of nociceptor is activated, the pain signal is then transmitted supraspinally, predominantly via the ascending pathways in the spinoreticular and spinothalamic tracts. These project into the medulla, brain stem, periaqueductal grey (PAG) and thalamus; structures that are involved in *modulation* of nociceptive transmission.

Within the nociceptive system there are two modulatory mechanisms involved in transmission, facilitation and inhibition, which are in equilibrium in the healthy state. Where *facilitation* is crucial for early and anticipatory detection of pain signals, regulation through *descending inhibition* is as important for an adaptive sensory system. The theoretical framework for this modulatory system was proposed in 1911 (Head and Holmes, 1911) and experimentally confirmed in the following years. This framework was expanded with the theory of gate control, approximately half a century later. (Melzack and Wall, 1965) Brain regions that have been identified to play a role in pain modulation are highly inter-related with the cognitive-affective dimensions of pain perception, which comprise for example the PAG, amygdala, hypothalamus, components of the anterior cingulate cortex, the insula and orbitofrontal the dorsolateral prefrontal cortex.

The aggregate of signalling within the sensory-discriminative and cognitive-affective systems results in pain *perception*: the subjective experience of becoming aware of a signal from the nociceptive system. It is the culmination of sensory and emotional input, and involves different cognitive aspects including casual attribution, appraisal, attention, context-specific meaning making, memory, etc. It is dependent not only on factors within the human body, but also on external factors including culture, upbringing, situation and numerous other factors.

Clearly, this unpleasant sensation is the most relevant aspect of the entire cascade for an individual patient, yet it is the most elusive.

Pain as symptom or as disease

There is a clear distinction between acute and chronic pain. Where acute pain is typically a symptom of an underlying pathophysiological source, chronic pain can to some degree be considered to be the disease itself. (Treede et al., 2019) Chronic pain persists for more than six months and results from a dysregulation within the nociceptive system. The International Association for the Study of Pain has classified chronic pain for the International Classification of Diseases (ICD-11) as a disease or symptom with either chronic primary pain syndromes (e.g. fibromyalgia, complex regional pain syndromes, irritable bowel syndromes, non-specific low-back pain, chronic migraine), or chronic

secondary pain syndromes (cancer-related pain, posttraumatic pain, musculoskeletal and neuropathic pain). (Treede et al., 2019)

In the case of neuropathic pain, a lesion secondary to the primary illness develops within the nociceptive system. Dependent on the localisation and type of the lesion in the nervous system this may induce different symptoms, which are either spontaneous (e.g. burning, cold, paraesthesia, hypoesthesia) or an extreme reaction to external stimuli. Sensitisation to evoked stimuli can result in the phenomena *allodynia* or *hyperalgesia*. Allodynia is the sensation of pain resulting from a low intensity stimulus due to hyperexcitability and lowered activation thresholds of the nociceptors, which in the healthy state would not induce pain. Hyperalgesia is a heightened pain sensation to a normally painful stimulus resulting from increased afferent firing after activation of a nociceptor. This phenomenon transpires not only in the context of neuropathic pain but also during localised inflammation of dermal tissue, for example after sunburn. This is known as *peripheral sensitisation* and results in increased sensitivity to thermal and mechanical stimulation at the primary area of injury.

When the lesion within the nociceptive system is located at the level of the dorsal horn or higher, *central sensitisation* may occur. This may not only lead to sensitisation in the primary area, but also to referred pain or secondary hyperalgesia or allodynia, i.e. sensitisation in an unaffected area. The exact underlying mechanisms are not fully understood, but different types of sensitisation occur. The activation thresholds to nociceptive afferents decreases, but also their receptive field increases to a level that even non-nociceptive (i.e. Aβ mechanoreceptors) can activate a nociceptive pathway. In the healthy state this protective mechanism ensures that damaged tissue is actively protected. However, in the pathological state this may result in (extremely) painful sensation to light touch of large body surfaces.

Precision pharmacology

Due to its multifaceted character, the treatment of chronic and neuropathic pain is a prototype indication where precision pharmacology should be applied. This refers to both clinical practice at bedside but also to drug development at large.

According to the Precision Medicine Initiative, precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person." (NIH) As a successor to "personalised medicine", the term precision medicine describes the use of various factors to categorise patients into accurate subcategories, thereby enabling evidence-based decision-making. Reversed, the failure to make the correct distinction between individual patients or groups of patients, may lead to errors in the selection of a treatment or treatment regimen.

Precision pharmacology requires an integrated approach that combines mechanistic information and clinical pharmacology in order to address inter-individual variability and to define subtypes where applicable.

Complex pain demands complex treatment

In 1986 the World Health Organisation (WHO) introduced the analgesic stepladder for cancer pain. (WHO, 1986) These recommendations have been the blueprint for the treatment of cancer pain but also other types of pain. The ladder that is proposed is a sequential list of different types of therapies that can be used. If a drug in the recommended dose regimen is not effective, a drug from the next group should be administered. Adjuvant drugs may be used as described. If a drug ceases to be effective, an alternative that is definitely stronger should be used instead. The steps of the ladder are as follows:

1. Non-opioids (e.g. aspirin, paracetamol, NSAIDS*
2. Weak opioids (e.g. codeine, hydrocodone*, oxycodone*, tramadol*,)
3. Strong opioids (e.g. morphine, buprenorphine, meperidine, hydromorphone, fentanyl, methadone, tapentadol)

** Not introduced in the initial ladder in 1986.*

Each step may be accompanied with adjuvant treatment (e.g. anticonvulsants, anxiolytics, antidepressants, gabapentinoids, NMDA receptor antagonists, steroids) Over time, this ladder, which was intended for terminally ill cancer patients, served as a standard to treat other persistent pains as well, even in the absence of evidence that this treatment is in fact reducing pain in the long term. For example, the effectiveness on quality of life of opioid therapy in the treatment of chronic pain is inconclusive (Noble et al., 2010), even though the risks and reduction in quality of life associated with opioid use are widely known. Although the ladder provides valid guidance in the treatment of acute and end of life pains, this is not necessarily the case for chronic pain, due to its unpredictable and capricious disease course. Neuroimaging studies have confirmed the clinical observation that in chronification of pain, plastic changes in cortical-limbic structures occur, inducing a shift from a somatosensorial experience to an affective experience. (Mansour et al., 2014) These affective components express into altered mood, anxiety, stress and a subsequent reduction in quality of life and doubling of suicide risk. (Racine, 2018) Consequently, the *perception* of pain, taking into account not only somatosensory but also the affective components, is the true target in the treatment of chronic pain.

Developing treatment that targets pain perception

The treatment of acute pain is characterised by symptom control, but chronic pain requires a more refined therapeutic approach. Due to the heterogeneity of chronic pain conditions and the abundance of potential pharmacological targets that involve most of the nervous system from peripheral nerves to higher brain centres, identifying new treatments that will be effective in large groups of different patients is challenging. It has been argued that clinicians today are not much more advanced than they were 20

years ago, in their capacity to either effectively treat or even diagnose their patients. (Vardeh et al., 2016) Apart from the hiatus in mechanistic understanding of underlying pathophysiology, the fact that the potential pharmacological targets are distributed throughout the entire nervous system creates a challenge in terms of the occurrence of undesirable effects resulting from either primary or secondary pharmacological action.

All these factors combined create the framework in which drug development for new therapies for chronic and neuropathic pain takes place. This is a complicated area where the chance of a "quick win" is minimal. However, given the disease burden of untreated and undertreated pain, there is a high need for effective analgesic drug development, for which the large diversity of targets provides ample opportunity. To treat pain perception, especially in the case of chronic neuropathic pain, it is crucial to approach the central nervous system in its totality. Although many peripheral causes of pain can be treated with single targeted pharmacological interventions, the most effective treatments for neuropathic pain do not target highly specific receptors, but aim to modulate the perception of pain at different levels in the cascade. Central pain modulation is the focus of this thesis, which mainly deals with two of the most widely distributed targets in the central nervous system. Cannabinoid and GABAergic transmission plays an important role in pain perception, but also in many other CNS functions, warranting an integrated approach. We will now present these two neurotransmitter systems as potential targets for the treatment of neuropathic pain, and subsequently proceed with a description of methods to measure their diverse effects.

CANNABINOID RECEPTORS

The two most important cannabinoid (CB) receptors are CB1 and CB2. As CB2 is predominantly found in the immune system, this thesis focuses on the CB1 receptor, which is distributed throughout the nervous system. These G-protein coupled receptors (GPCR) are chiefly expressed in neurons, mostly presynaptical. They function in modulating the release of different neurotransmitters including GABA, dopamine, noradrenaline, glutamate and serotonin. (Schlicker and Kathmann, 2001)

Anatomically, they are expressed in various brain regions: the cortex, basal ganglia, hippocampus, cerebellum, thalamus, amygdala, periaqueductal grey and medulla oblongata. Many of these regions are known to play a role in pain perception. Furthermore, CB1 receptors are found spinally, primarily in the interneurons of the dorsal horn. (Mackie, 2005). These receptors have not only been identified centrally, but are also expressed in the peripheral nervous system. (Hohmann and Herkenham, 1999; Veress et al., 2013) CB1 receptors are abundantly present in the sympathetic nerve endings and the peripheral nociceptive nerve fibres, mostly in the myelinated Aδ fibres, but also in a limited number of C fibres. (Bridges et al., 2003)

Apart from the two principal endogenous ligands for the cannabinoid receptors, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), other endocannabinoids include noladin ether, virodhamine, and N-arachidonoyldopamine (NADA). Unlike most other neurotransmitters, endocannabinoids are not stored in presynaptic vesicles, but are

synthesised and released from the cell membrane responding to physiological or pathological stimulation, including noxious stimuli. (Walker et al., 2002; De Petrocellis et al., 2004) The main psychoactive ingredient of *Cannabis sativa*, Δ9-tetrahydrocannabinol (Δ9-THC) is the most widely known exogenous ligand for the CB1 (and CB2) receptors.

The CB1 receptor does not only respond to different endogenous ligands, but these ligands (AEA and 2-AG) also have also been reported to interact with other receptors, in particular the Transient Receptor Potential (TRP) channel family, of which the TRPV1 is widely recognised for its role in (heat) pain sensation and regulation, and pathological pain states. (Ahluwalia et al., 2003)

As a result of its widespread distribution, the endocannabinoid system is associated with the regulation of a myriad of functions, e.g. learning, memory, mood, appetite, sleep, neuroprotection, gastrointestinal motility and pain perception. This wide spectrum of functions makes it an interesting and challenging opportunity for the treatment of various pathological states. Due to its undisputed association with pain and the modulation of pain sensation, this neurotransmitter system is of interest when developing new therapies for the treatment of chronic neuropathic pain.

GABA RECEPTORS

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the Central Nervous System. Similar to the cannabinoid receptors, two receptor types have been identified for GABA: $GABA_A$ and $GABA_B$. $GABA_A$ is a pentameric ionotropic receptor, mediating fast responses by opening chloride channels in the presence of GABA. Over 20 combinations of the subunits α_{1-6}, β1-4, γ1-3, δ, ε, σ have been identified, named subtypes. This results in a high degree of heterogeneity among the functional effects exhibited by the different subtypes. $GABA_B$ is a GPCR involved in slower cascades of second messenger systems. Activation of $GABA_B$ receptors is associated with activation of adenylyl cyclase, opening potassium channels, and inhibiting calcium channels.

GABA occurs in 30-40% of all synapses, and the specific types and subtypes of GABA receptors are distributed throughout the CNS. For example, the most prevalent $GABA_A$ receptors in the brain consist of a combination of $\alpha_1/\alpha_2/\alpha_3/\alpha_5$, and β_2/β_3, and γ_1. There is only little overlap in localisation of specific subtypes, consequently subtypes are associated with specific neurophysiological functions. Apart from its abundant distribution in the brain, GABA receptors are also widely expressed in the spinal cord. The highest concentration is found in the dorsal grey and ventral grey matter. In the dorsal horn, both $GABA_A$ receptors and $GABA_B$ receptors are localised in the presynaptic interneurons. (Malcangio and Bowery, 1996)

Behavioural and pharmacological investigations, ranging from *in vitro* to preclinical and clinical research, have assigned different functional roles to the different receptor subunits of $GABA_A$. For example, α1 agonism is mostly implicated with sedation and amnesia. The subtypes containing α2 and α3 have predominantly been associated with anxiolysis (Rudolph et al., 1999). In the last decade however, their role in the modulation of pain perception has also been established. (Knabl et al., 2008) Temporal

and special memory is processed among others in the hippocampus. Experiments have demonstrated that tonic inhibition of glutamatergic input via α5 regulates learning and memory. (Collinson et al., 2002b) When targeted with an agonist, the loss of inhibitory control results in a decrease in cognitive and memory performance.

Parallel to the endocannabinoid system, the GABA system is a comprehensive system involved in an extensive range of physiological functions. Based on this physiology, there are vast opportunities to intervene, when developing a treatment for neuropathic pain. However, both CB1 and GABA are important for the fine-tuning of neuronal cellular and network connections. The challenge for the development of drugs that target these neurotransmitters for the treatment of chronic pain is therefore to prevent further disruption of any (impaired) balance in pain regulation. Since these pharmacological mechanisms play similar regulatory roles in other CNS functions, inhibition can easily lead to adverse effects. Consequently, cannabinoid or GABAergic drug development for chronic pain should always consider the impact on comprehensive pain processing, in conjunction with other neurophysiological or psychological systems. This thesis contains several chapters where integrated or combined measurements were used for dose selection and optimisation, applying multimodal batteries for pain – the PainCart® – and for drug-sensitive CNS functions – the NeuroCart®.

Human evoked pain models as biomarkers for pain

In the changing landscape of analgesic drug development, the blockbuster model is likely to come to an end. With a stronger emphasis on identifying subpopulations of those that are likely to respond well to treatment, and those with higher risk of adverse events, more effective treatments could be developed and potentially with a lower chance of failure. This approach is for example precedented in the field of oncology. The concept that genotypical, phenotypical and mechanistic understanding is a prerequisite for the development of an effective therapy of one or more pharmacological interventions is now established, on account of the extensive efforts made to understand underlying pathophysiology.

An indispensible feature of effective analgesic drug development is to gain mechanistic knowledge on the mode of action of the pharmacological agent at each step of development. This is the only way to gain true understanding of the drug's pharmacology and the potential benefit and harm for the patient. This can be expected to lead to a more efficient and cost-effective development trajectory. In order to implement this already in the early clinical phase of research, biomarkers play a crucial role. A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention". (BiomarkersDefinitionsWorkingGroup, 2001). There is an inherent chasm between research in preclinical pain models of pain and a clinical, proof-of-concept study in patients with pain (Berge, 2011; Yekkirala et al.), strongly contributing to failed clinical trials at this stage.

Incorporating biomarkers for pain in early phase clinical studies in healthy subjects and patients has the potential to bridge this gap to a certain extent. Given the complexity of the pathophysiology of pain, it is highly improbable that a single biomarker can represent pain in its totality. However, it is achievable to identify mechanism-specific biomarkers that are: consistent in their response to pharmacological intervention; respond to therapeutic doses; demonstrate a dose-response relationship; and are valid in the sense that they reflect a plausible relationship with the pathophysiology under investigation. These criteria are defined as imperative for the selection of a biomarker, in addition to validation including an assessment of its sensitivity. (Cohen et al., 2015) In this light, a multi-modal approach, as applied in the battery of evoked pain tasks, referred to as the PainCart can play an important role in phase I clinical trials in healthy subjects. Even though the clinical presentation of a patient with chronic pain cannot be mimicked in any model, the battery of evoked pain tasks could provide valuable information on the mechanism of action of an analgesic compound under investigation. If aforesaid tool is utilised in a data-intensive phase I or IIa study, in conjunction with frequent pharmacokinetic and safety assessments, it is possible to obtain a comprehensive understanding of the human pharmacology of a new or already known compound. The value of this tool lies within the perception of the researcher or sponsor. Namely, where the limited resemblance between a highly heterogeneous chronic pain patient and evoking pain in a healthy volunteer in a controlled clinical research setting may be perceived as a constraint, it is actually a strong asset for early drug development. A few advantages for example are: homogenous study population, standardised stimulus modalities, controlled intensity and duration of painful stimuli, quantitative outcomes that can be compared over time. (Arendt-Nielsen et al., 2007) Therefore, this type of tool allows to thoroughly quantify specific elements of a system that is dysregulated in the pathophysiological state.

Gaining information in clinical pharmacology early during clinical development of a pharmacological agent provides the context for decision-making in subsequent steps, but needs to be interpreted prudently with the correct context.

Multimodal measurement of pain and central nervous system functions: PainCart® and NeuroCart®

Because many types of chronic pain ultimately lead to widespread dysregulations within the pain cascade, it is likely that drugs for these conditions will also have an impact throughout the central nervous system. To aid in the development of these drugs, integrated measurements of pain and other CNS functions should be used to determine the profile of desired and undesirable effects. This approach can help identify the optimal therapeutic window, as well as secondary pharmacological effects that can be used to treat concomitant conditions like insomnia or anxiety. To this end, the Centre for Human Drug Research (CHDR) developed two test batteries: the PainCart and the NeuroCart.

The PainCart is a test battery of human evoked pain tasks, which comprises different tasks, associated with different pain modalities. The individual pain tasks have been used extensively individually in clinical research. (Okkerse, 2018) A set of complementary tasks has been combined and validated into a composite, multi-modal biomarker. The operational characteristics are described in this thesis and elsewhere. (Hay et al., 2016)

Initially, the PainCart consisted of four tasks, each targeting different modalities of pain in different tissues, localised at different areas of the body. The following modalities were included: Pressure pain, Cold pain, Electrical pain and Heat pain. However, the test battery did not include a model for hyperalgesia, which is a common symptom of neuropathic pain. Therefore the literature review described in **Chapter 2** of this thesis was performed to identify a model for hyperalgesia in humans that meets the criteria for biomarkers in early phase clinical research.

TABLE 1 Overview of tasks and pharmacodynamics endpoints included in the PainCart® described in this thesis

PainCart Task	Endpoints
Cold Pressor (s)	PDT, PTT, AUC, VAS
Electrical Stimulation (mA)	PDT, PTT, AUC, VAS
CPM: Electrical stimulation * (mA)	PDT, PTT, AUC, VAS
Pressure Stimulation (kPa)	PDT, PTT, AUC, VAS
Normal Heat (°C)	PDT
UVB Heat (°C)	PDT

**(Difference Pre-Post Cold Pressor) / PDT - Pain Detection Threshold / PTT - Pain Tolerance Threshold / AUC - Area Under the Curve / VAS - Post-test Visual Analogue Scale*

The tasks in the PainCart are performed consecutively during a measurement round. Each measurement round is performed before administration of a treatment, and then multiple times after administration of a treatment (active or placebo). To account for bias resulting from inter-individual variability in pain reporting, a crossover design is recommended, as intra-individual variability is considerably lower. Variability from affects associated with fear of pain, is reduced by making the subjects themselves responsible for starting and ending each pain task. The risk of tissue damage is eliminated, as all pain tasks have a maximum safety cut-off.

The pain tasks in the battery are complementary in the sense that different elements of the nociceptive system are stimulated, resulting in the perception of pain. The distinction between the pain tasks arises from various phases of the pain cascade. The most obvious difference is observed on the level of the peripheral sensory nerve endings. Each pain task activates the nociceptive system through a specific receptor.

The Cold pressor task excites the nociceptive cascade through activation of the TRPM8 and TRPA1 receptors in the skin (Foulkes and Wood, 2007). Heat on the other hand is transduced into an action potential after exciting the TRPV1 receptor. Hyperalgesia, when induced for instance by UVB or a thermode burn, results from a local inflammatory reaction in response to tissue damage. In this process, a broad array of inflammatory factors are released, including bradykinin, protons (H+), Nerve Growth Factor (NGF) and various others, that either modulate or directly activate TRPV1 and TRP1 channels. Apart from the peripheral sensitisation that ensues, microglia activated by CASP6 can induce a spinal component to the observed hyperalgesia via sensitisation of nociceptor nerve terminals in the dorsal horn (Guan et al., 2016). Consequently, the activation threshold of thermal and mechanical nociceptors decreases in the injured area, causing allodynia and hyperalgesia. (Gustorff et al., 2004) The Pressure pain task does not activate superficial cutaneous mechanoreceptors, but reaches deeper high threshold mechanoreceptors in the muscle tissue. Muscle pain mainly originates from deep tissue group III and IV afferents. (Graven-Nielsen et al., 2004) In contrast with the other pain tasks, the Electrical Pain task bypasses the peripheral receptors and directly stimulates the nociceptive nerve.

TABLE 2 Overview of tasks and pharmacodynamics endpoints included in the NeuroCart® described in this thesis

NeuroCart Task	Endpoints
Saccadic eye movement	Saccadic Peak Velocity (deg/sec)
Smooth pursuit	Proportion in smooth pursuit (%)
Adaptive tracking	Average performance (%)
Body sway	Anteroposterior movement (mm)
VAS Bond and Lader	VAS Alertness (mm) VAS Mood (mm) VAS Calmness (mm)
VAS Bowdle	VAS Internal perception (mm) VAS External perception (mm) VAS 'Feeling High' (mm)
Visual Verbal Learning Test (VVLT)	Immediate recall: Number correct Delayed recall: Number correct Delayed recognition: Number correct
Pharmaco-EEG	Alpha Fz-Cz , Pz-Oz Beta Fz-Cz, Pz-Oz Delta Fz-Cz, Pz-Oz Theta Fz-Cz, Pz-Oz

VAS = Visual Analogue Scale

The noxious stimulus is transduced into an action potential that is transmitted via nociceptive nerve fibres. The Aδ fibres are responsible for the fast, immediate sensation of pain, whereas the C fibres cause the more dull, prolonged sensation of pain. Aδ fibres are specific for thermal and mechanical stimulation, and C fibres can also be polymodal. Cold pain is known to be mediated via specific subpopulations of Aδ fibres (Simone and Kajander, 1997) and C fibres, (Campero et al., 2009). Heat pain is initially mediated via Aδ fibres, but hyperalgesia resulting from the UVB model is thought sensitise both Aδ fibres and C fibres, both peripheral and centrally. (Neumann et al., 1996)

The NeuroCart, a multimodal cognitive and neurophysiological test battery, was also developed at CHDR. This test battery has been used extensively to characterise the specific, time- and dose-dependent, neurophysiological and/or neuropsychological effects of a compound, thereby confirming whether the compound reaches its intended target in the central nervous system. (Groeneveld et al., 2016) The NeuroCart consists of a diverse selection of validated tasks which provide information on various functional domains of the central nervous system and the effects of pharmacological agents thereon. In its full scope, the following functional domains are included in the NeuroCart: (visuo)motor coordination, alertness, memory, subjective drug effects and neurophysiological brain activity (electroencephalography). In this thesis, the test battery is limited to the tasks that have previously been demonstrated to be sensitive to cannabinoids and GABAergic compounds. Over the years, CHDR has gained extensive experience in assessing the pharmacodynamics of these agents with the NeuroCart.

Cannabinoids were the topic of two CHDR dissertations. Lineke Zuurman examined a newly developed standardised mode of intrapulmonary administration of Δ9-THC, which was thoroughly investigated with the NeuroCart in healthy subjects. (Zuurman, 2008) These studies provided reliable pharmacokinetic-pharmacodynamic models for a range of concentration-dependent CNS effects, in particular postural instability and visual analogue scales for alertness (Bond& Lader) and psychomimetic effects (Bowdle), but not eye movements or adaptive tracking. In a subsequent thesis, Linda Klumpers used these method validations to quantify the inhibitory effects of a range of selective CB1-antagonists. (Klumpers, 2014) Klumpers also published the first results with a novel oral formulation of Δ9-THC (Namisol®) in healthy volunteers. (Klumpers et al., 2012) In the current thesis the effects of this compound are described in **Chapters 3 and 4**. Chapter 3 describes a clinical trial in which an oral formulation of Δ9-THC is investigated in a four-week, placebo-controlled, proof-of-concept trial in a population of patients with progressive (primary or secondary) multiple sclerosis, suffering from spasticity in at least one of the lower limbs. Both objective and subjective endpoints for spasticity and pain, as well as objective and subjective endpoints for sedation and postural instability were included as endpoints to determine the efficacy of the therapy in relation to its undesirable effects. The mode of action of Δ9-THC in the treatment of pain is further characterised in healthy volunteers, as reported in **Chapter 4**. Here, the PainCart was utilised to determine the pharmacodynamic effects of an oral formulation of Δ9-THC and

oral paracetamol, compared with placebo and the negative control promethazine. In conjunction with the pain tasks, subjective effects were measured using the NeuroCart.

At CHDR, a considerable number of both nonselective and subtype-selective $GABA_A$ modulators have been examined in healthy subjects, using the NeuroCart, several of which were described in the thesis by Xia Chen (2017). (Chen, 2017). These studies consistently present a similar pattern in the pharmacodynamic effect profiles of α2/α3 subtype selective $GABA_A$ receptor modulators. In these studies, the subtype selectivity was demonstrated by the presence of significant treatment effects on tasks representative for specific $GABA_A$ receptor subtypes compared with the pharmacodynamic effect profile of nonselective positive allosteric modulators of the $GABA_A$ receptor. For example, a reduction in saccadic peak velocity (the primary endpoint in the task measuring saccadic eye movements) has been associated with modulation of the α2/α3 subunits of the $GABA_A$ receptor (Atack, 2010), whereas performance on adaptive tracking, subjective alertness and postural instability (body sway) has been related to α1 modulation (de Haas et al., 2010). Cognition and memory impairment as measured with the VVLT have been shown to be associated with α5 modulation (Collinson et al., 2002a; Crestani et al., 2002). When the findings of subtype selective $GABA_A$ receptor modulators were compared with the non-selective benzodiazepine lorazepam, a distinct fingerprint was observed. Building on this knowledge, **Chapter 5** presents a First-in-Human study in which the effects of PF-06372865, a novel α2/α3/α5 subtype-select partial $GABA_A$ positive allosteric modulator were characterised using the NeuroCart. Using an intricate study design, a wide dose range was explored as well as a head-to-head comparison of PF-06372865 alone and in combination with a positive control, lorazepam.

Even though the $GABA_A$ receptor system has been recognised for its role in the perception of pain, the clinical use of GABAergic therapies for the treatment of pain is very limited due to the sedative effects associated with their use. However, after establishing the clinical pharmacodynamic effect profile of PF-06372865, the analgesic potential also warrants further investigation. Due to its confirmed selectivity for the α2/α3/α5 $GABA_A$ receptor subtypes, PF-06372865 is a potential novel therapy in the treatment of (chronic) neuropathic pain, with a lower risk of sedation. This analgesic potential is investigated in **Chapter 6** of this thesis. In this study, the PainCart was used to investigate the analgesic effect profile of two pharmacologically active dose levels of PF-06372865, in a crossover study in healthy subjects.

In this thesis, both biomarker test batteries, the NeuroCart and PainCart are used to characterise the pharmacodynamic effects in relation to pharmacokinetics and safety measurements of novel and well-known potential analgesic and psychoactive compounds. This approach allows to investigate and characterise potentially desirable and undesirable effects of a pharmacological agent in relation to its pharmacokinetic profile, in phase I or Phase IIa studies. This, in turn, may result in identifying an optimal dose at which undesirable effects, e.g. sedation, reduced memory performance, or subjective drug effects do not occur, but analgesia is present.

REFERENCES

Ahluwalia J, Urban L, Bevan S, et al. (2003) Anandamide regulates neuropeptide release from capsaicin-sensitive primary sensory neurons by activating both the cannabinoid 1 receptor and the vanilloid receptor 1 in vitro. *Eur J Neurosci* 17: 2611-2618.

Arendt-Nielsen L, Curatolo M and Drewes A. (2007) Human experimental pain models in drug development: translational pain research. *Curr Opin Investig Drugs* 8: 41-53.

Atack JR. (2010) $GABA_A$ receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. *Curr Top. Behav Neurosci* 2: 331-360.

Berge O-G. (2011) Predictive validity of behavioural animal models for chronic pain. *British journal of pharmacology* 164: 1195-1206.

BiomarkersDefinitionsWorkingGroup. (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001/03/10 ed., 89-95.

Breivik H, Collett B, Ventafridda V, et al. (2006) Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 10: 287-333.

Bridges D, Rice AS, Egertova M, et al. (2003) Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. *Neuroscience* 119: 803-812.

Campero M, Baumann TK, Bostock H, et al. (2009) Human cutaneous C fibres activated by cooling, heating and menthol. *The Journal of physiology* 587: 5633-5652.

Chen X. (2017) Human pharmacology of current and novel GABA (A)-ergic treatments for anxiety. CHDR. Leiden, the Netherlands: Leiden University.

Cohen AF, Burggraaf J, van Gerven JM, et al. (2015) The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol* 55: 55-74.

Collinson N, Kuenzi FM, Jarolimek W, et al. (2002a) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci* 22: 5572-5580.

Collinson N, Kuenzi FM, Jarolimek W, et al. (2002b) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci* 22: 5572-5580.

Crestani F, Keist R, Fritschy JM, et al. (2002) Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. *Proc Natl Acad Sci U S A* 99: 8980-8985.

de Haas SL, Schoemaker RC, van Gerven JM, et al. (2010) Pharmacokinetics, pharmacodynamics and the pharmacokinetic/ pharmacodynamic relationship of zolpidem in healthy subjects. *J Psychopharmacol* 24: 1619-1629.

De Petrocellis L, Cascio MG and Di Marzo V. (2004) The endocannabinoid system: a general view and latest additions. *British journal of pharmacology* 141: 765-774.

Dureja GP, Jain PN, Shetty N, et al. (2014) Prevalence of chronic pain, impact on daily life, and treatment practices in India. *Pain Pract* 14: E51-62.

Foulkes T and Wood JN. (2007) Mechanisms of cold pain. *Channels (Austin)* 1: 154-160.

Graven-Nielsen T, Mense S and Arendt-Nielsen L. (2004) Painful and non-painful pressure sensations from human skeletal muscle. *Experimental brain research* 159: 273-283.

Groeneveld GJ, Hay JL and Van Gerven JM. (2016) Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol* 20: 27-34.

Guan Z, Hellman J and Schumacher M. (2016) Contemporary views on inflammatory pain mechanisms: TRPing over innate and microglial pathways. *F1000Research* 5: F1000 Faculty Rev-2425.

Gustorff B, Anzenhofer S, Sycha T, et al. (2004) The sunburn pain model: the stability of primary and secondary hyperalgesia over 10 hours in a crossover setting. *Anesth Analg* 98: 173-177, table of contents.

Hay JL, Okkerse P, van Amerongen G, et al. (2016) Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery. *J Vis Exp.*

Head H and Holmes G. (1911) Sensory Disturbances from Cerebral Lesions1. *Brain* 34.

Hohmann AG and Herkenham M. (1999) Cannabinoid receptors undergo axonal flow in sensory nerves. *Neuroscience* 92: 1171-1175.

Klumpers LE. (2014) Novel approaches in clinical development of cannabinoid drugs. CHDR. Leiden: Leiden University.

Klumpers LE, Beumer TL, van Hasselt JGC, et al. (2012) Novel Δ9-tetrahydrocannabinol formulation Namisol® has beneficial pharmacokinetics and promising pharmacodynamic effects. *Br J Clin Pharmacol* 74: 42-53.

Knabl J, Witschi R, Hosl K, et al. (2008) Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. *Nature* 451: 330-334.

Mackie K. (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol*: 299-325.

Malcangio M and Bowery NG. (1996) GABA and its receptors in the spinal cord. *Trends Pharmacol Sci* 17: 457-462.
Mansour AR, Farmer MA, Baliki MN, et al. (2014) Chronic pain: the role of learning and brain plasticity. *Restor Neurol Neurosci* 32: 129-139.
Melzack R and Wall PD. (1965) Pain mechanisms: a new theory. *Science* 150: 971-979.
Neumann S, Doubell TP, Leslie T, et al. (1996) Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature* 384: 360-364.
NIH. What Is Precision Medicine. *Genetics Home Reference.*
Noble M, Treadwell JR, Tregear SJ, et al. (2010) Long-term opioid management for chronic noncancer pain. *Cochrane Database of Systematic Reviews.*
Okkerse P. (2018) The use of a battery of evoked pain models in early phase drug development. CHDR. Leiden: Leiden University.
Racine M. (2018) Chronic pain and suicide risk: A comprehensive review. *Prog Neuropsychopharmacol Biol Psychiatry* 87: 269-280.
Rudolph U, Crestani F, Benke D, et al. (1999) Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* 401: 796-800.
Schlicker E and Kathmann M. (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* 22: 565-572.
Simone DA and Kajander KC. (1997) Responses of cutaneous A-fiber nociceptors to noxious cold. *J Neurophysiol* 77: 2049-2060.
Treede RD, Rief W, Barke A, et al. (2019) Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *PAIN* 160: 19-27.
Tsang A, Von Korff M, Lee S, et al. (2008) Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. *J Pain* 9: 883-891.
Vardeh D, Mannion RJ and Woolf CJ. (2016) Toward a Mechanism-Based Approach to Pain Diagnosis. *J Pain* 17: T50-69.
Veress G, Meszar Z, Muszil D, et al. (2013) Characterisation of cannabinoid 1 receptor expression in the perikarya, and peripheral and spinal processes of primary sensory neurons. *Brain Struct Funct* 218: 733-750.
Verhaak PF, Kerssens JJ, Dekker J, et al. (1998) Prevalence of chronic benign pain disorder among adults: a review of the literature. *PAIN* 77: 231-239.
Walker JM, Krey JF, Chu CJ, et al. (2002) Endocannabinoids and related fatty acid derivatives in pain modulation. *Chem Phys Lipids* 121: 159-172.
WHO. (1986) Cancer pain relief. *World Health Organization.*
Yekkirala AS, Roberson DP, Bean BP, et al. Breaking barriers to novel analgesic drug development.
Zuurman HH. (2008) Clinical pharmacology of cannabinoids in early phase drug development. CHDR. Leiden: Leiden University.

CHAPTER 2

A LITERATURE REVIEW ON PHARMACOLOGICAL SENSITIVITY OF HUMAN EVOKED HYPERALGESIA PAIN MODELS

G van Amerongen,[1] M W de Boer, G J Groeneveld,[1*] J L Hay[1*]

** joint last authors*

1 Centre for Human Drug Research (CHDR) / Leiden University Medical Centre (LUMC) Leiden, The Netherlands

ABSTRACT

AIMS Human evoked pain models can be used to determine efficacy of new and existing analgesics and to aid in the identification of new targets. Aspects of neuropathic pain can be simulated by inducing hyperalgesia resulting from provoked sensitisation. This literature review aims to provide insight into the sensitivity of different hyperalgesia and allodynia models to pharmacological treatment.

METHODS A literature search was performed to identify randomised, double-blind, placebo-controlled studies that included human hyperalgesia pain models and investigated the pharmacodynamic effect of different classes of drugs.

RESULTS Three hyperalgesia models (UVB, capsaicin and burn) have been used most extensively. Assessment of hyperalgesia/allodynia and pharmacological effect is measured using challenge tests, which generally comprise of thermal (heat/cold) or mechanical stimulation (pin prick, stroking or impact). The UVB model was sensitive to the antihyperalgesic effects of NSAIDS and opioids. The capsaicin model was somewhat sensitive to opioids. The burn model did not detect any antihyperalgesic effects when NSAIDS or local anaesthetics were administered, but responded to the effects of NMDA receptor antagonists by moderately reducing mechanical hyperalgesia.

CONCLUSIONS Based on pharmacological sensitivity, the UVB model adequately reflects inflammatory pain and was sensitive to NSAIDS and opioids. Findings from the capsaicin and burn models raised questions about the translatability of these models to the treatment of neuropathic pain. There is a need for a reproducible and predictive model of neuropathic pain, either in healthy subjects or in patients.

INTRODUCTION

Chronic pain is highly prevalent, it is estimated to range between 20-30% in Europe and the United States of America.[1,2] The nature of pain is complex as many different physiological and psychological mechanisms are at play. Commonly, pain is classified according to its supposed pathophysiology: nociceptive pain, neuropathic pain, psychogenic pain and mixed or unspecified pain.[3] These differ in terms of onset and expression: in general, nociceptive pain is associated with acute pain, whereas neuropathic pain is more frequently chronic in nature. Underlying mechanisms differ greatly: nociceptive pain results from activation by a noxious stimulus of the nociceptive afferents distributed throughout the body. Neuropathic pain has been defined as "Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system",[4] which results in sensitisation of the somatosensory system. Central sensitisation on the one hand results from an increased responsiveness of the neurons in the dorsal horn and thalamus (including nociceptive responses to the A-β mechanoreceptors). Peripheral sensitisation on the other hand is the consequence of increased sensitivity of nociceptors, resulting from lower activation thresholds and increased responsiveness, often associated with inflammation.[5–8] Central or peripheral sensitisation gives rise to the clinical presentation of neuropathic pain: allodynia (pain in response to a normally non-nociceptive stimulus) and/or hyperalgesia (more intense pain in response to a normally noxious stimulus). The treatment of neuropathic pain currently has a largely unmet medical need: analgesics are often ineffective or limited by side effects. In the development of new (analgesic) drugs, biomarkers can be a useful tool in early phase research.[9] Evoked pain models using biomarkers cannot describe the complexity of pain in a single parameter, yet using pain models rather than patients to test the efficacy of analgesic drugs can be advantageous in terms of standardization, proof-of-concept and to provide insight in pharmacological background. Furthermore, using pain models excludes confounding due to co-existing fever, general malaise and psychological cognitive and social aspects of illness. Various human evoked hyperalgesia models have been developed that induce central and/or peripheral sensitisation in healthy volunteers in a well-controlled manner. This level of sensitisation is subsequently measured and quantified using a normally non-painful thermal or mechanical challenge. Use of this challenge enables the assessment of analgesic efficacy of novel drugs.

To be able to benchmark the effects of novel pharmacological compounds and provide guidance in the selection of an appropriate biomarker, the present study's objective is to evaluate the capacity of each selected model to detect antihyperalgesic effects of different pharmacological subclasses of drugs. This review also aims to map the abundance of methods and degree of heterogeneity among the individual hyperalgesia models.

METHODS

Literature evaluation

A literature study was performed using MEDLINE, Web of Science and EMBASE up to the 21st of March 2016. MESH and free terms were used for the following search terms: 'hyperalgesia OR allodynia OR sensitisation'. Searches were limited to healthy human adults and manuscripts written in English. There was no limit to year of publication. To ensure clinical homogeneity, cutaneous hyperalgesia models were selected based on uniformity of methods, and thus comparability. Hyperalgesia models that were used in fewer than 10 individual clinical trials or hyperalgesia models that had been used to investigate fewer than three different classes of analgesics were excluded. This resulted in the selection of three cutaneous hyperalgesia models: the UVB model, the (thermode) burn model and the capsaicin model.

The UVB (or 'sunburn') model is regarded as a model for inflammatory pain and hyperalgesia is evoked by exposing an area of skin to an individualised dose of UVB on the leg, arm or back. Prior to the start of the study, the Minimal Erythemic Dose (MED) for each subject is determined, and subsequently a one-, two- or threefold of this dose is applied to the skin. Over the course of 2-96 hours a clearly discernible dose-related area of erythema becomes apparent, where lowered activation threshold for painful and non-painful stimuli (primary hyperalgesia) is observed.[10]

The thermode burn model is generally considered as a model for heat injury and pertaining inflammatory pain. Hyperalgesia evoked by inducing a first degree burn by exposing the subject to a specific heat paradigm, ranging from 100 seconds to 7 minutes, using a contact thermode at the skin. This procedure induces primary hyperalgesia on the site of exposure, but also secondary hyperalgesia in adjacent tissue, resulting from central sensitisation.

The capsaicin model is the most widely used model to mimic symptoms of neurogenic hyperalgesia as observed in neuropathic pain. Capsaicin exerts its hyperalgesic effects via Transient Receptor Potential cation channel subfamily V member 1 (TRPV1) receptor activation. Capsaicin is applied either topically, or as an intradermal or intramuscular injection. Since TRPV1 receptors are also activated by heat (>43°C), the method is also used in combination with heat exposure in order to potentiate the hyperalgesic effects of capsaicin. Topical absorption of capsaicin can be variable, therefore the extent of hyperalgesia can vary. When capsaicin is applied intradermally, acute severe stinging or burning pain occurs, followed by primary and secondary hyperalgesia up to 24 hours.[10,11]

A thermal or mechanical challenge was the predominant method used to determine the magnitude of hyperalgesia. Seldom, an electrical challenge was also used to quantify hyperalgesia or allodynia but findings from using this challenge were not included in this review due to the lack of standardization and the resulting difficulty in comparability.

Efficacy of the investigated pharmacological compound was quantified according to its effect on pain induced by a mechanical or thermal challenge. Studies lacking adequate blinding or randomisation were excluded from this review, as well as studies including fewer than 6 subjects. To address the temporal nature related to evoked hyperalgesia, either as a result of the body's adaptation to (mild) tissue damage, or resulting from pharmacokinetics of a chemical hyperalgesic agent, only studies using adequate (active or inactive placebo) control were included in this review. Studies solely reporting baseline controlled results were excluded. Finally, drugs that are still in the experimental phase of drug development were excluded, as the pharmacology of such drugs is not yet completely established.

This review categorised the selected randomised, double-blind, controlled trials investigating the efficacy of pharmacological compounds according to hyperalgesia model, corresponding challenge and class of pharmacological compound.

Other human evoked hyperalgesia models that were identified, but did not meet the entrance criterion regarding frequency of use for inclusion in this review, are, e.g freeze lesion,[12–14] mustard oil,[15–18] menthol[19–21] or substances including centrally acting opioids or local glutamate.[22–27]

Individual studies

All studies included in this review yielded the following outcomes, according to challenge: the effect of a pharmacological compound on thermal and mechanical Pain Detection Threshold (PDT), Pain Tolerance Threshold (PTT) and pain ratings (Visual Analogue Scales (VAS), Numeric Rating Scales (NRS)) in the hyperalgesic area and magnitude of area of hyperalgesia and allodynia. Besides provoked hyperalgesia, stimulus-independent hyperalgesia was also considered a relevant outcome, with outcomes including size and intensity of visual flare and spontaneous or ongoing pain.

For this review it was decided to use the term hyperalgesia in accordance with commonly used terminology in the reviewed literature referring to hyperalgesia as well as allodynia, even if the term allodynia would have been more appropriate based on definition. Pain responses to mild mechanical (punctate, brush) and thermal (heat/cold) challenge indicate a pain response to a normally non-noxious stimulus, and thus represent allodynia, rather than hyperalgesia.

Due to an anticipated variation in effect sizes, the individual results were ranked as "positive" (antihyperalgesic effect / (statistically) significant improvement compared with placebo), or "no effect" (no significant difference compared with placebo), per separate outcome, rather than quantifying the magnitude of effect of the pharmacological compound. Outcomes for different administration forms were regarded as separate outcomes. Differential dose or time effects were indicated with a note, and scored as a positive effect, as this model was apparently able to detect an antihyperalgesic effect, given the appropriate execution of the test.

Grouping of test results

The outcomes per challenge method were grouped according to type of outcome: thermal, mechanical and stimulus-independent. The category "thermal" was subdivided in the specific outcomes measured in the individual studies, e.g. heat / cold PDT or PTT. The category "mechanical" consisted of static (pin prick), dynamic (stroking with a brush, cotton gauze, etc), and impact (using an algometer) stimuli, providing the aforementioned outcomes. Stimulus-independent outcomes were related to spontaneous pain resulting from hyperalgesia and to intensity and size of flare. Results from the individual studies were subsequently grouped according to drug class to provide insight into the pharmacological effect of each class of drug on a specific hyperalgesia-challenge combination. Responsiveness of each model to each particular class of drugs was defined in this review as the pharmacological sensitivity.

RESULTS

Study designs

The literature study yielded 94 individual studies on the three selected hyperalgesia models: 16 used the UVB model to induce hyperalgesia, 48 studies explored the effects of various pharmacological compounds on capsaicin-induced hyperalgesia and 30 studies investigated thermode burn-induced hyperalgesia. Seven studies examined more than one hyperalgesia model. General study characteristics are presented in *Table 1*. The participants were aged between 17 and 65 years.

Even though the UVB, capsaicin and thermode burn models were selected based on high degree of standardization, there was considerable variation in the execution of the models as shown in *Table 2*. All studies utilizing UVB to induce hyperalgesia administered a dose of 1, 2 or 3 times the Minimal Erythemic Dose (MED). The administration of 1 MED was shown to be inconsistent at producing hyperalgesia[28] in one study. Larger variation was found among the methods of inducing hyperalgesia with capsaicin. Capsaicin was either injected intradermally or applied topically. Of the 16 capsaicin studies that used heat to further exacerbate/prolong the hyperalgesia, 2 studies kept the skin at a constant temperature, while the remainder used the method of rekindling: five minutes at a set temperature (40°C or 45°C at fixed time points) with a thermode placed directly on the skin or using a radiant heat lamp. The largest variation was seen in the thermode burn model: ten different heat administration regimens were identified, ranging from 100 seconds at 50°C (n=1) to 7 minutes at 47°C (n=14), causing blistering in one or more subjects in 20% of the studies. The thermode burn and UVB models were most often administered on one or both legs (68.8% and 83.3% respectively), whereas for administration of capsaicin to induce hyperalgesia, one or both arms were selected most often (89.4%). The frequency of use of challenge methods among the different hyperalgesia models is shown in *Table 3*.

Sensitivity of the UVB model

The use of the UVB model as a model for inflammation was relatively uncommon: 16 studies using this method were identified. Eight classes of drugs were investigated. Studies that investigated the effects of a combination of drugs are listed in a separate category. *Table 4* shows an overview of the pharmacological sensitivity of the UVB model per separate challenge method (mechanical, thermal or stimulus-independent) and grouped according to drug class.

A total of four studies investigating nonsteroidal anti-inflammatory drugs (NSAIDS), including ibuprofen[28–30] and Rofecoxib,[31] showed a significant effect by reducing hyperalgesia to thermal and mechanical stimuli. Two studies investigating the effects of ketorolac alone and in combination with paracetamol found mixed results.[32,33] Mixed results were also observed for the benzodiazepines clobazam and clonazepam.[34] Systemically administered opioids reduced hyperalgesia to thermal and mechanical stimuli.[35–38] Transdermal administration of either buprenorphine or fentanyl did not attenuate hyperalgesia to heat or static mechanical stimuli, but buprenorphine did have a significant effect on the PTT to impact stimuli.[38] Furthermore, remifentanil in combination with gabapentin showed no greater reduction in hyperalgesia than remifentanil alone. [36]

Lidocaine, a local anaesthetic, showed mixed results. One study found an attenuating effect on hyperalgesia to impact stimuli when lidocaine was injected intravenously.[39] Another study applied lidocaine topically and found a reduction of hyperalgesia to static and dynamic mechanical stimuli, but no attenuating effects on hyperalgesia to heat stimuli.[40] Studies investigating the voltage-gated Calcium channel α2–δ modulating anticonvulsant, gabapentin,[36] the neurotoxin botulinum toxin A[41] and paracetamol[33,37] found no significant effects on hyperalgesia. Tetrahydrocannabinol (Δ9-THC), a cannabinoid receptor agonist, also did not show significant positive effects on hyperalgesia to mechanical and thermal stimuli.[42] Noteworthy, Δ9-THC even showed significantly increased hyperalgesia at specific electrical stimuli intensities at specific time points.[42]

Sensitivity of the capsaicin model

The capsaicin model has been used extensively to test the efficacy of new and existing pharmacological compounds. This literature study yielded 48 articles eligible for inclusion in the current review. A total of 14 classes of pharmacological compounds were identified. For both analgesics and corticosteroids, there was only one study investigating a drug belonging to these classes. *Table 5* provides an overview of the findings of the individual studies using the capsaicin model grouped by class of drug and type of challenge/hyperalgesia.

Opioids, anaesthetics, N-methyl D-aspartate (NMDA) receptor antagonists, and to a lesser degree, the calcium channel α_2-δ ligands, appear to have an attenuating effect on capsaicin-induced hyperalgesia to mechanical stimuli,[43–46] although there are also

a number of studies for each of these drug classes where no effect could be found (e.g., 47-49). The α-2 adrenoreceptor agonist clonidine,[50,51] appeared to be effective in reducing hyperalgesia, particularly in response to mechanical stimuli in two studies.

Although NMDA receptor antagonists appear to be effective in reducing hyperalgesia, a number of the studies demonstrate a positive effect only at specific time points, mostly during infusion or measured immediately after infusion or bolus injection, particularly on mechanical hyperalgesia (e.g., 52-54 for ketamine, 55 for dextromethorphan and 56 for neramexane) (see the corresponding footnotes in *Table 5*). The remaining drug classes investigated showed no or very limited efficacy in attenuating capsaicin-induced hyperalgesia: NSAIDS),[32,57] analgesics,[56] cannabinoids,[42,58,59] tricyclic antidepressants[60,61] and antiarrhythmics.[62–64]

Sensitivity of the thermode burn model

This review included 30 studies investigating the efficacy of pharmacological compounds to attenuate hyperalgesia induced by the thermode burn model. Ten classes of pharmacological compounds to reduce hyperalgesia were found. Of these classes, five comprised a single compound. In addition, three studies investigating a combination of drugs are included. An overview of the results is shown in *Table 6*.

No class of drug showed clear efficacy when administered to completely reverse thermode burn-induced hyperalgesia. However, NMDA receptor antagonists were found to attenuate mechanical, but not thermal hyperalgesia to a moderate extent,[55,65–71] although there was also a number of studies that did not demonstrate this effect.[72,73] A similar reduction in mechanical hyperalgesia but not on thermal hyperalgesia was observed when ketamine was combined with the opioid receptor antagonist, naloxone,[67] indicating that co-administration of naloxone does not reduce the effects of ketamine.

Two studies were performed to investigate the presence of a synergistic effect of combined treatment of an opioid (morphine) and an NMDA receptor antagonist, but these showed inconclusive results.[69,72]

Opioids,[69,70,74,75] intracellular sodium channel blockers,[76,77] NSAIDs,[78–82] corticosteroids,[83,84] the calcium channel α_2-δ ligand, gabapentin,[85] the glutamate antagonist riluzole,[86] the opioid receptor antagonist naloxone[87] and P_1-receptor activator adenosine[88] were inconsistent at attenuating heat, mechanical and non-provoked hyperalgesia.

DISCUSSION

This literature review aimed to provide insight in the pharmacological sensitivity of three cutaneous hyperalgesia models: the UVB, capsaicin and thermode burn model, with the goal to determine the applicability of individual hyperalgesia models in early phase pharmacological pain research. The review of the identified randomised, double-blind, placebo-controlled trials investigating the efficacy of numerous pharmacological

compounds generated an overview of the classes of drugs that are investigated in pain paradigms and their efficacy at reducing specific hyperalgesia-challenge combinations.

The summarised findings of the included trials reflect the pharmacological sensitivity of three hyperalgesia models in combination with specific challenges, which were selected based on their standardised methodology and frequency of use.

The UVB model was only responsive to the pharmacological effects of NSAIDS and, to a lesser extent, opioids. The pharmacological sensitivity of the thermode burn model, used as a translational model for inflammatory pain as well as neuropathic pain, shows a different profile compared with the UVB model. First, NSAIDS and opioids do not seem to show antihyperalgesic effects when administered to reduce burn-induced hyperalgesia. Moderately effective at attenuating mechanical hyperalgesia were the NMDA receptor antagonists, whereas thermal hyperalgesia was largely unaffected. Some authors refer to the central mechanism involved in secondary mechanical hyperalgesia, in contrast with the peripheral sensitisation in primary (thermal) hyperalgesia as an explanation for the differential effect of NMDA receptor antagonists between heat and mechanical hyperalgesia.[70,71] Despite the general regard of capsaicin as a model for neuropathic pain, the model appeared insensitive to the classes of pharmacological compounds clinically prescribed in first-line treatment for neuropathic pain.[89] Calcium channel α_2-δ ligands (gabapentin and pregabalin), tricyclic antidepressants or topical lidocaine provided limited to no attenuation of hyperalgesia in the majority of the studies investigating this model. Most of the studies investigating the effects of opioids on mechanical hyperalgesia yielded positive results, however only a sparse number of studies investigated the effects of opioids on thermal hyperalgesia and therefore providing no evidence for responsiveness of thermal hyperalgesia induced by capsaicin, or the lack thereof, to opioids. A few positive studies investigating clonidine suggest that it exerts its effects by reducing spinal hypersensitivity through $\alpha 2$-adrenergic agonism in the dorsal horn. NMDA receptor antagonists exert their antihyperalgesic effects through inhibition of the glutamatergic signalling pathways. A limited number of studies demonstrated that the capsaicin model is sensitive to NMDA receptor antagonists. The results demonstrate a differential antihyperalgesic effect: mechanical hyperalgesia is attenuated in a small number of studies, but not thermal hyperalgesia. The capsaicin model appeared to be insensitive to the remainder of the pharmacological compounds that were investigated, including botulinum toxin A and cannabinoids.

For a number of classes of drugs investigated, this literature review included only one study and one compound per drug class. Therefore, for these drug classes, no strong recommendations can be made with respect to the suitability of the cutaneous hyperalgesia models, other than those based on face validity.

Limitations to this approach

In this review the pharmacological sensitivity of the selected hyperalgesia models is based on the capacity of the model to detect an antihyperalgesic effect for each class of

drug. Inherent to this approach is the assumption that the clinical trials are appropriately executed. The included clinical trials had to meet the following criteria: randomised, double-blind and placebo or active controlled. Only 6.7% to 18.5% of the studies used an active control (alone or in combination with true placebo). This may have introduced bias when investigating psychoactive pharmacological compounds compared to true placebo, as analgesia is known to be prone to placebo response.[90] This could be avoided by using an active placebo with known lack of analgesia but comparable psychoactive effects. Dosing regimens and administration forms are included in the tables to provide insight in potential differences, but for the studies that were included, clinically relevant dosing regimens were generally used.

Variability in reporting of the results was observed on different levels. Due to the bilateral nature of evoked hyperalgesia models, both induction and assessment of hyperalgesia potentially introduce variability. For example, some authors report absolute pain thresholds, whereas others report calculated hyperalgesia (compared to healthy control skin). Furthermore, to assess pharmacodynamic response, some groups compare a single post-dose measurement with a baseline in a paired t-test analysis, whereas other groups include multiple measurements in ANOVA. Consequently, a statistical meta-analysis of the results of the included clinical trials was not deemed feasible, nor does it fall within the scope of the present review.

Hyperalgesia models that were not included in this review, including the freeze lesion model, may eventually also prove to be useful tools to detect antihyperalgesic effects of novel compounds, given the reproducible and non-invasive methodology, but due to their limited use thus far no conclusion on pharmacological sensitivity can be made.

For ethical reasons, evoked hyperalgesia models are characterised by their temporal nature: either bodily adaptations to (mild) tissue damage or pharmacokinetics of a chemical hyperalgesic agent result in time-dependent hyperalgesia, which attenuates over time without intervention. To overcome this, a protocol needs be designed with an appropriate control. Nonetheless, this potentially interferes with interpretation of the results of analgesics or antihyperalgesics with a prolonged pharmacological effect.

Implications for pain research

In early phase drug development, research in healthy subjects can form the bridge between animal models and clinical application and provide the basis for proof-of-concept of new compounds or techniques. Furthermore, experiments can be used to investigate the basic mechanisms to characterise sensory dysfunction in patients.[91] The main concern of human pain research is to appraise the value of a model in terms of translation to clinical practice. In this respect the UVB model is a highly satisfactory model for inflammation, as it is highly reproducible and responds well to NSAIDS. The thermode burn model responds well to NMDA receptor antagonists in the attenuation of mechanical hyperalgesia. This might reflect a specific component of neuropathic pain, so-called 'wind-up' pain, which is also reduced by NMDA receptor antagonists in clinical

practice.[92] However, as the model does not respond well to the other medications that are efficacious in the treatment of neuropathic pain; this model appears to be solely capable of mimicking this specific element of neuropathic pain. As a model for inflammatory pain, the thermode burn model is unsuitable, as it is insensitive to anti-inflammatory drugs. The capsaicin model shows most sensitivity to the antihyperalgesic effects of opioids compared with other drug classes. The established drugs for the treatment of neuropathic pain, such as the calcium channel α_2-δ ligands only show antihyperalgesic effects on specific endpoints, indicating that finetuning of the model in combination with the correct challenge could potentially provide a pharmacologically sensitive model for these classes of compounds. Although sensitisation is present in the capsaicin-challenge model, it is due to different mechanisms than those involved in clinical presentation of neuropathic pain. Nonetheless, finetuning of this model may render it a useful tool for early phase drug research, as no single model can completely replicate the clinical presentation of neuropathic pain. Alternatively, the capsaicin model may only mimic the features of clinical (neuropathic) pain in certain healthy subjects.[93] and just like for the UVB model where subjects are generally pre-screened for 'responders' and the model is individualised per subject, this may be necessary for the capsaicin model. Pre-screening for 'responders', as is occasionally done,[94,95] ensures homogeneity and thereby reduces variability. In early phase research for a compound with a novel mechanism of action for the indication of neuropathic pain, one needs to keep these limitations in mind. As such the capsaicin model is not suitable for go/no-go decision-making, but can be a useful tool to aid clinical development of novel analgesic treatments.

CONCLUSIONS

This literature review demonstrates the importance of carefully considering the appropriate design in early phase pharmacological research. Due to the abundance of possible working mechanisms, no single human evoked pain model is capable to detect antihyperalgesic or analgesic effects of each class of drugs. Therefore, the appropriateness and translatability of the model has to be taken into account when designing an early phase proof-of-concept study. In this respect, the UVB model can be considered a predictive model for inflammatory pain based on its capacity to detect antihyperalgesic effects from NSAIDS. The thermode burn model is considered to reflect a specific aspect of neuropathic pain; still, as a whole, the model lacks sensitivity to serve as a complete model for neuropathic pain. The capsaicin model in its current form also lacks pharmacological sensitivity to be used as a model for neuropathic pain. It may, however, provide important insight in mechanisms involved in hyperalgesia, including signal transduction and pain perception. In our opinion, further standardization and validation is needed before the capsaicin model can be used as a model to screen drugs for their effect on symptoms of neuropathic pain.

While investigating the pharmacological sensitivity of hyperalgesia pain models, we revealed the lack of robust models for neuropathic pain. Current hyperalgesia models evidently do not reflect the clinical presentation of neuropathic pain. Asserting that a certain model is representative of neuropathic is overstating the confidence of the models. Neuropathic pain is a heterogeneous entity and further research is needed to investigate the link between the evoked pain models and the different types of neuropathic pain. Carefully selecting appropriate biomarkers and understanding their merits and limitations for early phase drug research is essential for effective and efficient drug development.

REFERENCES

1 Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur.J.Pain 2006; 10: 287-333.

2 Johannes CB, Le TK, Zhou X, Johnston JA, Dworkin RH. The prevalence of chronic pain in United States adults: results of an Internet-based survey. J Pain 2010; 11: 1230-1239.

3 Pharmacological management of persistent pain in older persons. Pain Med 2009; 10: 1062-1083.

4 Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology. 2008; 70: 1630-1635.

5 Sandkuhler J, Benrath J, Brechtel C, Ruscheweyh R, Heinke B. Synaptic mechanisms of hyperalgesia.In: Progress in Brain Research Nervous System Plasticity and Chronic Pain, Volume 129 Edition, ed Sandkuhler J, Elsevier, 2000: 81-100.

6 Woolf CJ. An overview of the mechanisms of hyperalgesia. Pulm.Pharmacol. 1995; 8: 161-167.

7 Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011; 152: S2-15.

8 Willis WD. Role of neurotransmitters in sensitization of pain responses. Ann N Y Acad Sci 2001; 933: 142-156.

9 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. Annu Rev Pharmacol Toxicol 2015; 55: 55-74.

10 Bishop T, Ballard A, Holmes H, Young AR, McMahon SB. Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain 2009; 13: 524-532.

11 LaMotte RH, Lundberg LE, Torebjork HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol 1992; 448: 749-764.

12 Chassaing C, Schmidt J, Eschalier A, Cardot JM, Dubray C. Hyperalgesia induced by cutaneous freeze injury for testing analgesics in healthy volunteers. Br J Clin Pharmacol 2006; 61: 389-397.

13 Schmidtko A, Burian M, Altis K, Hardt K, Angioni C, Schmidt R, Podda M, Geisslinger G. Pharmacological and histopathological characterization of a hyperalgesia model induced by freeze lesion. Pain 2007; 127: 287-295.

14 Tegeder I, Meier S, Burian M, Schmidt H, Geisslinger G, Lotsch J. Peripheral opioid analgesia in experimental human pain models. Brain. 2003; 126: 1092-1102.

15 Sjolund KF, Segerdahl M, Sollevi A. Adenosine reduces secondary hyperalgesia in two human models of cutaneous inflammatory pain. Anesth.Analg. 1999; 88: 605-610.

16 Olausson B. Recordings of human polymodal single C-fiber afferents following mechanical and argon-laser heat stimulation of inflamed skin. Exp Brain Res 1998; 122: 55-61.

17 Ward L, Wright E, McMahon SB. A comparison of the effects of noxious and innocuous counterstimuli on experimentally induced itch and pain. Pain 1996; 64: 129-138.

18 Magerl W, Koltzenburg M, Schmitz JM, Handwerker HO. Asymmetry and time-course of cutaneous sympathetic reflex responses following sustained excitation of chemosensitive nociceptors in humans. J Auton.Nerv Syst 1996; 57: 63-72.

19 Andersen HH, Poulsen JN, Uchida Y, Nikbakht A, Arendt-Nielsen L, Gazerani P. Cold and L-menthol-induced sensitization in healthy volunteers–a cold hypersensitivity analogue to the heat/capsaicin model. Pain 2015; 156: 880-889.

20 Binder A, Stengel M, Klebe O, Wasner G, Baron R. Topical high-concentration (40%) menthol-somatosensory profile of a human surrogate pain model. J Pain 2011; 12: 764-773.

21 Mahn F, Hullemann P, Wasner G, Baron R, Binder A. Topical high-concentration menthol: reproducibility of a human surrogate pain model. Eur J Pain 2014; 18: 1248-1258.

22 Angst MS, Koppert W, Pahl I, Clark DJ, Schmelz M. Short-term infusion of the mu-opioid agonist remifentanil in humans causes hyperalgesia during withdrawal. Pain 2003; 106: 49-57.

23 Cairns BE, Svensson P, Wang K, Castrillon E, Hupfeld S, Sessle BJ, Arendt-Nielsen L. Ketamine attenuates glutamate-induced mechanical sensitization of the masseter muscle in human males. Exp.Brain.Res. 2006; 169: 467-472.

24 Laulin JP, Maurette P, Corcuff JB, Rivat C, Chauvin M, Simonnet G. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. Anesth.Analg. 2002; 94: 1263-9.

25 Luginbuhl M, Gerber A, Schnider TW, Petersen-Felix S, Arendt-Nielsen L, Curatolo M. Modulation of remifentanil-induced analgesia, hyperalgesia, and tolerance by small-dose ketamine in humans. Anesth. Analg. 2003; 96: 726-32.

26 Pappagallo M, Gaspardone A, Tomai F, Iamele M, Crea F, Gioffre PA. Analgesic effect of bamiphylline on pain induced by intradermal injection of adenosine. Pain 1993; 53: 199-204.

27 Sandrini G, Tassorelli C, Cecchini AP, Alfonsi E, Nappi G. Effects of nimesulide on nitric oxide-induced hyperalgesia in humans-a neurophysiological study. European Journal of Pharmacology 2002; 450: 259-262.

28 Bickel A, Dorfs S, Schmelz M, Forster C, Uhl W, Handwerker HO. Effects of antihyperalgesic drugs on experimentally induced hyperalgesia in man. Pain 1998; 76: 317-325.

29 Angst MS, Clark JD, Carvalho B, Tingle M, Schmelz M, Yeomans DC. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: A microdialysis study. Pain 2008; 139: 15-27.

30 Sycha T, Gustorff B, Lehr S, Tanew A, Eichler HG, Schmetterer L. A simple pain model for the evaluation of analgesic effects of NSAIDS in healthy subjects. Br J Clin Pharmacol 2003; 56: 165-172.

31 Sycha T, Anzenhofer S, Lehr S, Schmetterer L, Chizh B, Eichler HG, Gustorff B. Rofecoxib attenuates both primary and secondary inflammatory hyperalgesia: a randomized, double-blinded, placebo controlled crossover trial in the UV-B pain model. Pain 2005; 113: 316-322.

32 Eisenach JC, Curry R, Tong C, Houle TT, Yaksh TL. Effects of intrathecal ketorolac on human experimental pain. Anesthesiology 2010; 112: 1216-1224.

33 Ing LK, Besson M, Daali Y, Salomon D, Dayer P, Desmeules J. A randomized, controlled trial validates a peripheral supra-additive antihyperalgesic effect of a paracetamol-ketorolac combination. Basic Clin Pharmacol Toxicol. 2011; 109: 357-364.

34 Besson M, Matthey A, Daali Y, Poncet A, Vuilleumier P, Curatolo M, Zeilhofer HU, Desmeules J. GABAergic modulation in central sensitization in humans: a randomized placebo-controlled pharmacokinetic-pharmacodynamic study comparing clobazam with clonazepam in healthy volunteers. Pain 2015; 156: 397-404.

35 Koppert W, Likar R, Geisslinger G, Zeck S, Schmelz M, Sittl R. Peripheral antihyperalgesic effect of morphine to heat, but not mechanical, stimulation in healthy volunteers after ultraviolet-B irradiation. Anesth. Analg. 1999; 88: 117-122.

36 Gustorff B, Hoechtl K, Sycha T, Felouzis E, Lehr S, Kress HG. The effects of remifentanil and GABApentin on hyperalgesia in a new extended inflammatory skin pain model in healthy volunteers. Anesth.Analg. 2004; 98: 401-407.

37 Ortner CM, Steiner I, Margeta K, Schulz M, Gustorff B. Dose response of tramadol and its combination with paracetamol in UVB induced hyperalgesia. Eur J Pain 2012; 16: 562-573.

38 Andresen T, Staahl C, Oksche A, Mansikka H, Arendt-Nielsen L, Drewes A. Effect of transdermal opioids in experimentally induced superficial, deep and hyperalgesic pain. Br J Pharmacol 2011; 164: 934-935.

39 Koppert W, Brueckl V, Weidner C, Schmelz M. Mechanically induced axon reflex and hyperalgesia in human UV-B burn are reduced by systemic lidocaine. Eur J Pain 2004; 8: 237-244.

40 Gustorff B, Hauer D, Thaler J, Seis A, Draxler J. Antihyperalgesic efficacy of 5% lidocaine medicated plaster in capsaicin and sunburn pain models–two randomized, double-blinded, placebo-controlled crossover trials in healthy volunteers. Expert Opin Pharmacother. 2011; 12: 2781-2790.

41 Sycha T, Samal D, Chizh B, Lehr S, Gustorff B, Schnider P, Auff E. A lack of antinociceptive or antiinflammatory effect of botulinum toxin A in an inflammatory human pain model. Anesth.Analg. 2006; 102: 509-516.

42 Kraft B, Frickey NA, Kaufmann RM, Reif M, Frey R, Gustorff B, Kress HG. Lack of analgesia by oral standardized cannabis extract on acute inflammatory pain and hyperalgesia in volunteers. Anesthesiology 2008; 109: 101-110.

43 Dirks J, Petersen KL, Rowbotham MC, Dahl JB. GABApentin suppresses cutaneous hyperalgesia following heat-capsaicin sensitization. Anesthesiology 2002; 97: 102-107.

44 Gottrup H, Juhl G, Kristensen AD, Lai R, Chizh BA, Brown J, Bach FW, Jensen TS. Chronic oral GABApentin reduces elements of central sensitization in human experimental hyperalgesia. Anesthesiology 2004; 101: 1400-1408.

45 Mathiesen O, Imbimbo BP, Hilsted KL, Fabbri L, Dahl JB. CHF3381, a N-methyl-D-aspartate receptor antagonist and monoamine oxidase-A inhibitor, attenuates secondary hyperalgesia in a human pain model. J Pain 2006; 7: 565-574.

46 Wanigasekera V, Mezue M, Andersson J, Kong Y, Tracey I. Disambiguating Pharmacodynamic Efficacy from Behavior with Neuroimaging: Implications for Analgesic Drug Development. Anesthesiology 2016; 124: 159-168.

47 Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. The effect of systemic lidocaine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Anesth.Analg. 2000; 91: 967-972.
48 Wallace MS, Braun J, Schulteis G. Postdelivery of alfentanil and ketamine has no effect on intradermal capsaicin-induced pain and hyperalgesia. Clin J Pain 2002; 18: 373-379.
49 Wallace MS, Schulteis G. Effect of chronic oral GABApentin on capsaicin-induced pain and hyperalgesia: a double-blind, placebo-controlled, crossover study. Clin J Pain 2008; 24: 544-549.
50 Eisenach JC, Hood DD, Curry R. Intrathecal, but not intravenous, clonidine reduces experimental thermal or capsaicin-induced pain and hyperalgesia in normal volunteers. Anesth.Analg. 1998; 87: 591-596.
51 Eisenach JC, Hood DD, Curry R. Relative potency of epidural to intrathecal clonidine differs between acute thermal pain and capsaicin-induced allodynia. Pain 2000; 84: 57-64.
52 Gottrup H, Hansen PO, Arendt-Nielsen L, Jensen TS. Differential effects of systemically administered ketamine and lidocaine on dynamic and static hyperalgesia induced by intradermal capsaicin in humans. Br J Anaesth. 2000; 84: 155-162.
53 Koppert W, Zeck S, Blunk JA, Schmelz M, Likar R, Sittl R. The effects of intradermal fentanyl and ketamine on capsaicin-induced secondary hyperalgesia and flare reaction. Anesth.Analg. 1999; 89: 1521-1527.
54 Park KM, Max MB, Robinovitz E, Gracely RH, Bennett GJ. Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects. Pain 1995; 63: 163-172.
55 Duedahl TH, Dirks J, Petersen KB, Romsing J, Larsen NE, Dahl JB. Intravenous dextromethorphan to human volunteers: relationship between pharmacokinetics and antihyperalgesic effect. Pain 2005; 113: 360-368.
56 Klein T, Magerl W, Hanschmann A, Althaus M, Treede RD. Antihyperalgesic and analgesic properties of the N-methyl-D-aspartate (NMDA) receptor antagonist neramexane in a human surrogate model of neurogenic hyperalgesia. Eur J Pain 2008; 12: 17-29.
57 Burns D, Hill L, Essandoh M, Jarzembowski TM, Schuler HG, Janicki PK. Effect of valdecoxib pretreatment on pain and secondary hyperalgesia: a randomized controlled trial in healthy volunteers [ISRCTN05282752, NCT00260325]. BMC.Anesthesiol. 2006; 6: 3.
58 Kalliomaki J, Philipp A, Baxendale J, Annas P, Karlsten R, Segerdahl M. Lack of effect of central nervous system-active doses of nabilone on capsaicin-induced pain and hyperalgesia. Clin Exp Pharmacol Physiol. 2012; 39: 336-342.
59 Wallace M, Schulteis G, Atkinson JH, Wolfson T, Lazzaretto D, Bentley H, Gouaux B, Abramson I. Dose-dependent effects of smoked cannabis on capsaicin-induced pain and hyperalgesia in healthy volunteers. Anesthesiology 2007; 107: 785-796.
60 Eisenach JC, Hood DD, Curry R, Tong C. Alfentanil, but not amitriptyline, reduces pain, hyperalgesia, and allodynia from intradermal injection of capsaicin in humans. Anesthesiology 1997; 86: 1279-1287.
61 Wallace MS, Barger D, Schulteis G. The effect of chronic oral desipramine on capsaicin-induced allodynia and hyperalgesia: a double-blinded, placebo-controlled, crossover study. Anesth. Analg. 2002; 95: 973-8.
62 Ando K, Wallace MS, Braun J, Schulteis G. Effect of oral mexiletine on capsaicin-induced allodynia and hyperalgesia: A double-blind, placebo-controlled, crossover study. Regional Anesthesia and Pain Medicine 2000; 25: 468-474.
63 Dirks J, Petersen KL, Rowbotham MC, Dahl JB. Effect of systemic adenosine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Reg Anesth.Pain Med 2001; 26: 414-419.
64 Eisenach JC, Curry R, Hood DD. Dose response of intrathecal adenosine in experimental pain and allodynia. Anesthesiology 2002; 97: 938-942.
65 Ilkjaer S, Petersen KL, Brennum J, Wernberg M, Dahl JB. Effect of systemic N-methyl-D-aspartate receptor antagonist (ketamine) on primary and secondary hyperalgesia in humans. Br.J.Anaesth. 1996; 76: 829-834.
66 Ilkjaer S, Dirks J, Brennum J, Wernberg M, Dahl JB. Effect of systemic N-methyl-D-aspartate receptor antagonist (dextromethorphan) on primary and secondary hyperalgesia in humans. Br.J.Anaesth. 1997; 79: 600-605.
67 Mikkelsen S, Ilkjaer S, Brennum J, Borgbjerg FM, Dahl JB. The effect of naloxone on ketamine-induced effects on hyperalgesia and ketamine-induced side effects in humans. Anesthesiology. 1999; 90: 1539-1545.
68 Pedersen JL, Galle TS, Kehlet H. Peripheral analgesic effects of ketamine in acute inflammatory pain. Anesthesiology. 1998; 89: 58-66.

69 Schulte H, Sollevi A, Segerdahl M. The synergistic effect of combined treatment with systemic ketamine and morphine on experimentally induced windup-like pain in humans. Anesth.Analg. 2004; 98: 1574-80.
70 Warncke T, Stubhaug A, Jørum Ellen. Ketamine, an NMDA receptor antagonist, suppresses spatial and temporal properties of burn-induced secondary hyperalgesia in man: a double-blind, cross-over comparison with morphine and placebo. Pain 1997; 72: 99-106.
71 Warncke T, Stubhaug A, Jørum Ellen. Preinjury treatment with morphine or ketamine inhibits the development of experimentally induced secondary hyperalgesia in man. Pain 2000; 86: 293-303.
72 Frymoyer AR, Rowbotham MC, Petersen KL. Placebo-controlled comparison of a morphine/dextromethorphan combination with morphine on experimental pain and hyperalgesia in healthy volunteers. J Pain 2007; 8: 19-25.
73 Mikkelsen S, Jørgensen H, Larsen PS, Brennum J, Dahl JB. Effect of oral ketamine on secondary hyperalgesia, thermal and mechanical pain thresholds, and sedation in humans. Regional Anesthesia and Pain Medicine 2000; 25: 452-458.
74 Lilleso J, Hammer NA, Pedersen JL, Kehlet H. Effect of peripheral morphine in a human model of acute inflammatory pain. Br.J.Anaesth. 2000; 85: 228-232.
75 Robertson LJ, Drummond PD, Hammond GR. Naloxone Antagonizes the Local Antihyperalgesic Effect of Fentanyl in Burnt Skin of Healthy Humans. The Journal of Pain 2007; 8: 489-493.
76 Holthusen H, Irsfeld S, Lipfert P. Effect of pre- or post-traumatically applied i.v. lidocaine on primary and secondary hyperalgesia after experimental heat trauma in humans. Pain 2000; 88: 295-302.
77 Pedersen JL, Callesen T, Moiniche S, Kehlet H. Analgesic and anti-inflammatory effects of lignocaine-prilocaine (EMLA) cream in human burn injury. Br.J.Anaesth. 1996; 76: 806-810.
78 Lundell JC, Silverman DG, Brull SJ, O'Connor TZ, Kitahata LM, Collins JG, LaMotte R. Reduction of post-burn hyperalgesia after local injection of ketorolac in healthy volunteers. Anesthesiology 1996; 84: 502-509.
79 Moiniche S, Dahl JB, Kehlet H. Short-term topical piroxicam has no anti-inflammatory or antinociceptive effects after burn injury. Current Therapeutic Research 1993; 53: 466-472.
80 Moiniche S, Pedersen JL, Kehlet H. Topical ketorolac has no antinociceptive or anti-inflammatory effect in thermal injury. Burns 1994; 20: 483-486.
81 Petersen KL, Brennum J, Dahl JB. Experimental evaluation of the analgesic effect of ibuprofen on primary and secondary hyperalgesia. Pain 1997; 70: 167-174.
82 Warncke T, Brennum J, Arendt-Nielsen L, Branebjerg PE. Effects of local and systemic ibuprofen on primary and secondary hyperalgesia in man. Curr Ther Res Clin Exp 1996; 57: 937-949.
83 Pedersen JL, Moiniche S, Kehlet H. Topical glucocorticoid has no antinociceptive or anti-inflammatory effect in thermal injury. Br.J.Anaesth. 1994; 72: 379-382.
84 Werner MU, Lassen B, Kehlet H. Analgesic effects of dexamethasone in burn injury. Reg.Anesth.Pain Med 2002; 27: 254-260.
85 Werner MU, Perkins FM, Holte K, Pedersen JL, Kehlet H. Effects of GABApentin in acute inflammatory pain in humans. Reg.Anesth.Pain Med 2001; 26: 322-328.
86 Hammer NA, Lilleso J, Pedersen JL, Kehlet H. Effect of riluzole on acute pain and hyperalgesia in humans. Br.J.Anaesth. 1999; 82: 718-722.
87 Brennum J, Kaiser F, Dahl JB. Effect of naloxone on primary and secondary hyperalgesia induced by the human burn injury model. Acta Anaesthesiol.Scand. 2001; 45: 954-960.
88 Sjolund KF, Segerdahl M, Sollevi A. Adenosine reduces secondary hyperalgesia in two human models of cutaneous inflammatory pain. Anesth.Analg. 1999; 88: 605-610.
89 O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am J Med 2009; 122: S22-S32.
90 Wager TD, Atlas LY. The neuroscience of placebo effects: connecting context, learning and health. Nat Rev Neurosci 2015; 16: 403-418.
91 Arendt-Nielsen L. Translational human pain research. European Journal of Pain Supplements 2007; 1: 38-40.
92 Blonk MI, Koder BG, van den Bemt PM, Huygen FJ. Use of oral ketamine in chronic pain management: a review. Eur J Pain 2010; 14: 466-472.
93 Lötsch J, Dimova V, Hermens H, Zimmermann M, Geisslinger G, Oertel BG, Ultsch A. Pattern of neuropathic pain induced by topical capsaicin application in healthy subjects. Pain 2015; 156: 405-414.
94 Hughes A, Macleod A, Growcott J, Thomas I. Assessment of the reproducibility of intradermal administration of capsaicin as a model for inducing human pain. Pain 2002; 99: 323-331.
95 Gustafsson H, Akesson J, Lau CL, Williams D, Miller L, Yap S, Rolan P. A comparison of two formulations of intradermal capsaicin as models of neuropathic pain

in healthy volunteers. Br J Clin Pharmacol 2009; 68: 511-517.
96 Bauer M, Schwameis R, Scherzer T, Lang-Zwosta I, Nishino K, Zeitlinger M. A double-blind, randomized clinical study to determine the efficacy of benzocaine 10% on histamine-induced pruritus and UVB-light induced slight sunburn pain. J Dermatolog.Treat. 2015; 26: 367-372.
97 Wang H, Bolognese J, Calder N, Baxendale J, Kehler A, Cummings C, Connell J, Herman G. Effect of morphine and preGABAlin compared with diphenhydramine hydrochloride and placebo on hyperalgesia and allodynia induced by intradermal capsaicin in healthy male subjects. J Pain 2008; 9: 1088-1095.
98 Wallace MS, Ridgeway B, III, Leung A, Schulteis G, Yaksh TL. Concentration-effect relationships for intravenous alfentanil and ketamine infusions in human volunteers: effects on acute thresholds and capsaicin-evoked hyperpathia. J.Clin Pharmacol 2002; 42: 70-80.
99 Petersen KL, Jones B, Segredo V, Dahl JB, Rowbotham MC. Effect of remifentanil on pain and secondary hyperalgesia associated with the heat–capsaicin sensitization model in healthy volunteers. Anesthesiology 2001; 94: 15-20.
100 Petersen KL, Maloney A, Hoke F, Dahl JB, Rowbotham MC. A randomized study of the effect of oral lamotrigine and hydromorphone on pain and hyperalgesia following heat/capsaicin sensitization. J Pain 2003; 4: 400-406.
101 Koppert W, Ostermeier N, Sittl R, Weidner C, Schmelz M. Low-dose lidocaine reduces secondary hyperalgesia by a central mode of action. Pain 2000; 85: 217-224.
102 Wallace MS, Laitin S, Licht D, Yaksh TL. Concentration-effect relations for intravenous lidocaine infusions in human volunteers: effects on acute sensory thresholds and capsaicin-evoked hyperpathia. Anesthesiology 1997; 86: 1262-1272.
103 Gottrup H, Bach FW, Arendt-Nielsen L, Jensen TS. Peripheral lidocaine but not ketamine inhibits capsaicin-induced hyperalgesia in humans. Br J Anaesth. 2000; 85: 520-528.
104 Lam VY, Wallace M, Schulteis G. Effects of lidocaine patch on intradermal capsaicin-induced pain: a double-blind, controlled trial. J.Pain 2011; 12: 323-330.
105 Zheng Z, Gibson SJ, Helme RD, McMeeken JM. The effect of local anaesthetic on age-related capsaicin-induced mechanical hyperalgesia - A randomised, controlled study. Pain 2009; 144: 101-109.
106 Kilo S, Forster C, Geisslinger G, Brune K, Handwerker HO. Inflammatory models of cutaneous hyperalgesia are sensitive to effects of ibuprofen in man. Pain 1995; 62: 187-193.
107 McCormack K, Kidd BL, Morris V. Assay of topically administered ibuprofen using a model of post-injury hypersensitivity. A randomised, double-blind, placebo-controlled study. Eur.J.Clin Pharmacol 2000; 56: 459-462.
108 Andersen OK, Felsby S, Nicolaisen L, Bjerring P, Jensen TS, Arendt-Nielsen L. The effect of Ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin–a double-blind, placebo-controlled, human experimental study. Pain 1996; 66: 51-62.
109 Poyhia R, Vainio A. Topically administered ketamine reduces capsaicin-evoked mechanical hyperalgesia. Clin J Pain 2006; 22: 32-36.
110 Kauppila T, Grönroos M, Pertovaara A. An attempt to attenuate experimental pain in humans by dextromethorphan, an NMDA receptor antagonist. Pharmacol.Biochem.Behav. 1995; 52: 641-644.
111 Vuilleumier PH, Besson M, Desmeules J, Arendt-Nielsen L, Curatolo M. Evaluation of antihyperalgesic and analgesic effects of two benzodiazepines in human experimental pain: a randomized placebo-controlled study. PLoS One 2013; 8: e43896.
112 Wallace MS, Quessy S, Schulteis G. Lack of effect of two oral sodium channel antagonists, lamotrigine and 4030W92, on intradermal capsaicin-induced hyperalgesia model. Pharmacol Biochem.Behav. 2004; 78: 349-355.
113 Mikkelsen S, Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. Effect of intravenous magnesium on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Br J Anaesth. 2001; 86: 871-873.
114 Voller B, Sycha T, Gustorff B, Schmetterer L, Lehr S, Eichler HG, Auff E, Schnider P. A randomized, double-blind, placebo controlled study on analgesic effects of botulinum toxin A. Neurology 2003; 61: 940-944.
115 Schulte-Mattler WJ, Opatz O, Blersch W, May A, Bigalke H, Wohlfahrt K. Botulinum toxin A does not alter capsaicin-induced pain perception in human skin. J Neurol.Sci 2007; 260: 38-42.
116 Gazerani P, Staahl C, Drewes AnM, Arendt-Nielsen L. The effects of Botulinum Toxin type A on capsaicin-evoked pain, flare, and secondary hyperalgesia in an experimental human model of trigeminal sensitization. Pain 2006; 122: 315-325.

117 Michaux GP, Magerl W, Anton F, Treede RD. Experimental characterization of the effects of acute stresslike doses of hydrocortisone in human neurogenic hyperalgesia models. Pain 2012; 153: 420-428.
118 Schulte H, Sollevi A, Segerdahl M. Dose-dependent effects of morphine on experimentally induced cutaneous pain in healthy volunteers. Pain 2005; 116: 366-374.
119 Moiniche S, Dahl JB, Kehlet H. Peripheral antinociceptive effects of morphine after burn injury. Acta Anaesthesiol.Scand. 1993; 37: 710-712.
120 Stubhaug A, Romundstad L, Kaasa T, Breivik H. Methylprednisolone and ketorolac rapidly reduce hyperalgesia around a skin burn injury and increase pressure pain thresholds. Acta Anaesthesiol.Scand. 2007; 51: 1138-1146.
121 Andersen LP, Gogenur I, Fenger AQ, Petersen MC, Rosenberg J, Werner MU. Analgesic and antihyperalgesic effects of melatonin in a human inflammatory pain model: a randomized, double-blind, placebo-controlled, three-arm crossover study. Pain 2015; 156: 2286-2294.

TABLE 1 Characteristics of randomised, double-blind, (active) placebo controlled studies specified according to hyperalgesia model

	Design		Control		Subjects		
	Crossover (%)	**Parallel (%)**	**Inactive placebo (%)**	**Active control (%)**	**N (Median/ range)**	**Age in years (range)**	**Sex (%) Males / Mixed / Females / Unknown**
UVB (n = 16)	93.7	6.3	81.3	18.7	16 (6 – 42)	18 - 55	31.3 / 62.5 / 6.2 / 0
Capsaicin (n = 48)	97.9	2.1	89.6	12.5	16.5 (6 – 50)	18 - 65	31.3 / 60.4 / 2.1 / 6.3
Burn (n = 30)	100	0	93.3	6.7	17 (6 – 29)	17 - 52	50.0 / 46.7 / 0 / 3.3

TABLE 2 Frequency of use (%) of general methods for the induction of hyperalgesia specified according to hyperalgesia model

Hyperalgesia model	Specific methods		Frequency of use (%)
UVB (n = 16)	UVB Dose	1MED	6.3
		2MED	18.8
		3MED	75.0
	Location	Leg	68.8
		Arm	18.8
		Back	6.3
	Time between exposure and hyperalgesia assessment	12 hours	6.3
		20 hours	18.8
		24 hours	62.5
		20-26 hours	6.3
		Not specified	6.3
Capsaicin (n = 48)	Formulation and duration of application	Topical	41.7
		30 minutes	65.0
		40 minutes	5.0
		60 minutes	15.0
		90 minutes	5.0
		Not specified	10.0
		Intradermal injection	58.3
	Administration form and dose	Topical	41.7
		0.075%	55.0
		0.1%	5.0
		1%	20.0
		Other / not specified	20.0
		Intradermal injection	59.6
		10µg	3.6
		20µg	14.3
		40µg	7.1
		100µg	67.9
		250µg	7.1
	Applying heat	No heat applied	70.8
		Rekindling ≥1 time *	25.0
		Constant temperature	4.2
	Location	Leg	12.5
		Arm	87.5
		Foot	2.1
		Forehead	2.1

Burn (n = 30)	Application	1:40 minute at 50°C	3.3
		2 minutes at 48°C	3.3
		3 minutes at 45°C	6.7
		3-5minutes at 45°C	3.3
		4 minutes at 49°C	3.3
		5 minutes at 47°C	10
		5 minutes at 49°C	13.3
		6 minutes at 47°C	3.3
		7 minutes at 46°C	6.7
		7 minutes at 47°C	46.7
	Surface area	3.75 cm^2	10.0
		4.5 cm^2	3.3
		12.5 cm^2	73.3
		22.8 cm^2	3.3
		Unknown	6.7
	Blistering in any subject	Yes	20.0
		No	47.0
		Unknown	33.0
	Location	Leg	83.3
		Arm	13.3
		Abdomen	3.3

** All studies that used rekindling also pre-heated before capsaicin application for 5 minutes at 45°C.*

TABLE 3 Frequency of use (%) of main challenge methods specified according to hyperalgesia model

Challenge	Method	Frequency of use (%) *		
		UVB (n = 16)	Capsaicin (n = 48)	Burn (n = 30)
Thermal – Heat	Thermode	68.8	50.0	76.7
	Halogen bulb	12.5	2.1	3.3
Thermal – Cold	Thermode	25.0	8.3	3.3
Mechanical (static) – Pin prick	Von Frey	56.3	77.1	80.0
	Custom-made / Other	12.5	6.3	10.0
Mechanical (dynamic) – Stroking	Brush	12.5	43.8	13.3
	Cotton	18.8	35.4	6.7
	Fingertip	0	0	6.7
	Von Frey	0	0	3.3
Mechanical – impact stimulus	Algometer (static)	6.3	0	0
	Algometer (dynamic)	18.8	2.1	3.3

** Frequencies of use exceed 100% because most studies make use of more than one method.*

TABLE 4 Schematic summary of results of randomised controlled trials investigating hyperalgesia induced by UVB, according to type of challenge

Class	Drug (administration) form / dose	Challenge type	Challenge/ outcome	Overall effect	
				Effective	No effect
OPIOIDS	**Morphine** (IV / 4 mg) [35] **Remifentanil** (IV / 0.8 µg/ kg/min) [36]A[1] **Fentanyl** (Transdermal / 25µg/h, 72h) [38]A **Buprenorphine** (Transdermal / 20µg/h, 144h) [38]B **Tramadol** (IV / 0.3 mg/kg, 0.6 mg/kg, 1 mg/kg) [37][2] **Remifentanil** (IV infusion/0.8µg/kg/min) & **Gabapentin** (Oral/ 600 mg) [36]B[1]	T	Heat/PDT	[35],[36] A,[36]B	[37]
			Heat/PTT	[36]A,[36]B	[38]B
			Cold/PDT		[37]
		M	Pin prick/Area	[36]A,[36]B	[38]A,[38] B,[37]
			Pin prick/Pain score	[37][2]	
			Impact stimulus/Pain score		[35]
			Impact stimulus/PTT	[38]B[3]	[38]A
ANAESTHETICS	**Lidocaine** (Topical patch / 5% medicated plaster) [40], (IV-bolus / 2 mg/kg in 10 minutes, then 2 mg/kg/h for 30 minutes) [39]; **Benzocaine** (topical / 10 % ointment) [96]	T	Heat/PDT		[40]
			Heat/PTT		[40]
			Cold/PDT	[40]	
			Cold/PTT		[40]
		M	Impact/Pain score	[39]	
			Pin prick/Area	[40]	
			Stroking/Pain score	[40]	
		S-I	Flare/intensity	[39]	
			Flare/Area		[40]
			Spontaneous pain		[96]
NSAIDS	**Ibuprofen** (Oral / 400 mg – 800 mg) [28-30] **Rofecoxib** (Oral / 50 mg, 250 mg, 500 mg) [31] **Ketorolac** (Oral / 20 mg) [33]A; (Intrathecal / 2 mg) [32] **Ketorolac** (Oral / 20 mg) & **Paracetamol** (Oral / 1 mg) [33]B	T	Heat/PDT	[31], [30], [28], [29], [33] B	[33]A
			Heat /PTT	[31],[30]	
		M	Impact stimulus/Pain score	[28]	
			Pin prick/Area	[31], [32]	[33]A,[33]B
			Pin prick/PDT	[29],[33]B	[33]A
			Stroking/Area		[32]
		S-I	Flare/ intensity	[31],[28]	

CALCIUM CHANNEL α2-δ LIGAND	**Gabapentin** (Oral / 600 mg) [36]C[1]	T	Heat/PDT		[36]C
			Heat/PTT		[36]C
		M	Pin Prick/ Pain score		
			Pin prick/Area		[36]C
CANNABINOIDS	**Δ-9-THC** (Oral / 20 mg) [42][4,5]	T	Heat/PDT		[42]
			Heat/PTT		[42]
		M	Pin prick/Area		[42]
			Stroking/Area		[42]
BENZODIAZEPINES	**Clobazam** (Oral / 20 mg) [34][6]A **Clonazepam** (Oral / 1 mg) [34][6]B	T	Heat/PDT	[34]A	[34]B
			Heat/PTT	[34]B	[34]A
			Cold/PDT		[34]A
		M	Pin prick/Area	[34]A,[34]B	
			Pin prick/PDT	[34]B	[34]A
NEUROTOXINS	**Botulinum Toxin A** (Intracutaneous / 100 Mouse Units (MU)) [41]	T	Heat/PDT		[41]
			Cold/PDT		[41]
		M	Stroking/ Pain score		[41]
			Pin prick/Area		[41]
			Pin prick/PDT		[41]
ANALGESICS	**Paracetamol** (Oral / 1 g) [33]C; (IV / 330 mg) [37]	M	Pin prick/PDT		[33]C
			Pin prick/Area		[33]C,[37]
			Pin prick/Pain score		[37]
		T	Heat/PDT		[33]C,[37]
			Cold/PDT		[37]

T=Thermal, M=Mechanical, S-I=Stimulus-Independent / 1 Effect compared with active placebo: diazepam (2 mg). / 2 Significant effect found only at 1 mg/kg dose of tramadol, not at 0.3 mg/kg or 0.6 mg/kg doses. / 3 Significant effect found at 48 and 72 hours post dosing, but not at 24 or 144 hours post dosing: neither short nor long term effect. / 4 Also electrical stimuli administered, results not shown here. / 5 Compared with active placebo: diazepam (5 mg) / 6 Compared with active placebo: tolterodine (1.37 mg)

TABLE 5 Schematic summary of results of randomised controlled trials investigating hyperalgesia induced by capsaicin, according to type of challenge

Class	Drug (administration form/dose)	Challenge Type	Challenge/ outcome	Overall effect	
				Effective	No effect
OPIOIDS	**Morphine** (Oral / 30 mg) [72]A; (IV infusion / 10 mg) [97] **Morphine** (Oral / 30 mg) & **Dextrometorphan** (Oral / 30 mg) [72]B **Alfentanil** (IV / plasma concentration 50 ng/mL or 200 ng/mL) [60]A[1]; (IV / 3.075 mg [54]; (IV infusion / 1.9 ± 0.5 mg) [48][1]; (IV infusion / 3.33 ± 0.42 mg) [98] **Alfentanil** (IV / plasma concentration 50 ng/mL or 200 ng/mL) & **Amitriptyline** (Intramuscular injection / 25 mg) [60]B[1] **Remifentanil** (IV / 0.05 µg/kg/min for 5 minutes, then 0.1 µg/kg/min for 35 minutes) [99]; (IV / 0.05 µg/kg/min for 10 minutes, then 0.1 µg/kg/min for 25 minutes) [100]A **Fentanyl** (Transdermal / 25µg/h, 72h) [38]A; (Intradermal injection / 1 µg, 10 µg) [53] **Buprenorphine** (Transdermal / 20µg/h, 144h) [38]B **Hydromorphone** (Oral / 8 mg) [100]B	T	Heat/PDT		[48]
			Heat/Rating	[99]	[48]
			Heat/Area		[48], [98]
			Cold/PDT		[48]
			Cold/Rating		[48]
		M	Pin prick/Area	[97], [60]A, [60]B, [54], [98], [99], [100]A, [100]B	[72]A, [72]B, [48], [38]A, [38]B, [53]
			Pin prick/Pain rating	[54], [97][2]	[53], [48]
			Pin prick/PDT	[98]	[48]
			Stroking/Area	[72]A, [60]A, [60]B, [98], [99], [100]A, [100]B, [54], [97][1]	[72]B, [48], [38]A, [38]B, [53]
			Stroking/Pain score	[97][1]	[48], [53]
		S-I	Flare/area		[97], [98], [48]
			Flare/intensity	[53][3]	
			Spontaneous pain	[60]A, [60]B, [54], [98]	[97], [48], [53]
ANAESTHETICS	**Lidocaine** (IV / bolus of 2 mg/kg in 10 minutes, then infusion of 2 mg/kg/h for another 50 minutes) [101]A; (IV / 2 mg/kg/min for 10 minutes, then 3 mg/kg/h) [47]; (5 mg / kg in 30 minutes) [52]; (IV infusion / 1 µg per mL, 2 µg per mL, 3 µg per mL [102][1] (Intradermal injection / 20 µg per 40 mL) [101]B; (Subcutaneous infiltration / 20 mg per 2 mL) [103]; (Transdermal patch / dose unknown) [104]; (Topical patch / 5% medicated plaster) [40] **EMLA** (Topical cream / 2 g of 2.5% Lignocaine and 2.5% Procain) [105]	T	Heat/PDT		[47], [102], [40], [104], [101]A, [101]B
			Heat/PTT		[40], [52]
			Heat/Rating	[105]	[47]
			Heat/Area	[102]	
			Cold/PDT		[102]
		M	Pin prick/Area	[52], [105][4], [40], [101]A, [103]	[47], [102], [104], [101]B
			Pin prick/Pain rating	[105], [103]	[52]
			Pin prick/PDT	[105], [103]	[102], [104]
			Stroking/Area	[105], [40], [103]	[47], [52], [102], [101]A, [101]B, [104]
			Stroking/Pain score		[52]
		S-I	Flare/area	[102], [101]A, [101]B	[104], [40]
			Spontaneous pain		[102], [40], [101]A, [101]B, [104]

NSAIDS	**Ibuprofen** (Oral / 1200 mg, 2400 mg) [106]; (Oral / 600 mg) [46]A; (Topical cream / 0.5 g in 100 mg of gel containing 5% Ibuprofen) [107] **Valdecoxib** (Oral / 40 mg) [57] **Ketorolac** (Intrathecal / 2 mg per 2 mL) [32]	M	Pin prick/Area		[57], [32]
			Pin prick/Pain rating		[46]A
			Stroking/Area	[107]	[106], [57], [32]
			Stroking/Pin prick		[46]A
		S-I	Spontaneous pain		[46]A
ANALGESICS	**Flupirtine** (Oral / 100 mg) [56]	M	Pin prick/Pain rating		[56]
			Stroking/Pain rating		[56]
		S-I	Flare		[56]
			Spontaneous pain	[56]	
NMDA RECEPTOR ANTAGONISTS	**Ketamine** (IV infusion / 20 µg/kg/min for 10 minutes, then 5 µg/kg/min) [108]; (IV infusion / 28 mg, 375 mg in 30 minutes) [52]; (IV / 32 mg in 35 minutes) [54]; (IV infusion / 15.8 ± 4.4 mg) [48][1]; (IV infusion / 35 mg in 20 minutes) [98]; (Subcutaneous infiltration / 5 mg per 2 mL) [103]; Intradermal injection / 0.1 mg, 1 mg) [53]; (Topical 50 mg / 1 mL) [109] **Dextrometorphan** (IV / 0.5mg/kg) [55]; (Oral / 30 mg) [72]; (Oral / 100 mg, 200 mg) [110] **Neramexane** (Oral / 40 mg) [56]	T	Heat/PDT	[108]	[48], [55]
			Heat/Rating	[109]	[48]
			Heat/PTT		[52]
			Heat/Area		[48], [98]
			Cold/PDT		[48]
			Cold/Rating		[48]
		M	Pin prick/Area	[52][5], [54][6], [55][7]	[72], [48], [98], [103], [53]
			Pin prick/Pain rating	[54], [53][8], [109] , [56]	[52], [48], [103]
			Pin prick/PDT	[98]	[48]
			Stroking/Area	[52][9]	[72], [48], [98], [103], [54], [53]
			Stroking/Pain rating	[53][8], [56][10]	[52], [48], [103]
		S-I	Flare/area		[48], [98]
			Flare/intensity		[53], [56]
			Spontaneous pain	[56][11], [54], [98]	[48], [109], [110], [53]
CALCIUM CHANNEL α2-δ LIGANDS	**Gabapentin** (Oral / 1200 mg) [45]; (Oral / 1200 mg) [43]; (Oral – Chronic / 2400 mg per day on day 15) [44]; (Oral / 1800 mg per day on day 10) [49]; (Oral / 1200 mg) [46]B **Pregabalin** (Oral / 300 mg) [97]	T	Heat/PDT	[43]	[45]
			Heat/Rating		[43]
		M	Pin prick/Area	[45], [43], [97][2]	[44], [49]
			Pin prick/Pain rating	[97][1], [46]B	[49], [44]
			Stroking/Area	[43], [44]	[49], [97]
			Stroking/Pain rating		[49], [97], [44], [46]B
		S-I	Flare/Area		[49], [97]
			Spontaneous pain	[97][12]	[49], [44], [46]

TABLE 5

Class	Drug (administration form/dose)	Challenge Type	Challenge/outcome	Overall effect	
				Effective	No effect
BENZO-DIAZEPINES	**Clobazam** (Oral / 20 mg) [111]A **Clonazepam** (Oral / 1 mg) [111]B	T	Heat/PDT		
		M	Pin prick/Area	[111]A, [111]B	
			Pin prick/pain rating		[111]A, [111]B
			Stroking/Area		[111]A, [111]B
		S-I	Spontaneous pain		[111]A, [111]B
ANTICONVULSANTS	**Lamotrigine** (Oral / 400 mg) [100]; (Oral / 300 mg) [112]; **Magnesium sulfate** (IV infusion / 0.2 mmol/kg in 15 minutes, then 0.2 mmol/kg/h for 90 minutes) [113]	T	Heat/PDT		[113]
			Heat/Rating		[112], [113]
			Heat/Area		[112]
		M	Pin prick/Area		[112], [113], [100]
			Pin prick/Pain rating		[112]
			Stroking/Area		[100], [112], [113]
			Stroking/Pain rating		[112]
		S-I	Spontaneous pain		[112]
CANNABINOIDS	**Δ-9-THC** (Inhalation / 2%, 4%, 8%) [59][13]; (Oral / 1 – 3 mg) [58] **Δ-9-THC** + **Cannabidiol** (Oral / 20 mg) [42][15]	T	Heat/PDT		[59], [58]
			Cold/PDT		[59]
		M	Pin prick/Area		[59], [42]
			Pin prick/PDT		
			Pin prick/Pain rating		[59]
			Stroking/Area	[59][14]	[58]
			Stroking/Pain rating		[59]
		S-I	Flare/Area		[59], [42]
			Flare/Intensity		
			Spontaneous pain	[59][16]	[42], [58]
TRICYCLIC ANTIDEPRESSANTS	**Amitriptyline** (Intramuscular injection / 25 mg) [60] **Desipramine** (Oral – Chronic / 300 mg per day on day 14) [61]	T	Heat/Rating		[61]
			Heat/Area		[61]
		M	Pin prick/Area		[60], [61]
			Pin prick/Pain rating		[61]
			Stroking/Area		[60], [61]
			Stroking/Pain rating		[61]
		S-I	Spontaneous pain		[61], [60]

NEUROTOXINS	**Botulinum toxin A** (Intradermal / 30 MU) [114]; (Intradermal / 100 MU) [115]; (Intramuscular / 150 MU) [116]	T	Heat/PDT		[115]
			Cold/PDT		[115]
		M	Pin prick/Area	[116][17]	[114]
			Impact stimulus/Area	[116]	
		S-I	Flare/Are		[114], [115]
			Spontaneous pain		[114]
ANTIARRYHTMICS	**Adenosine** (IV bolus / 5.1 mg/kg) [63]; (Intrathecal / 0.5 mg, 2 mg) [64] **Mexiletine** (Oral – Chronic / Increasing dose: 1350 mg per day on days 13 - 17) [62]	T	Heat/PDT		[63]
			Heat/Rating	[64][18]	[63], [62]
			Heat/Area		[62]
		M	Pin prick/Area	[62]	[63], [64]
			Pin prick/Pain rating		[62]
			Stroking/Area	[64]	[62], [63]
			Stroking/Pain rating		[62]
		S-I	Flare/Area		[62]
			Spontaneous pain		[62]
ANTIHYPER-TENSIVES	**Clonidine** (IV bolus / 50µg, 150µg) [50]A; (Intrathecal / 50µg, 150µg) [50]B; (Intrathecal / 75µg, 150µg, 300µg) [51]A; (Epidural / 150µg, 300µg, 600µg) [51]B	T	Heat/Rating	[50]B[19]	[50]A
		M	Pin prick/Pain rating	[51]A, [51]B	
			Stroking/Pain rating	[51]A, [51]B	
			Pin prick/Area	[50]B[19]	[50]A
CORTICO-STEROIDS	**Hydrocortisone** (Oral / 40 mg) [117]	M	Stroking/Pain score		[117]
			Pin prick/Pain score	[117]	
		S-I	Spontaneous pain	[117]	
			Flare/Area		[117]

T=Thermal, M=Mechanical, S-I=Stimulus-Independent / 1. Compared with active placebo: diphenhydrate hydrochloride. / 2. Only significant effect when compared with active placebo (diphenhydrate) group, not when compared with true placebo. / 3. Only significant different in flare intensity at high dose (10 µg/200 µl). / 4. No effect shown in elderly subpopulation (mean age 74.9±4.4years). / 5. Effect only seen during infusion, no significant differences from 15 minutes post infusion onwards. / 6. Effect only when dosed after capsaicin, no significant difference when dosed during capsaicin. / 7. Effect only after 135 min. / 8. Effect only in high dose (1 mg), no significant difference in lower dose (0.1 mg). / 9. Effect only seen during infusion, no significant differences from 15 minutes post infusion onwards. / 10. Only significant when measured 30 minutes post capsaicin, and not when up to 1.5 hours measured. / 11. Only significant effect when measured 1 minute post capsaicin, not 2 – 5 minutes. / 12. Only significant effect when compared with true placebo group, not when compared with active placebo (diphenhydrate). / 13. A reverse effect was demonstrated at high dose (8% (Δ9-THC) at 65 minute post dosing: increased spontaneous pain and medium dose (4% Δ9-THC) at 65 minutes post dosing: reduction of PDT to impact stimuli. / 14. Only short term effect, significant difference up to 30 minutes post dosing. / 15. Compared with active placebo: diazepam (2mg/5mg) / 16. Only significant difference in medium dose (4% (Δ9-THC), not in low (2%) or high (8%) dose, only at 65 minutes post dosing. / 17 Only significant after 1 and 4 weeks. / 18. Only significant difference at 80 & 120 minutes post dosing. / 19. Only significant effect at high dose (150 µg).

TABLE 6 Schematic summary of results of randomised controlled trials investigating hyperalgesia induced by thermode burn, according to type of challenge

Class	Drug (administration form / dose)	Challenge Type	Challenge / outcome	Overall effect	
				Effective	No effect
OPIOIDS	**Morphine** (IV injection / 2 mg) [74]A; (IV infusion / 0.14 mg per kg, 0.28 mg per kg) [118]A[2]; (IV infusion / 0.1 mg per kg) [69]A; (IV infusion / 0.15 mg per kg) [70] A; (IV infusion / 0.205 mg per kg in 80 minutes) [71] A; (Oral / 30 mg) [72]A; (Subcutaneous injection in burn / 2 mg) [74]B; (Subcutaneous injection in burn / 2 mg) [119] **Morphine** (IV Infusion / 0.1 mg per kg) & **Ketamine** (IV Infusion / 0.405 mg per kg) [69]B; **Morphine** (Oral / 30 mg) & **Dextrometorphan** (Oral / 30 mg) [72]B **Fentanyl** (Local injection / 10 µg) [75] **Alfentanil** (IV infusion / 73 µg per kg) [118]B[2]	T	Heat/PDT	[119],[75][1]	[74]A,[74]B,[71]A,[70]A
			Cold/PDT		[70]A
			Heat/Rating		[74]A,[74]B,[75]
		M	Pin prick/Area	[71]A, [72]B,[118]B[3]	[74]A,[74]B,[118]A,[69]A, [69]B,[70]A,[72]A
			Pin prick/Pain rating	[69]B[5]	[74]A,[74]B,[69]A,[75]
			Pin prick/PDT	[69]B,[118]A4,[118]B[3]	[74]A,[74]B,[69]A,[71]A
			Stroking/Area	[71]A	[72]A,[72]B,[70]A
			Impact stimulus/PDT		[119]
		S-I	Spontaneous pain		[74]A,[74]B
OPIOID ANTAGONISTS	**Naloxone** (IV Bolus / 0.4 mg) [87]	T	Heat/PDT		[87]
			Heat/Rating		[87]
		M	Pin prick/Area		[87]
			Stroking/Area		[87]
ANAESTHETICS	**Lidocaine** (IV infusion / 317.5 mg) [76] **EMLA** (Topical cream / 2 g) [77]	T	Heat/PDT		[76],[77]
			Heat/Pain rating		
		M	Pin prick/Area		[77],[76]
			Pin prick/PDT		[77],[76]
		S-I	Flare/area		[76]
			Flare/intensity		[77]
NSAIDS	**Ibuprofen** (Oral / 500 mg) [81]; (Oral / 600 mg) [82]A; (Topical cream / 3 g) [82]B **Ketorolac** (Local injection / 0.3 mg) [78]; (Topical gel / 0.075 g) [80]; (IV injection / 60 mg) [120]; **Piroxicam** (Topical gel / 5 mg) [79]	T	Heat/PDT		[82]A,[82]B,[80],[79]
			Heat/Rating		[78]
			Heat/PTT		[82]A,[82]B,[80],[79]
		M	Pin prick/Area	[120]	[81],[82]A,[82]B,[80],[79]
			Pin prick/PDT		[80],[79]
			Stroking/Area		[81]
			Stroking/Pain rating	[81]	
		S-I	Flare/intensity		[80],[79]
			Spontaneous pain		[81]

CALCIUM CHANNEL α2-δ LIGANDS	**Gabapentin** (Oral / 1200 mg) [85] [45]	T	Heat/PDT		[85]
		M	Pin prick/Area	[45]	[85]
			Pin prick/Pain rating		[85]
			Pin prick/PDT	[85]	
			Stroking/Area		[85]
		S-I	Spontaneous pain		[85]
NMDA RECEPTOR ANTAGONISTS	**Ketamine** (IV infusion / 0.49 mg per kg in 150 minutes) [65]A; (IV infusion / 0.98 mg per kg in 150 minutes) [65]B; (IV infusion / 0.405 mg per kg in 45 min) [69]B; (IV infusion / 0.15 mg per kg) [70]B; (IV Infusion / 0.39 mg per kg in 80 min) [71]B; (Oral / 0.5 mg per kg, 1.0 mg per kg) [73]; (IV infusion / 0.3 mg per kg per 15 minutes, then 0.3 mg per kg per hour for 15 minutes) [67]A; (Systemic subcutaneous injection / 15 mg) [68]A; (Local subcutaneous injection / 7.5 mg) [68]B; **Naloxone** (IV infusion / 0.8 mg per kg per 15 minutes) & **Ketamine** (IV Infusion / 0.375 mg per kg per 30 minutes) [67]B **Dextrometorphan** (Oral / 60 mg, 120 mg) [66]; (IV infusion / 0.5 mg per kg) [55]; (Oral / 30 mg) [72]	T	Heat/PDT	[65]B	[65]A,[73],[71]B,[70]B,[68]A,[68]B,[67]A, [67]B,[66]
			Heat/Rating		[68]A,[68]B
			Cold/PDT		[70]B
		M	Pin prick/Area	[65]B,[70]B[7],[71]B,[67]A,[67]B[6], [66][8],[55][9],[69]B[10]	[65]A,[73],[68]A,[68]B,[72]
			Pin prick/PDT	[71]B[11],[68]B[12],[69]B	[68]A
			Pin prick/Pain rating		[68]A,[68]B,[69]B
			Stroking/Area	[65]A[11],[65]B,[70]B[12],[71]B[15],[67]A,[67]B[6]	[73],[66],[72],[68]A,[68]B
		S-I	Spontaneous pain	[65]B,[68]B	[65]A,[73],[68]A,[66]
GLUTAMATE RECEPTOR ANTAGONISTS	**Riluzole** (Oral / 300 mg) [86]	T	Heat/PDT		[86]
			Heat/Rating		[86]
		M	Pin prick/Area		[86]
			Pin prick/PDT		[86]
			Pin prick/Pain rating		[86]
		S-I	Spontaneous pain		[86]
CORTICOSTEROIDS	**Clobetasol** propionate (Topical cream / 0.05 g) [83]; **Dexamethasone** (IV infusion / 8 mg) [84]; **Methylprednisolone** (IV injection / 125 mg) [120]	T	Heat/PDT		[83],[84]
			Heat/PTT		[83]
			Heat/Pain rating		[84]
		M	Pin prick/Area	[120]	[83],[84]
			Pin prick/PDT		[83],[84]
			Pin prick/Pain rating		[84]
		S-I	Flare/intensity		[83],[84]
			Spontaneous pain		[84]

TABLE 6 -

Class	Drug (administration form / dose)	Challenge Type	Challenge / outcome	Overall effect	
				Effective	No effect
HORMONE	**Melatonin** (IV infusion / 100 mg[16]) [121]	T	Heat/PDT		[121]
		M	Pin prick/area		[121]
			Pin prick/PDT		[121]
			Impact stimulus/PDT		[121]
			Impact stimulus/PTT		[121]
		S-I	Flare/intensity		[121]
			Spontaneous pain		[121]
ANTI-ARRYTHMICS	**Adenosine** (IV infusion / 7.2 mg per kg) [88]	T	Heat/PDT		[88]
			Heat/Rating		[88]
		M	Pin prick/PDT		[88]
			Pin prick/Area	[88]	
			Pin prick/Pain rating		[88]

T=Thermal, M=Mechanical, S-I=Stimulus-Independent / 1. Concomitant treatment with naloxone (80 µg) reversed this statistically significant reduction in pain score. / / 2. Compared with saline or active placebo: midazolam (2 mg per kg per minute). / 3. Only significant effect when measured during infusion 85 minutes post burn, and not 80 minutes post infusion 205 minutes post burn. / 4. Significant difference only seen at high dose (0.28 mg/kg) at late phase (80 minutes post dosing), not in earlier measurements. / 5. Only significant difference up to 45 minutes post dosing. / 6. Only effect at late phase (135 minutes post burn). / 7. Only short term effect: no significant difference from 15 minutes post dosing onwards. / 8. Only at specific time point (180 minutes post burn, not before or after) at high dose (120mg). / 9. Significant difference from 90 to 180 minutes post dosing, not earlier. / 10. Only significant effect at 45 minutes post dosing, not at 75 minutes post dosing. / 11. Only short term effect during infusion, no significant difference at 80 or 120 minutes post dosing. / 12. Only significant effect at 0 minutes post burn, no significant difference at 60 or 120 minutes post burn. / 13. Only significant effect at 100 minutes post dosing, not at 60 or 160 minutes post dosing. / 14. Only short term effect: no significant difference from 15 minutes post dosing onwards. / 15. Only short term effect during infusion, no significant difference at 80 or 120 minutes post dosing. / 16. Administration of melatonin 10 mg IV infusion demonstrated equal lack of analgesic effect on all parameters.

CHAPTER 3

EFFECTS ON SPASTICITY AND NEUROPATHIC PAIN OF A NOVEL ORAL FORMULATION OF DELTA-9-TETRAHYDRO-CANNABINOL IN PATIENTS WITH PROGRESSIVE MULTIPLE SCLEROSIS

G van Amerongen,[1] K Kanhai,[1] AC Baakman,[1] J Heuberger,[1] E Klaassen,[1] TL Beumer,[2] RLM Strijers,[3] J Killestein,[3] JMA van Gerven,[1] AF Cohen,[1] GJ Groeneveld[1,3]

1 Centre for Human Drug Research (CHDR) / Leiden University Medical Centre (LUMC) Leiden, The Netherlands
2 Echo Pharmaceuticals, Weesp, The Netherlands
3 Department of Neurology and Clinical Neurophysiology, VU University Medical Center, Amsterdam, the Netherlands

ABSTRACT

PURPOSE Cannabinoids have been shown to improve symptoms of Multiple Sclerosis (MS) including muscle spasticity and pain through modulation of neuronal excitability via presynaptic cannabinoid receptors. Previous formulations of Δ9-THC are notorious for variable pharmacokinetic profiles, thereby demanding cumbersome uptitration. The current formulation was developed to overcome this and improve clinical application of Δ9-THC in the treatment of spasticity and pain in MS. The aim of the present study was to evaluate the efficacy of a novel oral formulation of Δ9-THC (ECP002A) in patients with progressive MS.

METHODS This accelerated proof-of-concept study consisted of two phases: a crossover challenge ('dose-finding') phase and a 4-week parallel randomized, placebo-controlled treatment phase. Twenty-four patients with progressive MS and moderate spasticity were enrolled. During the treatment phase biomarkers for efficacy and secondary pharmacodynamic effects were measured at baseline, and after two and four weeks of treatment. Serum samples were collected to determine pharmacokinetic parameters and perform population modelling. Safety and tolerability was assessed based on Adverse Events and safety measurements.

FINDINGS Pain was significantly reduced when measured directly after administration of ECP002A in the clinic, but not when measured in a daily diary. A similar pattern was observed in subjective muscle spasticity. Other clinical outcomes were not significantly different between active treatment and placebo. Cognitive testing indicated there was no decline in cognition after 2 or 4 weeks of treatment due to ECP002A compared to placebo.

IMPLICATIONS This study specifically underlines the added value of thorough investigation of PK/PD relationships in the target population. Despite the complex interplay of psychoactive effects and analgesia, the current oral formulation of Δ9-THC may play a role in the treatment of spasticity and pain associated with MS, as it was well-tolerated and showed a stable pharmacokinetic profile.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the nervous system characterized by highly variable clinical aspects and an unpredictable course[1]. Of the many symptoms encountered in MS, muscle spasticity and spasms occur in up to 75% of patients[2]. These symptoms often lead to considerable distress from reduced mobility, and interference with activities of daily living. Other disabling features include sensory symptoms (e.g. pain), present in up to 86% of the patients[3]. Spasticity refers to feelings of stiffness and a wide range of involuntary muscle spasms (sustained muscle contractions or sudden movements). Spasticity may be as mild as the feeling of tightness of muscles or may be so severe as to produce painful, uncontrollable spasms of extremities. Spasticity may also produce feelings of pain or tightness in and around joints and can cause lower back pain. Although spasticity can occur in any limb, it is much more common in the legs.

The endogenous cannabinoid system appears to be tonically active in the control of spasticity[4,5] and cannabinoids have been proposed in MS because of their ability to reduce the subjective feeling of spasticity[6]. Cannabinoids have been shown to modulate motor cortical excitability probably through presynaptic cannabinoid receptors CB1 that control the release of neurotransmitters from axonal terminals[7,8]. Delta-9-tetrahydrocannabinol (Δ9-THC) is one of the cannabinoids in the Cannabis sativa plant and a direct partial agonist of the cannabinoid receptor CB1.

Several studies have examined the effect of (synthetic forms of) Δ9-THC in the treatment of multiple sclerosis. No significant effects of doses between 10 mg to 25 mg (total daily dose, twice daily dosing) of oral Δ9-THC were observed on spasticity as measured on the Ashworth scale in a large population. However, a small, but clinically relevant benefit of treatment with cannabis extract or Δ9-THC capsules of dosages up to 25 mg/day was found in secondary outcome measures of perception of spasticity and mobility[9]. Several other studies have also found an effect of Δ9-THC on subjective measures of spasticity[10,11] and pain in patients with MS[9,12] at different dosing regimens. Another study comparing the effects of an oral formulation of Δ9-THC to a cannabis plant extract and to placebo did not demonstrate efficacy in the treatment of spasticity of either product.[13]

Oral bioavailability of Δ9-THC is variable due to significant first pass effect and the current formulation of Δ9-THC, ECP002A (Namisol®), was shown in a phase I study to have superior pharmacokinetic properties to previous formulations, leading to more stable Δ9-THC plasma levels without high peaks and thus expected early onset of treatment effects.[14] It has a novel tablet formulation of pure Δ9-THC that was produced using Alitra™ (Echo Pharmaceuticals b.v., Nijmegen, the Netherlands), an emulsifying drug delivery technology. This technology was designed to improve the uptake of poorly soluble lipophilic compounds, using less surfactant (less than 10% w/w). The current study was designed to investigate the pharmacokinetics (PK) and safety and the effects on spasticity and pain of this novel formulation in a cohort of 24 patients suffering from primary and secondary progressive MS, using a crossover challenge ('dose-finding') phase and a 28-day parallel treatment period.

METHODS

This study was designed as a hybrid between a typical multiple dose study to investigate PK, PD and safety, and a first-in-patient study to establish proof-of-concept, and hence considered to be an accelerated proof-of-concept study that consisted of two phases. The challenge phase was designed as a randomized, double-blind, placebo-controlled, two-way crossover design to determine the optimal effective dose of ECP002A to treat spasticity of each individual and limit the risk of Adverse Events, using pharmacokinetic/pharmacodynamic (PK/PD) modelling. Each of the two visits in the challenge phase consisted of uptitration of three consecutive drug administrations with a 100-minute time interval in ascending order. If well-tolerated, the three dose levels were predetermined to be 3 mg, 5 mg and 8 mg, leading to a total daily dose of 16 mg, which was based on the PK and PD findings in the previous study.[14] In between the administrations of Δ9-THC or placebo, different measurements for safety, tolerability and biomarkers for were performed. In between the two visits was a wash-out period of 7-14 days.

The four week treatment phase was performed in a randomized, double-blind, placebo-controlled parallel fashion to determine safety, tolerability and efficacy of ECP002A in patients with multiple sclerosis suffering from spasticity and pain. Based on the findings of the challenge phase, patients start with a predetermined daily dose divided over three intakes. After two weeks of treatment the dose for each subject was evaluated, and increased when considered appropriate. The study was approved by the Medical Ethics Committee of the VU University Medical Center (Amsterdam, 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 study is registered in the EU Clinical Trials Register (EUDRACT) under protocol number 2010-022033-28 and in the Dutch clinical trial registry (www.toetsingonline.nl) under dossier number NL34443.029.10. The study was performed by the Centre for Human Drug Research (Leiden, the Netherlands) and VU University Medical Center (Amsterdam, The Netherlands) and was funded by Echo Pharmaceuticals.

Twenty-four patients aged 18 years or older with a diagnosis of progressive (primary or secondary) Multiple Sclerosis according to the revised McDonald criteria [15], who had a disease duration of >1 year and were clinically stable for at least 30 days prior to the start of the challenge phase were to be enrolled. In addition, patients had to have moderate spasticity as defined by an Ashworth score of 2 or higher (range 0-4) and a Kurtzke Expanded Disability Status Scale (EDSS) score between 4.5 and 7.5 at baseline (range 0-10). Spasmolytic therapy was allowed, given that dosage and treatment regimen was stable for at least 30 days prior to study participation and remained stable throughout study participation. Current use of Δ9-THC was exclusionary, as confirmed per urine drug screen. All patients provided written informed consent prior to participation. ECP002A and matching placebo tablets were manufactured and provided under the

responsibility of Echo Pharmaceuticals B.V. Tablets were available in the strengths 1.5 mg and 5 mg Δ9-THC and contained no other active ingredients. Based on the observed pharmacokinetic profile in the First-in-Human study, the dosing regimen for the treatment phase was fixed on intake thrice daily of the starting dose as determined during the challenge phase.

Both the challenge- and treatment phase included biomarkers for efficacy and secondary pharmacodynamic effects. Both types consisted of objective and subjective measurements. The endpoints for the challenge phase were a set of biomarkers for efficacy (objective spasticity: the ratio of the maximum amplitude of the Hoffmann reflex to the maximum M response, recorded over the soleus muscle after electrophysiological stimulation of the popliteal nerve (H/M ratio)[16,17]; subjective spasticity and pain expressed using a Numerical Rating Scale (NRS)); and biomarkers for secondary pharmacodynamic effects (changes in internal / external perception ('feeling high') as measured with the VAS Bowdle[18], changes in alertness, mood and calmness as measured with the VAS Bond & Lader[19] and postural instability), as well as PK endpoints. The primary endpoint for the treatment phase was the H/M ratio[16,17]. Secondary endpoints were biomarkers for efficacy that were either measured in the clinic: Ashworth[20], subjective spasticity (NRS), number of spasms[21] and pain using an NRS [22] and the McGill Pain Questionnaire[23–25]); or measured at home using a daily diary: subjective spasticity (NRS), number of spasms and pain (NRS). Furthermore, a set of functional outcome measures was selected to assess treatment effects: EDSS.[26] the patient's global impression of change (PGIC),[22] quality of sleep as determined by the Pittsburgh sleep quality index (PSQI)[27], walking distance recorded the Timed 25 Foot Walk test (T25FW),[2], the Fatigue Severity Scale (FSS).[29] Finally, the same biomarkers for secondary pharmacodynamic effects were included, namely VAS Bowdle, VAS Bond & Lader and postural instability, in addition to a test to assess visual perception, attention and working-memory, the Symbol Digit Substitution Test (SDST)[30] and Heart Rate.[31]

Statistics

A sample size of 24 patients (including active and placebo treatment) was determined to have 90% power to detect a difference in mean H/M ratio (change from baseline) between placebo and ECP002A of 20%, assuming a standard deviation of differences of 21%, using a paired t-test with a 0.050 two-sided significance level.

For both the challenge and the treatment phase, a randomization schedule was prepared under the responsibility of an independent statistician within CHDR, but not involved in the execution of the study. All staff involved in the clinical execution of the study was blinded until all data was collected and the database was locked. For the treatment phase block randomization was applied. The schedule was sent to the hospital pharmacy and sealed envelopes for code breaking were available for the investigator. Treatment allocation was performed on basis of the date of eligibility of the subject as the subject identification numbers are assigned at that moment.

The results of the pharmacodynamic endpoints were compared between the ECP002A and placebo treated group with an analysis of (co)variance with treatment, time and treatment by time as fixed factor and subject as random factor and, if available the (average) baseline measurement as covariate. Within the model contrasts are calculated over all measurements, only the measurements of week 2 and only the measurements of week 4. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters were estimated using the restricted maximum likelihood method. The general treatment effect and specific contrasts were reported with the estimated difference and the 95% confidence interval, the Least Square Mean (LSM) estimates and the p-value. Graphs of the LSM estimates over time by treatment were presented with 95% confidence intervals as error bars.

As body sway and T25FW data were not normally distributed, the data were log-transformed before analysis and back-transformed after analysis. VAS Bowdle subscale scores were log transformed (10log) after a value of 2 was added to each score, to avoid log transformation from zero. Combined internal, external and feeling high scores were calculated on log transformed data.

All calculations of the pharmacodynamic parameters were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, NC, USA). No adjustments for multiple comparisons were employed.

Post-hoc analysis

Upon review of the data the authors noted that there were patients that indicated to experience no subjective spasticity or pain at the start of the treatment phase, due to the erratic nature of these symptoms of MS. Therefore a subgroup analysis was performed which only included patients indicating to experience subjective spasticity (N=17) or pain (N=17) at the start of treatment. Additionally, in order to differentiate acute from chronic treatment effects, an additional analysis was performed in which the measurements immediately after the first dosing at the start of the treatment phase were excluded from the model and only measurements of week 2 and 4 were used to estimate contrasts.

Pharmacokinetic modelling

The population PK analysis focused on identifying 1 and 2 compartmental structural models with first-order absorption and elimination to describe the data. The random effects structure that was applied included a proportional residual error distribution, and log-normal distributions for the inter-individual variability (IIV) of the PK parameters. The latter was established using an exponential transformation of a normal random effects distribution. Various types of variance-covariance matrices were tested for the inter-individual variability. The estimated population values (both fixed and random effects), were used to determine individual empirical Bayes' estimates (post hoc estimates) of the pharmacokinetic parameters, and related values such as after single dose: area under

the plasma curve from zero to infinity ($AUC_{0-\infty}$), maximal plasma concentration (C_{max}) and terminal half-life ($T_{½}$). Calculations were performed using R v2.12.0 (R Foundation for Statistical Computing, Vienna, Austria). The analyses closely followed the guidelines of the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for performing and reporting population pharmacokinetic analyses.

RESULTS

During the clinical execution a total of 213 potential patients were identified (*Figure 1*). Seventy-three patients were found eligible for screening after telephone prescreening, of which 66 were screened. Between August 2011 and January 2013 a total of 24 patients were enrolled. Baseline characteristics are described in *Table 1*. There were no relevant differences between the treatment groups. All randomized patients completed the challenge phase and were subsequently enrolled in the treatment phase. One subject (randomized to placebo) dropped out during treatment phase due to intolerable Adverse Events.

Challenge phase

None of the measurements included to assess acute effects on spasticity or pain improved significantly after three consecutive dose administrations of ECP002A during the challenge phase (*Table 2*). H/M ratio, NRS for pain and spasticity, were not significantly different between ECP002A and placebo treatment.

Several biomarkers for pharmacodynamic effects were measured during the challenge phase. On a group level, postural instability, heart rate and internal/external perception were significantly affected by ECP002A administration compared to placebo. VAS scores for alertness, calmness and mood were not significantly affected differently by ECP002A than by placebo.

PK/PD modelling

To overcome individual differences in tolerability, this study protocol was designed with PK/PD modelling implemented as an aid to determine individual dose per patient. Results from the crossover challenge phase were modelled to assess the individual dose at which desired effects would occur, in the absence of Adverse Events. However, due to a lack of a robust pharmacodynamic response during the challenge phase (spasticity in H/M ratio or NRS) or other secondary pharmacodynamic effects (e.g. VAS for feeling high), a PK/PD model could not be established on an individual level as intended. Due to large variability in acute pharmacodynamic response, a prediction of plasma concentrations needed to exert a desired pharmacodynamic effect could not be made. The pragmatic approach that we chose instead was uptitration to the level of tolerability to Adverse Events.

During the challenge phase, the highest consecutive dose of 8 mg was not reached in two patients due to Adverse Events. Twelve patients did not experience any Adverse Events during the challenge phase and were dosed at the maximum allowed starting dose per protocol of 24 mg/day (intake 8 mg thrice daily). Seven of these patients were randomized to active treatment. The remaining five patients randomized to active treatment started at a dose of 15 mg/day (intake 5 mg thrice daily), as they experienced intolerable Adverse Events after administration of 8 mg during the challenge phase. Daily doses prescribed in the treatment phase are presented in *Figure 2*. After two weeks of treatment the daily dose was increased with 4.5 mg in all patients, except one. For two patients the dose was subsequently decreased to the starting dose (15 mg/day and 24 mg/day, respectively), due to Adverse Events, indicating that the maximum tolerated dose was reached for these patients.

Treatment phase

Treatment effects were measured using different types of outcome measures, which we categorized as objective or subjective measures of efficacy or secondary pharmacodynamic response results. The results of the measures of efficacy are summarized in *Table 3*. No significant treatment effect was observed on the objective endpoints for spasticity: H/M ratio and Ashworth score. Measures of subjective spasticity did demonstrate a chronic treatment effect in a post-hoc analysis that included patients who reported spasticity at the start of the treatment phase (N=18): a non-significant reduction of 0.94 point (95%CI: -2.05 – 0.17, p=0.0910). Additionally, in a post-hoc analyses of chronic treatment effects in patients who reported pain at the start of treatment (N=17), pain rating was significantly reduced overall during four weeks of treatment with ECP002A (LSM 2.74 for active treatment versus 4.25 for placebo, LSM estimated difference -1.51 (95%CI: -2.75 – -0.28, p=0.0198) (*Figure 3*). When spasticity and pain were measured with a daily diary at home, no significant treatment effect was observed for either pain (-0.47 (95%CI: -2.66 – 1.71, p=0.6581) or spasticity (-0.09 (95%CI -1.99 – 1.81, p=0.9195). Fatigue, measured using the FSS was significantly reduced after 2 weeks of ECP002A treatment, compared with placebo, LSM estimated difference -0.74 (95%CI: -1.43 – -0.04, p=0.0382). This difference was not significant overall: -0.42 (95%CI: -1.03 –0.20, p=0.1769). Other functional outcome measures for efficacy including EDSS, T25FW, PGIC, PSQI did not significantly improve during four weeks of treatment (*Table 3*).

Other secondary pharmacodynamic effects were assessed using two objective biomarkers and two subjective questionnaires. The results are described in *Table 4*. None of the tests evidenced a clinically relevant or statistically significant decline of postural stability, cognitive functioning, mood or psychotomimetic effects. During each treatment visit, the patients were asked which treatment they assumed to be receiving in order to assess possible bias amongst patients. At the end of the treatment, 5 (41.7%) patients in the placebo arm guessed correctly that they had received placebo treatment

and 5 (41.7%) patients were not sure, whereas 8 (66.7%) patients on active treatment guessed correctly that they had been receiving active treatment. Presumed treatment allocation was included in the statistical analyses, but was not a significant factor in treatment response.

A responder analysis was performed, in which responders for spasticity and pain (NRS) and were identified and compared in terms of baseline characteristics. This analysis did not yield significant differences in baseline characteristics between responders and non-responders.

Pharmacokinetics

Pharmacokinetic parameters were derived from the data collected during the challenge phase and during the treatment phase. An overview of the population parameter estimates of the final one compartment PK model for Δ9-THC is shown in *Table 5*. The model includes inter-individual variability on the elimination rate constant (K_{20}) (ω2 Estimate 0.038, Standard Error (SE) 0.018) and inter-occasion variability on the absorption rate constant (K_a) (ω2 Estimate 0.47, SE 0.087). The parameter estimate for K_a is 0.0033 min-1 (95%CI= 0.0025 – 0.0042). The parameter estimate for K_{20}: 0.036 min-1 (95%CI = 0.022 – 0.058). The apparent volume of distribution (V_{app}) is estimated at 285 L. (95%CI = 170-479).

Safety

In total, 200 Adverse Events were recorded, the vast majority of which were classified as mild. Nine (4.5%) Treatment Emergent Adverse Events (TEAEs) were considered moderate and one (0.5%) diagnosis of euphoric mood was judged as severe, as it lead to inability to work or perform daily activity. A summary table of all Adverse Events that were observed more than once is provided in *Table 6*. The most commonly reported Adverse Events were dizziness and euphoric mood, followed by headache, somnolence and fatigue. Whenever a subject reported to be 'feeling high' this was recorded as 'Euphoric mood' in accordance with MEDDRA coding, regardless of whether or not the subject reported euphoria. 'Feeling abnormal' was used to describe changes in internal or external perception, without a patient specifically mentioning the word 'high'. Adverse Events related to disease state and commonly present in this population, including muscular weakness, muscle spasticity, tremor or paraesthesia were recorded only if there was an increase compared to prior to the start of the study, as experienced by the patient. During the treatment phase, five Adverse Events lead to a dose adjustment or omission of dose increase after two weeks of treatment. No Serious Adverse Events (SAEs) occurred during this study. Individual patients reported psychiatric symptoms including confusion, disorientation, irritability or apathy, but this was not endemic for treatment with ECP002A. One subject reported Adverse Events that ultimately led to termination of the participation of this subject after six days of placebo treatment. Four

out of twelve patients on active treatment (33.3%) reported an increase in muscular weakness, of which one was considered moderate. The safety profile observed during active treatment in this study corresponds with the expected Adverse Event profile for this class of drugs.

DISCUSSION

This was a phase II accelerated proof-of-concept study to investigate the adverse effect profile and tolerability, pharmacodynamics and pharmacokinetics of a novel oral formulation of Δ9-THC in patients suffering from progressive MS and spasticity. The current study was performed immediately following the First-in-Human study in healthy volunteers. This study was designed as a hybrid between a typical multiple dose study to investigate PK, PD and safety, and a first-in-patient study to establish proof-of-concept. In this small-sized study (N=24), we were able to meet these objectives. The challenge phase in the study design proved to be an elegant way to investigate PK, PD and safety and decide on an appropriate starting dose per individual patient, to avoid cumbrous and inefficient uptitration during the treatment phase. Moreover, the placebo-controlled crossover setting reduced the risk of bias. Even though dose selection for the treatment phase on an individual level was less refined than initially intended due to variability in acute PD response which impeded determination of a starting dose using PK/PD modelling, the challenge phase still lead to an effective treatment phase of 4 weeks in which a pharmacodynamic treatment effect could be demonstrated in the target population. The effect sizes in terms of treatment effect of the novel oral formulation of Δ9-THC on the clinical endpoints of subjective spasticity, pain and various other clinical endpoints are consistent with the findings of earlier studies on this topic.[11,32]

Overall, treatment with ECP002A was well-tolerated. The most frequently observed events, dizziness, somnolence and changes in mood, including euphoric mood, were related to the primary pharmacological mechanism of action. As such these events were in line with what was expected. One-third (N=4) of the patients treated with ECP002A reported muscular weakness during the treatment phase. This muscular weakness may be a part of the causal pathway of reduced muscle tension leading to the intended treatment of spasticity.

Subjective spasticity measured with an NRS repeatedly during the treatment visits on week 0, 2 and 4 showed an improvement after two and four weeks of treatment, which was significant at two weeks of treatment. The same pattern was observed in a more pronounced way for the NRS for pain measured as an NRS repeatedly during each treatment visit demonstrated an overall improvement in favour of treatment compared to placebo. For both spasticity and pain post-hoc analyses were performed, which only included patients with any subjective spasticity and pain at the start of treatment, as patients with an NRS spasticity or pain of 0 at baseline would not have been susceptible to improvement, thus leading to a statistical floor effect. For NRS spasticity this analysis

in 18 patients emphasized the pattern that was already seen in the Intention-To-Treat (ITT) analysis: a reduction in subjective spasticity, which was significant after two weeks of treatment, but not after four weeks of treatment and no overall significant treatment effect. The NRS for pain (N=17) showed a significant overall treatment effect and an overall reduction in pain of 1.27 point. To differentiate between acute and (sub)chronic treatment effects, an additional analysis was performed that included the results of the baseline and week 2 and 4, and omitted those measurements taken immediately after the first dosing administration of the treatment phase. These analyses accentuated the pattern that was observed in the ITT analysis. No significant treatment effect was observed for the objective measurements of spasticity: H/M ratio and Ashworth. In addition, subjective spasticity and pain measured with an NRS using a daily diary during the treatment phase showed a limited decrease in level of subjective spasticity and pain in patients treated with active treatment compared to placebo, which was not statistically significant.

The data-intensive study design allowed for a thorough investigation of the relationship between acute and chronic pharmacodynamics and pharmacokinetics in the target population. The observed difference between acute and daily treatment effects brought to light the importance of timing of measuring subjective treatment effects.

The discrepancy between the objective and subjective measures of spasticity seen in this study has previously also been observed in phase II and III trials of cannabinoids and even occasionally for currently first-line spasmolytics in patients with MS[33]. According to the reviews by Rog et al. and Lahkan et al.[10,11], only one study[34] reported an improvement in Ashworth score, whereas multiple studies reported only subjective improvement of spasticity. In the aforementioned reviews and clinical studies the validity of the Ashworth scale as an outcome measure for clinically relevant improvement has been questioned, partially due to its limited sensitivity for detecting small changes, as is the case for any objective measure of spasticity[35]. With the goal to further elucidate the pharmacological mechanism of action of Δ9-THC on spasticity in patients suffering from MS in this data-intensive clinical study, this endpoint was included in the protocol nonetheless. Even though the study was performed in a double-blind fashion, cannabinoids are known to induce subjective psychoactive effects, which are potentially undermining blinding of study treatment allocation. This could introduce bias, especially when measuring subjective outcome measures. However, it is impossible to disentangle desired spasmolytic treatment effects from psychoactive "unblinding" effects, as they both result from modulation of the cannabinoid system, and even possibly share the same pathway.

In two out of the three other studies where the effects of Δ9-THC on H/M ratio were investigated, no significant treatment effects were seen after 4-6 weeks treatment with oromucosal cannabis-based therapy[36–38]. In the current study, the baseline H/M ratio values observed in the in the m. soleus, were relatively low compared to what is generally considered hyperreflexia or muscle spasticity[39]. This can possibly be explained by the extent of muscle tone observed in these patients: if muscle tone is increased for a prolonged period of time, reflexes are often diminished due to reduced excitability of

the muscle. This phenomenon was distributed unevenly among the treatment groups, as it was observed at the start of treatment and during the challenge phase preceding the treatment phase and is thus considered a group difference resulting from chance.

During the challenge phase objective (postural stability) and subjective (alterations in internal/external perception or mood) pharmacodynamic effects were affected by ECP002A compared to placebo. However, these pharmacodynamic effects were not observed during the four week treatment phase: patients receiving active treatment did not demonstrate an increase in postural instability after two or four weeks of treatment compared to placebo. In addition, the minor psychoactive effects observed after acute administration of ECP002A during the challenge phase were not observed during the treatment phase. A comparable pattern was observed in the SDST, a measure of attention, short-term memory and psychomotor speed, which demonstrated a slight deterioration after two weeks of treatment with ECP002A compared to placebo. This difference however, was reversed after 4 weeks of treatment, suggesting an improvement in reaction time. This slim statistical difference was skewed due to a ceiling effect, and is considered not clinically relevant. It does indicate, however, that no clinically relevant deterioration in attention and cognitive functioning had taken place during 4 weeks of treatment with ECP002A.These findings appear to imply habituation to the (undesirable) psychoactive effects, which was also observed in previous studies investigating the potential for cannabinoids in therapeutic applications[40].

To our knowledge the first PK model for Δ9-THC in this patient population was created based on the data that was collected during the challenge and treatment phase. The current PK model exhibits the flip-flop kinetics phenomenon, where the $K_a < K_{20}$ and therefore the terminal phase is determined by KA. Although resulting in the best model fit, it is known from previously published PK models [41] that this is not true for Δ9-THC. The reason for this discrepancy is that the mathematical description of the data with a one-compartment oral absorption model can be identical when the value for K_a and K_{20} are interchanged, and the value for V_{app} is then scaled. Such a more physiologically plausible fit with $K_a > K_{20}$ could not be accomplished with the current data, and therefore this should be taken into consideration when interpreting the values for K_a, K_{20} and V_{app}. In line with the variability in pharmacodynamic outcomes observed in this trial, moderate variability in pharmacokinetics was observed during both the challenge and treatment phase, compared to the phase 1 trial investigating the PK and PD of ECP002A [14]. Thus, this increased variability is most likely attributable to an increase in variability observed in a heterogeneous patient population, compared to healthy volunteers. Pharmacokinetic modelling demonstrates a relatively high typical apparent clearance (10.27 L/min) and typical apparent (central) distribution volume compared to previous findings, which is most likely related to a lower bioavailability (previously estimated between 4%-12% [42]. In addition, a slower absorption rate was observed compared to what was observed in a previous study investigating ECP002A in healthy subjects[14]. This was most likely caused by a reduced gastro-intestinal motility, which has previously been reported in patients with MS.[43]

In this four week study, the subjective measures of the severity of experienced spasticity and pain demonstrated a treatment effect compared to placebo. These findings are in line with what has been previously reported on the effects of cannabinoids in patients with MS, when measured in the clinic. However, assessment of subjective effects using a daily diary yielded discrepant results, underlining the importance of selecting the appropriate method for determining treatment effects, when patients are treated at home. The mild Adverse Event profile indicates overall good tolerability for this formulation of Δ9-THC. Pharmacokinetic modelling provided insight in the relatively large variability in absorption between and within patients, thereby underlining the rationale for this combined crossover and parallel study design.

According to recent reviews[44,45] there currently is moderate evidence supporting the use of cannabinoids (Δ9-THC alone or in combination with cannabidiol), for the treatment of spasticity and pain in patients suffering from MS. Even though research thus far has focused on different formulations of cannabinoids (e.g. nabiximols), the findings of the present study demonstrate that the current formulation has the potential to play a role in the treatment of symptoms including spasticity and pain associated with MS.

In conclusion, this study demonstrates that the current formulation of ECP002A exerts a similar effect on spasticity and pain as other Δ9-THC formulations that was detectable after two weeks of treatment and was safe and well-tolerated in the target population. In line with previous reports, spasticity and pain appear to be influenced by Δ9-THC through higher-level CNS modulation of perception of spasticity rather than electrophysiological muscle spasticity itself. Accordingly, ECP002A may have a role in symptomatic treatment of spasticity and pain in MS.

REFERENCES

1 Compston A., Ebers G., Lassmann H., McDonald I., Werkele H. McAlpine's multiple sclerosis, third Edition, London: Churchill Livingstone, 1998.

2 Paisley S, Beard S, Hunn A, Wight J. Clinical effectiveness of oral treatments for spasticity in multiple sclerosis: a systematic review. Mult.Scler. 2002; 8: 319-329.

3 Solaro C, Brichetto G, Amato MP, Cocco E, Colombo B, D'Aleo G, Gasperini C, Ghezzi A, Martinelli V, Milanese C, Patti F, Trojano M, Verdun E, Mancardi GL. The prevalence of pain in multiple sclerosis: a multicenter cross-sectional study. Neurology 2004; 63: 919-921.

4 Jean-Gilles L, Feng S, Tench CR, Chapman V, Kendall DA, Barrett DA, Constantinescu CS. Plasma endocannabinoid levels in multiple sclerosis. J Neurol Sci 2009; 287: 212-215.

5 Scotter EL, Abood ME, Glass M. The endocannabinoid system as a target for the treatment of neurodegenerative disease. Br J Pharmacol 2010; 160: 480-498.

6 Collin C, Davies P, Mutiboko IK, Ratcliffe S. Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. Eur J Neurol. 2007; 14: 290-296.

7 Iversen L. Cannabis and the brain. Brain 2003; 126: 1252-1270.

8 Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci. 2001; 22: 565-572.

9 Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A, Thompson A. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. Lancet 2003; 362: 1517-1526.

10 Rog DJ. Cannabis-based medicines in multiple sclerosis–a review of clinical studies. Immunobiology. 2010; 215: 658-672.

11 Lakhan SE, Rowland M. Whole plant cannabis extracts in the treatment of spasticity in multiple sclerosis: a systematic review. BMC Neurol 2009; 9: 59.

12 Svendsen KB, Jensen TS, Bach FW. Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double-blind placebo controlled crossover trial. BMJ 2004; 329: 253.

13 Killestein J, Hoogervorst EL, Reif M, Kalkers NF, Van Loenen AC, Staats PG, Gorter RW, Uitdehaag BM, Polman CH. Safety, tolerability, and efficacy of orally administered cannabinoids in MS. Neurology 2002; 58: 1404-1407.

14 Klumpers LE, Beumer TL, van Hasselt JG, Lipplaa A, Karger LB, Kleinloog HD, Freijer JI, de Kam ML, van Gerven JM. Novel Delta(9) -tetrahydrocannabinol formulation Namisol(R) has beneficial pharmacokinetics and promising pharmacodynamic effects. Br J Clin Pharmacol 2012; 74: 42-53.

15 Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann.Neurol. 2005; 58: 840-846.

16 Eisen A. Electromyography in disorders of muscle tone. Can J Neurol Sci 1987; 14: 501-505.

17 Matthews WB. Ratio of maximum H reflex to maximum M response as a measure of spasticity. J Neurol Neurosurg Psychiatry 1966; 29: 201-204.

18 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998; 88: 82-88.

19 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-218.

20 Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys. Ther 1987; 67: 206-207.

21 Penn RD. Intrathecal baclofen for severe spasticity. Ann.N.Y.Acad.Sci. 1988; 531: 157-166.

22 Farrar JT, Young JP, Jr., LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 2001; 94: 149-158.

23 Melzack R. The short-form McGill Pain Questionnaire. Pain 1987; 30: 191-197.

24 Wright KD, Asmundson GJ, McCreary DR. Factorial validity of the short-form McGill pain questionnaire (SF-MPQ). Eur J Pain 2001; 5: 279-284.

25 Bolton JE, Wilkinson RC. Responsiveness of pain scales: a comparison of three pain intensity measures in chiropractic patients. J Manipulative.Physiol Ther 1998; 21: 1-7.

26 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33: 1444-1452.

27 Buysse DJ, Reynolds CF, III, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193-213.

28 Cohen JA, Fischer JS, Bolibrush DM, Jak AJ, Kniker JE, Mertz LA, Skaramagas TT, Cutter GR. Intrarater and interrater reliability of the MS functional composite outcome measure. Neurology 2000; 54: 802-806.
29 Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch.Neurol. 1989; 46: 1121-1123.
30 Wilson WH, Ellinwood EH, Mathew RJ, Johnson K. Effects of marijuana on performance of a computerized cognitive-neuromotor test battery. Psychiatry Res 1994; 51: 115-125.
31 Strougo A, Zuurman L, Roy C, Pinquier JL, van Gerven JM, Cohen AF, Schoemaker RC. Modelling of the concentration–effect relationship of THC on central nervous system parameters and heart rate – insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 2008; 22: 717-726.
32 Chohan H, Greenfield AL, Yadav V, Graves J. Use of Cannabinoids for Spasticity and Pain Management in MS. Curr Treat.Options Neurol 2016; 18: 1.
33 Beard S, Hunn A, Wight J. Treatments for spasticity and pain in multiple sclerosis: a systematic review. Health Technol.Assess. 2003; 7: iii, ix-iii,111.
34 Vaney C, Heinzel-Gutenbrunner M, Jobin P, Tschopp F, Gattlen B, Hagen U, Schnelle M, Reif M. Efficacy, safety and tolerability of an orally administered cannabis extract in the treatment of spasticity in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled, crossover study. Mult.Scler. 2004; 10: 417-424.
35 Thaera GM, Wellik KE, Carter JL, Demaerschalk BM, Wingerchuk DM. Do cannabinoids reduce multiple sclerosis-related spasticity? Neurologist. 2009; 15: 369-371.
36 Centonze D, Mori F, Koch G, Buttari F, Codeca C, Rossi S, Cencioni MT, Bari M, Fiore S, Bernardi G, Battistini L, Maccarrone M. Lack of effect of cannabis-based treatment on clinical and laboratory measures in multiple sclerosis. Neurol Sci 2009; 30: 531-534.
37 Leocani L, Nuara A, Houdayer E, Schiavetti I, Del CU, Amadio S, Straffi L, Rossi P, Martinelli V, Vila C, Sormani MP, Comi G. Sativex((R)) and clinical-neurophysiological measures of spasticity in progressive multiple sclerosis. J Neurol 2015; 262: 2520-2527.
38 Russo M, Calabro RS, Naro A, Sessa E, Rifici C, D'Aleo G, Leo A, De LR, Quartarone A, Bramanti P. Sativex in the management of multiple sclerosis-related spasticity: role of the corticospinal modulation. Neural Plast. 2015; 2015: 656582.
39 Kumru H, Murillo N, Samso JV, Valls-Sole J, Edwards D, Pelayo R, Valero-Cabre A, Tormos JM, Pascual-Leone A. Reduction of Spasticity With Repetitive Transcranial Magnetic Stimulation in Patients With Spinal Cord Injury. Neurorehabil.Neural Repair 2010; 24: 435-441.
40 Croxford JL. Therapeutic potential of cannabinoids in CNS disease. CNS Drugs 2003; 17: 179-202.
41 Heuberger JA, Guan Z, Oyetayo OO, Klumpers L, Morrison PD, Beumer TL, van Gerven JM, Cohen AF, Freijer J. Population pharmacokinetic model of THC integrates oral, intravenous, and pulmonary dosing and characterizes short- and long-term pharmacokinetics. Clin Pharmacokinet 2015; 54: 209-219.
42 McGilveray IJ. Pharmacokinetics of cannabinoids. Pain Res Manag. 2005; 10 Suppl A: 15A-22A.
43 el-Maghraby TA, Shalaby NM, Al-Tawdy MH, Salem SS. Gastric motility dysfunction in patients with multiple sclerosis assessed by gastric emptying scintigraphy. Can.J Gastroenterol. 2005; 19: 141-145.
44 Chohan H, Greenfield AL, Yadav V, Graves J. Use of Cannabinoids for Spasticity and Pain Management in MS. Curr Treat.Options Neurol 2016; 18: 1.
45 Whiting PF, Wolff RF, Deshpande S, Di NM, Duffy S, Hernandez AV, Keurentjes JC, Lang S, Misso K, Ryder S, Schmidlkofer S, Westwood M, Kleijnen J. Cannabinoids for Medical Use: A Systematic Review and Meta-analysis. JAMA 2015; 313: 2456-2473.

TABLE 1 Baseline characteristics

		Total (N=24)	Δ9-THC (N=12)	Placebo (N=12)
Age (years)	Mean (SD)	54.3 (8.9)	57.3 (9.0)	51.4 (8.0)
	Range	38-73	41 – 73	38 – 64
Sex (n)	Male	8 (33.3%)	4 (33.3%)	4 (33.3%)
	Female	16 (66.7%)	8 (66.7%)	8 (66.7%)
Disease Duration (years)	Mean (SD)	11.5 (5.8)	10.3 (6.5)	12.6 (4.9)
	Range	3 - 27	3 – 27	6 – 21
Spasticity (n)	Modified Ashworth score of 2	16 (66.7%)	8 (66.7%)	8 (66.7%)
	Modified Ashworth score of 3	8 (33.3%)	4 (33.3%	4 (33.3%
EDSS (total)	Mean (SD)	6.2 (0.9)	6.2 (1.2)	6.3 (0.5)
	Range	4.5-7.5	4.5 – 7.5	5.5 – 7.5

SD = standard deviation / EDSS = Kurtzke Expanded Disability Status Scale

TABLE 2 Summary of analysis of measures of pharmacological effects during the challenge phase

	LS Means		Contrasts	LS Means change from baseline	
Parameter	**Placebo**	**Active**	**Estimate of difference (95%CI)**	**Placebo**	**Active**
OBJECTIVE AND SUBJECTIVE MEASURES FOR EFFICACY					
H/M Ratio	0.333	0.326	-.007 (-.070, 0.057) p=0.8238	-0.002	-0.008
NRS: spasticity	3.35	3.64	0.28 (-0.01, 0.58) p=0.0595	-0.73	-0.45
NRS: neuropathic pain	2.75	2.71	-0.03 (-0.41, 0.34) p=0.8470	-0.23	-0.27
OBJECTIVE MEASUREMENTS FOR SECONDARY PHARMACODYNAMIC EFFECTS					
Body sway (mm)	775.3	919.4	18.6% (5.9%, 32.8%) p=0.0067	-2.0%	16.3%
Heart rate (supine) (bpm)	71.1	74.6	3.5 (1.4, 5.7) p=0.0025	-1.0	2.5
SUBJECTIVE MEASUREMENTS FOR SECONDARY PHARMACODYNAMIC EFFECTS					
VAS External log (mm)	0.323	0.384	0.061 (0.028, 0.094) p=0.0009	-0.022	0.040
VAS Internal log (mm)	0.321	0.352	0.030 (0.003, 0.057) p=0.0295	-0.030	0.000
VAS feeling high log (mm)	0.322	0.542	0.220 (0.067, 0.373) p=0.0070	0.019	0.239
VAS Alertness (mm)	54.5	52.7	-1.7 (-4.1, 0.6) p=0.1342	1.3	-0.4
VAS Calmness (mm)	53.8	54.7	0.9 (-1.5, 3.3) p=0.4289	1.8	2.7
VAS Mood (mm)	55.2	56.0	0.8 (-0.4, 2.0) p=0.1699	0.5	1.4

H/M ratio = ratio of the maximum amplitude of the Hoffmann reflex to the maximum M response / NRS: Numerical Rating Scale / mm = millimeter / bpm = Beats Per Minute / VAS = Visual Analogue Scale / LS Means = Least Square Means / 95%CI = 95% Confidence Interval

TABLE 3 Summary of analyses of measures for efficacy during treatment phase

	LS Means		Contrasts			LS Means change from baseline	
Parameter	**Placebo**	**Active**	**Overall Estimate of difference (95%CI)**	**Week 2 Estimate of difference (95%CI)**	**Week 4 Estimate of difference (95%CI)**	**Placebo**	**Active**
OBJECTIVE EFFICACY							
H/M ratio (Score 0-1)	0.385	0.386	0.001 (-.178, 0.179) p=0.9929	-.009 (-.198, 0.180) p=0.9216	0.031 (-.069, 0.131) p=0.5269	0.052	0.053
Ashworth (Score 1-4)	1.70	1.60	-0.11 (-0.41, 0.19) p=0.4615	-0.10 (-0.52, 0.31) p=0.6142	-0.11 (-0.53, 0.30) p=0.5888	-0.21	-0.32
SUBJECTIVE EFFICACY							
NRS: spasticity (Score 1-10)	3.61	3.23	-0.38 (-1.30, 0.53) p=0.3907	-1.00 (-1.98, -0.03) p=0.0445	-0.31 (-1.29, 0.66) p=0.5176	-0.22	-0.61
NRS: spasticity (Score 1-10): subgroup (N=18)	4.50	3.81	-0.69 (-1.79, 0.42) p=0.2038	-1.23 (-2.39, -0.07) p=0.0387	-0.84 (-2.00, 0.32) p=0.1450	-0.46	-1.15
NRS: spasticity (Score 1-10): subgroup (N=18) Chronic treatment	4.52	3.57	-0.94 (-2.05, 0.17) p=0.0910				
NRS: pain (Score 1-10)	2.95	2.15	-0.81 (-1.66, 0.04) p=0.0618	-1.09 (-1.98, -0.20) p=0.0183	-0.85 (-1.74, 0.04) p=0.0612	-0.21	-1.02
NRS: pain (Score 1-10): subgroup (N=17)	4.26	2.99	-1.27 (-2.50, -0.04) p=0.0439	-1.69 (-2.96, -0.41) p=0.0124	-1.38 (-2.65, -0.10) p=0.0360	-0.27	-1.54
NRS: pain (Score 1-10): subgroup (N=17) Chronic treatment	4.25	2.74	-1.51 (-2.75, -0.28) p=0.0198				
Diary: spasticity (Score 1-10)	3.65	3.56	-0.09 (-1.99, 1.81) p=0.9195				

Diary: pain (Score 1-10)	2.57	2.10	-0.47 (-2.66, 1.71) p=0.6581				
EDSS (Score 1-10): subanalysis	6.42	6.39	-0.03 (-0.22, 0.17) =0.7650	-0.12 (-0.34, 0.11) =0.2935	0.06 (-0.16, 0.28) p=0.5759	-0.05	-0.08
T25 feet walk (ft/sec): subanalysis	3.70	3.87	4.8% (-7.8, 19.1%) =0.4425	4.0% (-9.1, 19.0%) =0.5481	5.6% (-7.8, 21.0%) p=0.4080	7.5%	12.6%
PGIC (Score 1-7)	4.08	3.59	-0.49 (-1.19, 0.21) p=0.1632	-0.58 (-1.33, 0.16) p=0.1213	-0.39 (-1.14, 0.35) p=0.2900	-0.22	-0.71
PSQI (Score 0-21)	4.15	5.15	1.00 (-0.83, 2.84) p=0.2688	0.36 (-1.62, 2.33) p=0.7147	1.64 (-0.33, 3.62) p=0.0996	-1.41	-0.41
FSS (Score 1-7)	4.33	3.92	-0.42 (-1.03, 0.20) p=0.1769	-0.74 (-1.43, -0.04) p=0.0382	-0.44 (-1.13, 0.25) p=0.2065	-0.13	-0.55

H/M ratio = ratio of the maximum amplitude of the Hoffmann reflex to the maximum M response / NRS: Numerical Rating Scale / EDSS = Kurtzke Expanded Disability Status Scale / T25FW = Timed 25 foot walk / PGIC = Patients Global Impression of Change / PSQI = Pittsburgh Sleep Quality Index / FSS = Fatigue Severity Scale / LS Means = Least Square Means / 95%CI = 95% Confidence Interval

TABLE 4 Summary of analyses of measures for secondary pharmacodynamic effects during treatment phase

	LS Means			Contrasts			LS Means change from baseline	
Parameter	**Placebo**	**Active**	**Treatment P-value**	**Overall Estimate of difference (95%CI)**	**Week 2 Estimate of difference (95%CI)**	**Week 4 Estimate of difference (95%CI)**	**Placebo**	**Active**
OBJECTIVE MEASUREMENTS								
Body sway (mm)	861.8	887.4	0.7024	3.0% (-12.5%, 21.2%) p=0.7024	9.8% (-7.7%, 30.7%) p=0.2722	0.5% (-15.8%, 20.0%) p=0.9527	-6.4%	-3.7%
SDST: Total correct responses (N)	55.5	55.8	0.0671	0.3 (-0.0, 0.6) p=0.0671	-1.2 (-2.7, 0.3) p=0.1298	2.5 (0.9, 4.0) p=0.0017	-0.1	0.2
SDST: Percentage correct responses (%)	97.73	97.84	0.0275	0.11 (0.01, 0.20) P=0.0275	0.16 (-0.92, 1.23) p=0.7745	1.58 (0.50, 2.66) p=0.0042	-0.08	0.03
SDST: Average reaction time (msec)	2944.1	2929.9	0.1521	-14.2 (-34.2, 5.77) p=0.1521	137.1 (30.01, 244.2) p=0.0123	-147 (-255, -39.0) p=0.0078	-4.29	-18.50
SUBJECTIVE MEASUREMENTS								
VAS Bowdle: External log(mm)	0.312	0.340	0.2153	0.028 (-.018, 0.075) p=0.2153	0.038 (-.013, 0.089) p=0.1421	0.034 (-.018, 0.085) p=0.1890	-0.000	0.028
VAS Bowdle: Internal log(mm)	0.325	0.320	0.6331	-.005 (-.026, 0.016) p=0.6331	-.002 (-.026, 0.021) p=0.8285	-.010 (-.033, 0.013) p=0.4011	-0.010	-0.015
VAS Bowdle: feeling high log(mm)	0.365	0.475	0.2020	0.110 (-.063, 0.283) p=0.2020	0.113 (-.074, 0.300) p=0.2277	0.159 (-.028, 0.346) p=0.0935	0.064	0.174
VAS Bond and Lader: Alertness (mm)	51.2	52.1	0.3295	0.8 (-0.9, 2.6) p=0.3295	0.7 (-1.2, 2.7) p=0.4550	0.9 (-1.0, 2.9) p=0.3348	-0.7	0.2
VAS Bond and Lader: Calmness (mm)	50.4	52.6	0.1422	2.2 (-0.8, 5.3) p=0.1422	3.3 (0.1, 6.6) p=0.0462	2.1 (-1.2, 5.3) p=0.2046	0.1	2.4
VAS Bond and Lader: Mood (mm)	52.0	53.9	0.2469	1.9 (-1.4, 5.2) p=0.2469	2.0 (-1.5, 5.4) p=0.2470	2.6 (-0.8, 6.0) p=0.1283	-0.1	1.8

mm = millimeter / SDST = Symbol Digit Substitution Test / VAS = Visual Analogue Scale / msec = millisecond

TABLE 5 Population parameter estimates of one compartment PK model for Δ9-THC.

	PK Parameter	95% CI	CV (%)
Primary parameter			
k_a (min^{-1})	0.0033	(0.0025;0.0042)	77.7
Lag time (min)	5.26	(5.11;5.41)	-
V_{app} (L)	285	(170;479)	
k_{20} (min^{-1})	0.036	(0.022;0.058)	19.6
CL_{app} ($L*min^{-1}$)	10.27	(8.72; 12.1)	19.6
$T_{1/2}$ (min)	213.6	(165; 275)	77.8
Inter-individual variability	*W^2 Estimate*	*S.E.*	*Shrinkage (%)*
k_{20}	0.038	0.018	32.2
k_a (IOV)	0.47	0.087	6.8 - 43.1
Residual error	*σ^2 Estimate*	*S.E.*	*Shrinkage (%)*
Proportional	0.18	0.018	9.4

*SE = Standard Error / K_a = absorption rate constant / V_{app} = apparent volume of distribution / K_{20} = elimination rate constant / CL_{app} = apparent clearance / IOV = inter-occasion variability / Clearance (CL) = aVd * k_{20}*

TABLE 6 Overview of Adverse Events and incidence of events reported more than once.

	Dose finding phase		Treatment phase	
	Δ9-THC N=24 n (%)	**Placebo N=24 n (%)**	**Δ9-THC N=12 n (%)**	**Placebo N=12 n (%)**
Number of subjects with at least one Adverse Event	20 (83.3%)	10 (41.7%)	10 (83.3%)	7 (58.3%)
Number of different Adverse Events	15	9	34	15
OVERVIEW OF ADVERSE EVENTS (INCIDENCE >1)				
Nervous system				
Dizziness	6 (25.0%)	1 (4.2%)	7 (58.3%)	1 (8.3%)
Headache	3 (12.5%)	2 (8.3%)	6 (50.0%)	3 (25.0%)
Somnolence	6 (25.0%)	-	3 (25.0%)	2 (16.7%)
Muscular weakness	1 (4.2%)	1 (4.2%)	4 (33.3%)	1 (8.3%)
Muscle spasticity	-	-	3 (25.0%)	3 (25.0%)
Paresthesia	-	1 (4.2%)	2 (16.7%)	-
Tremor	1 (4.2%)	-	2 (16.7%)	-
Tinnitus	-	-	2 (16.7%)	-
Psychiatric / mood				
Euphoric mood	5 (20.8%)	1 (4.2%)	4 (33.3%)	2 (16.7%)
Disturbance in attention	1 (4.2%)	1 (4.2%)	-	-
Insomnia	-	-	1 (8.3%)	1 (8.3%)
General disorders and administration site conditions				
Fatigue	3 (12.5%)	2 (8.3%)	2 (16.7%)	3 (25.0%)
Feeling abnormal	4 (16.7%)	-	1 (8.3%)	2 (16.7%)
Feeling hot	1 (4.2%)	-	2 (16.7%)	2 (16.7%)
Gastrointestinal				
Dry mouth	1 (4.2%)	-	2 (16.7%)	-
Nausea	1 (4.2%)	-	-	1 (8.3%)
Increased appetite	1 (4.2%)	-	1 (8.3%)	-

AE = Adverse Event

FIGURE 1 Disposition of patients

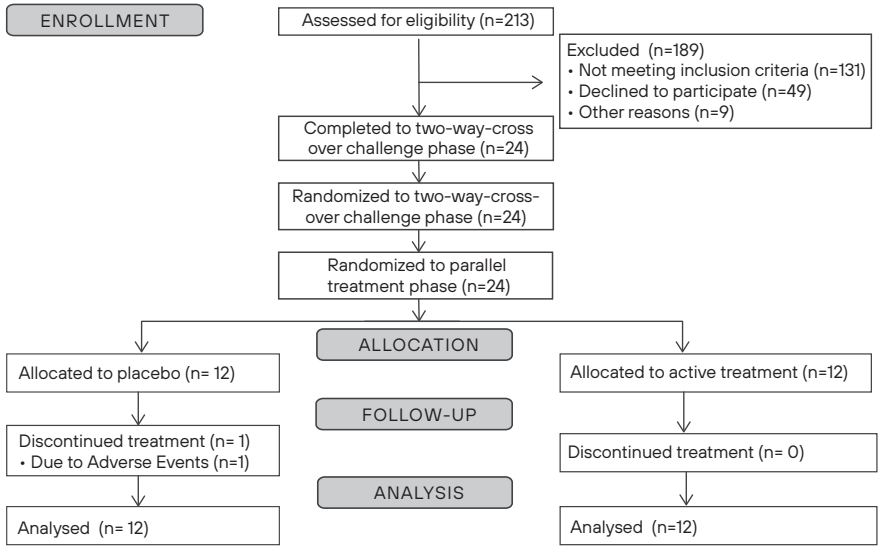
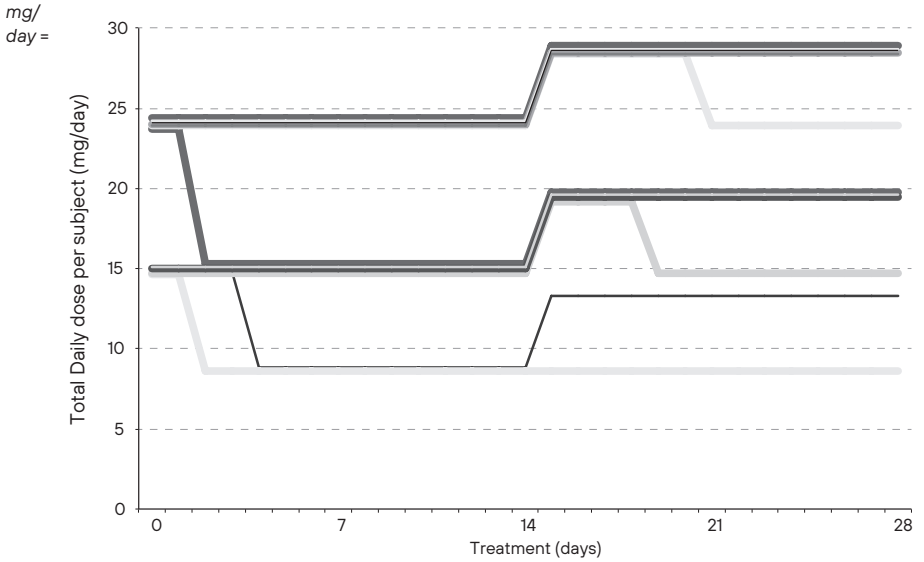
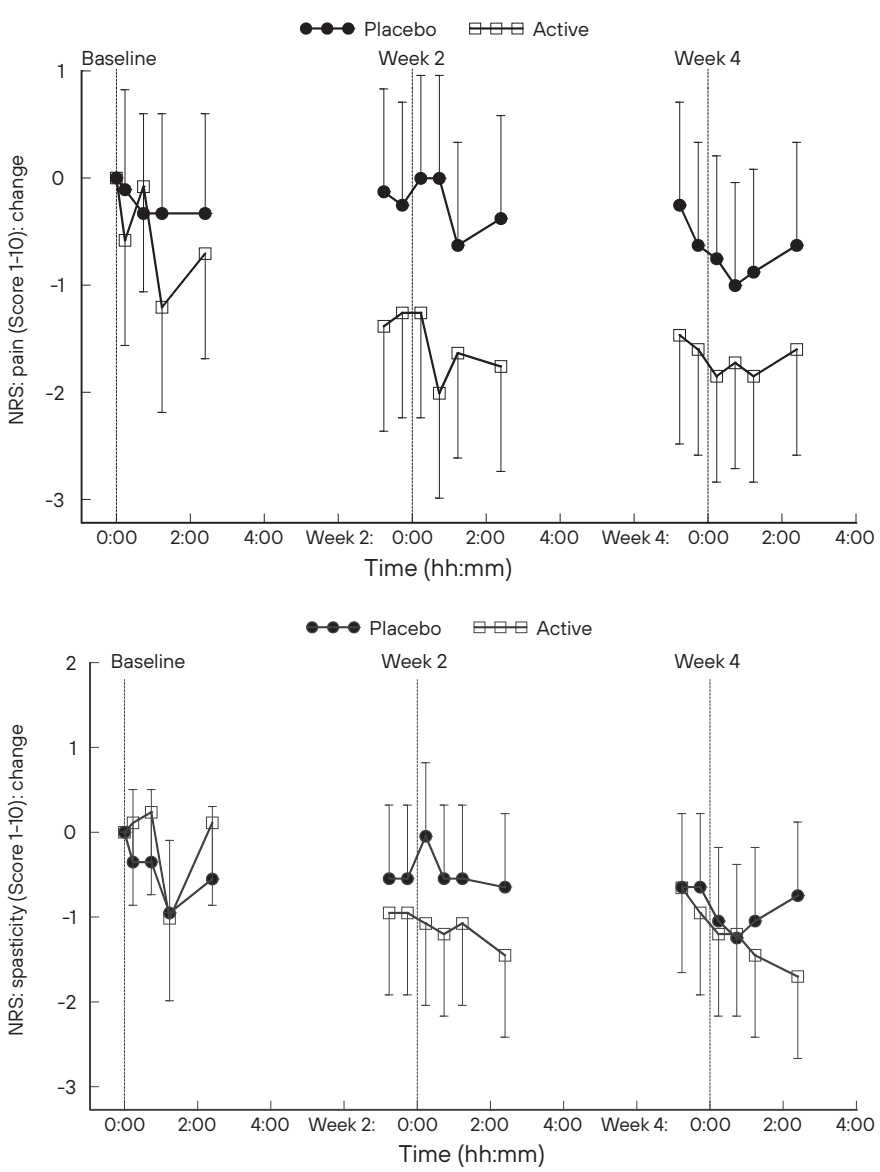


FIGURE 2 Total daily dose of Δ9-THC prescribed per subject in the treatment phase (intake thrice daily) (N=12)



milligram per day.

FIGURE 3A AND B Post-hoc analyses: LSM change from baseline time profile for NRS for (A) spasticity (N=18) and (B) pain (N=17).



NRS = Numerical Rating Scale / 95%CI = 95% Confidence Interval

CHAPTER 4

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

G van Amerongen,[1] P Siebenga,[1] M L de Kam,[1] J L Hay,[1]* G J Groeneveld[1]*
** joint last authors*

1 Centre for Human Drug Research (CHDR) / Leiden University Medical Centre (LUMC) Leiden, The Netherlands

ABSTRACT

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

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

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

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

INTRODUCTION

The complex clinical reality of pain medicine demands novel therapeutics. A multi-modal battery of evoked pain tasks could be a useful tool to investigate the analgesic properties of novel compounds, but needs to be pharmacologically validated for specific classes of compounds. In the present study the effects of three oral drugs were investigated and compared to placebo: delta-9-tetrahydrocannabinol (Δ9-THC), paracetamol and promethazine.

Different cannabinoids have previously been shown to be effective in various pain conditions, including neuropathic pain related to oncological disease. (Vadalouca et al. 2012) Δ9-THC has been shown to be an effective analgesic in preclinical studies and clinical trials. However, previous formulations of cannabinoid Δ9-THC are also known for variable pharmacokinetic profiles and pharmacodynamic responses. (Huestis 2007) To overcome barriers in clinical application, novel formulations and cannabinoids are under development. (Klumpers et al. 2012)

Even though paracetamol is one of the most widely used medications in the world, there is still debate regarding its exact mechanism of action. Paracetamol is thought to be a weak inhibitor of prostaglandins (PG) synthesis. The subsequent main driving mechanism of paracetamol analgesia is not completely understood. It has been proposed that it exerts most of its effects through COX-2 inhibition, but also inhibition of endocannabinoids has been proposed. In addition, various neurotransmitter systems (e.g. serotonergic, opioid and noradrenaline) are thought to be involved. (Bertolini et al. 2006; Boychuk et al. 2015; Graham et al. 2013; Koppel et al. 2014)

To investigate the role of sedation rather than analgesic effects of psychoactive compounds a negative control was included in the current study in the form of the H1 antihistaminergic promethazine (50 mg). Even though it has been observed in preclinical research that H1 antihistaminergic drugs may have analgesic potential, this has not been replicated in clinical practice for oral formulations administered alone. (Rumore and Schlichting 1985; Raffa 2001) Therefore we considered this sedative compound suitable as a comparator drug without analgesic effects

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

The battery of evoked pain tasks has been pharmacologically validated by investigating a broad range of analgesics from various classes, with diverse but well-known mechanisms of action. (Okkerse, van Amerongen, et al. 2016) This first pharmacological validation study demonstrated the necessity of utilising a range of pain tasks in

early-phase drug research. Namely, each compound provided a unique fingerprint of effects on the test battery. These findings emphasized the importance of utilising a range of pain tasks, rather than a single pain task, when determining the profile of analgesic effects of a compound in early phase drug development. Building on this knowledge, the present study investigated the effects of two (classes of) analgesics, paracetamol and Δ9-THC, and additionally the effects of sedation using promethazine as a negative control.

METHODS

Subjects and study design

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

Each subject provided written informed consent before any screening procedures were performed. A total of 24 healthy subjects (12 males and 12 females) between 18 and 45 years of age with a body mass index of 18 to 30 kg/m^2 were enrolled. The subjects underwent a full medical screening, including medical history anamnesis, a physical examination, blood chemistry and haematology, urinalysis, electrocardiogram (ECG) and assessment of the minimal erythema dose (MED) for ultraviolet B (UVB) light to assess eligibility. Subjects with a clinically significant known medical condition, in particular any existing condition that would affect sensitivity to cold or pain were excluded. Subjects with Fitzpatrick skin type V or VI, widespread acne, tattoos or scarring on the back were excluded due to the inability to accurately assess MED. Also any subject, who was a regular user of any illicit drugs, had a history of drug abuse or a positive drug screen at screening was excluded. Smoking and the use of xanthine-containing products were not allowed during dosing days. Alcohol was not allowed at least 24 hours before each scheduled visit and during the stay in the research unit.

Study drugs

Paracetamol (1000 mg), Δ9-THC (10 mg), promethazine (50 mg) or placebo was given as a single dose. Paracetamol 1000 mg is within the labelled dose range in the European

Union (EU) and has been shown to be effective in reducing various types of pain. The currently used formulation of Δ9-THC (Namisol®, Echo Pharmaceuticals) has been administered in multiple studies including healthy volunteers (Klumpers et al. 2012) and different patient populations. (Ahmed et al. 2014; Utomo et al. 2015; van Amerongen et al. 2017) Δ9-THC has potential side effects, but is generally considered well-tolerated, even in high dosages. Promethazine is a classic H1 antihistamine with some anticholinergic effects. Daily doses up to 150 mg are prescribed for the treatment of allergic rhinitis and motion sickness. Single doses up to 50 mg are prescribed to induce mild sedation.

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

Pharmacodynamic assessments

Pain detection and tolerance thresholds were measured using a battery of evoked pain tasks, as described previously. (Hay et al. 2016; Okkerse, Alvarez-Jimenez, et al. 2016; Okkerse, Hay, Sitsen, et al. 2016; Okkerse, Hay, Versage, et al. 2016; Okkerse, van Amerongen, et al. 2016) The test battery consists of an integrated range of pain tasks for measuring different modalities of pain. Assessments were conducted twice pre-dose (double baseline) and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours post-dose by trained personnel. Each measurement round was performed in a fixed order and took approximately 30 minutes to complete. To eliminate the risk of tissue damage, all pain tasks had a maximum safety cut-off. The aim of the test battery is to assess as objectively as possible the levels of pain induced by different noxious mechanisms in human subjects. A training session was included as part of the screening examination to reduce learning effects during the study and exclude non-responders (i.e. subjects who reach PDT at >80% of the maximum at any of the nociceptive tasks, excluding the heat pain task) or extreme responders (subjects indicating to be intolerable to any of the nociceptive tasks). All measurements were performed in a quiet room with ambient illumination. Per session, there was only one subject present in the same room. To reduce variability from affects associated with fear of pain, the subjects themselves were responsible for starting and ending each pain task.

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

For the electrical stimulation task, the pressure stimulation task and the cold pressor task, pain intensity was measured continuously (beginning from when the first stimulus was applied until the end of the test) using an electronic visual analogue scale

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

In addition to the evoked pain tasks, subjective assessment of sedation and psychotomimetic effects were included as PD outcome measures. Visual analogue scales (VAS) as originally described by Norris (Norris 1971) have often been used previously to quantify subjective effects of a variety of sedative agents. (de Visser et al. 2001; Norris 1971) A set of VAS scales assessing alertness, mood, and calmness (Bond and Lader 1974) were used for subjective assessment of sedation. The VAS allows the subjects to evaluate their current subjective states. Each VAS scale consists of 2 words representing opposite feelings placed to the left and right of a horizontal line. The subject is asked to mark his/her current feelings. Subjective psychotomimetic (psychedelic) effects were evaluated using VAS Bowdle. This scale has been used extensively to quantify subjective psychotomimetic effects of psychoactive compounds, including ketamine. (Bowdle et al. 1998) Bowdle Psychotomimetic Effects Scores consist of thirteen visual analogue lines ranging from 0 ('not at all) to 100 ('extremely') (van Steveninck 1993), addressing various (abnormal) states of mind.

Sample size and randomisation

Based on literature, PDT for the cold pressor assessment was used for the sample size calculation as this assessment has been shown sensitive to the effects of Δ9-THC in previous research. (Cooper, Comer, and Haney 2013a) For the cold PDT, a sample size of 24 subjects has 80% power to detect a difference in means of 35%, assuming a standard deviation of differences of 0.5, using a paired t-test with a 0.05 two-sided significance level. For the sample size calculation, placebo data from a previous study with the battery of pain tasks were used to determine variability. (Okkerse, van Amerongen, et al. 2016) The balanced Williams design randomization code was generated using SAS version 9.1.3 by a study-independent statistician.

Statistical analysis

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

RESULTS

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

Pharmacodynamics

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

Furthermore, the results for the subjective scales for cognitive functioning and psychotomimetic symptoms are presented in *Table 3*. Paracetamol did not show a

significant reduction in pain sensation compared to placebo. A small increase in AUC (p=0.0314) was observed for the pressure pain task, indicating a slight increase in perceived pain sensation. Treatment with paracetamol did not lead to any observable changes in subjective cognitive functioning or mood. Promethazine demonstrated a statistically significant reduction in PTT for the cold pressor pain task (p=0.0189) and for the pressure stimulation task (p=0.0149), as well as an increase in AUC (p=0.0032), indicating an increase in pain sensation. In addition to the pharmacodynamic effects of promethazine on the pain task battery, a reduction in subjective alertness (p=0.0002) was observed. Δ9-THC did not show a statistically significant analgesic effect on any of the pain tasks. For the electrical stimulation task, the PTT was significantly decreased by -12.7%, (p=0.0134), also indicating an increase in pain sensation. Furthermore, a significant reduction was observed for the pressure stimulation task PTT (p=0.0126) and AUC (p=0.0001). In addition to the effects observed on the pain task battery, Δ9-THC also demonstrated other pharmacodynamic effects, including a reduction on the composite scale for alertness (-p=<.0001) and an increase on the composite scale for calmness (p=<.0001) compared to placebo. Moreover, significant psychotomimetic effects were observed expressed in changes in internal perception (p=<.0001) and external perception (p=<.0001), measured using the VAS Bowdle, as well as VAS Feeling high (p=<.0001). Of note, psychotomimetic effects were virtually absent after placebo treatment, thereby leading to high significance levels even at small effect sizes.

Safety

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

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

DISCUSSION

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

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

At first glance it may have been surprising that the battery of evoked pain tasks was not sensitive to detect analgesic effects of paracetamol over a period of 4 hours post-dose, as it is among the most widely used analgesics worldwide. It has been shown to be effective in the treatment of different types of clinical pain, although not all. While it is effective at reducing postoperative pain (McNicol et al. 2016; Weil et al. 2007), episodic tension headache (Stephens, Derry, and Moore 2016) and acute migraine (Derry, Moore, and McQuay 2010), there is no evidence for its effectiveness in treating lower back pain (Williams et al. 2014; Saragiotto et al. 2016) or pain related to osteoarthritis. (Machado et al. 2015) However, when looking at available literature on human evoked pain tasks in healthy volunteers, the image becomes more diffuse. For each of the pain tasks that were investigated in more than one clinical trial, positive as well as negative results have been reported: mixed results were obtained using the Cold pressor (Miner 2009; Munsterhjelm et al. 2005; Tiippana et al. 2013; Yuan et al. 1998), there was a single negative study for contact heat (Tiippana et al. 2013), and again mixed

results for electrical pain (Bandschapp et al. 2011; Filitz et al. 2008; Olesen et al. 2007; Tiippana et al. 2013), mixed results for pressure pain (Olesen et al. 2007; Pickering et al. 2008; Romundstad et al. 2006) and only a single study showing analgesic effects on inflammatory pain using the UVB model. (Ortner et al. 2012) Interestingly, the published studies measuring pain experience (post-test NRS or post-test VAS) tend to be more likely to show analgesia by paracetamol than studies measuring the more objective pain thresholds. This may indicate that paracetamol exerts its analgesic effect on the aspect of subjective pain experience by means of pain modulation rather than exerting changes in nociceptive pain perception thresholds. This differential effect was not observed in the present study. Additionally, the analgesic effects of paracetamol in human evoked pain models tend to be more subtle than the effect sizes that were used for the power calculation, therefore the study may have been underpowered. This applies specifically to for the Cold pressor task, where a non-significant increase in pain thresholds was observed. Summarising, based on the findings in literature and the aforementioned hypothesis, the outcome might have been different if a two-way crossover compared to placebo design was used in which different endpoints, i.e. Laser Evoked Potentials (Arendt-Nielsen, Nielsen, and Bjerring 1991; Nielsen, Bjerring, and Arendt-Nielsen 1991; Nielsen et al. 1992), were investigated.

Medicinal use of cannabis dates back tens of thousands of years. (Abel 1980) In the last decade the role for (plant-derived or synthetic) cannabinoids has shifted from complementary medicine to regular care for pain related to oncology (Abrams 2016) and neuropathic pain resulting to spinal cord injury (Wilsey et al. 2016) or Multiple Sclerosis (MS). (Turcotte et al. 2015; Russo et al. 2016) The oral formulation of Δ9-THC (Namisol®) that was used in the current study has been shown to be effective in reducing neuropathic pain in a recently performed study in 24 patients suffering from progressive MS after 4 weeks of chronic treatment. (van Amerongen et al. 2017) However, given its interaction with the endocannabinoid system it cannot be considered an "antinociceptive" analgesic, even if it may have analgesic effects in some conditions. This is reflected in the results of clinical studies using human evoked pain models to investigate pharmacology and mechanism of action. Only two studies investigating the effects of either inhaled cannabis or oral Δ9-THC showed a statistically significant reduction in pain sensation on the cold pressor task (Cooper, Comer, and Haney 2013b) or the heat pain task. (Greenwald and Stitzer 2000) Two other studies investigating the effects on heat pain alone, did not demonstrate this improvement. (Redmond et al. 2008; Roberts, Gennings, and Shih 2006) The results of the present study are in line with the results of Naef et al. (Naef et al. 2003) and Kraft et al. (Kraft et al. 2008), who showed lack of analgesia on a set of pain tasks and even a significant or non-significant increase in pain sensation for electrical pain and cold pressor. The finding of Δ9-THC induced hyperalgesia has also been observed in the clinic. (Beaulieu 2006) A possible explanation is that this effect is dose-related, due to a bell-shaped effect curve. As proposed by Walter et al. (Walter, Oertel, and Lotsch 2015), this narrow therapeutic window may be the result of co-activation of TRPA1 and TRPV1 channels along with CB1 receptors by Δ9-THC at higher concentrations. The dose of 10 mg of the oral formulation

of Δ9-THC was the highest single dose that is administered to healthy volunteers of this formulation to date. (Klumpers et al. 2012) Due to inter-subject variability this dose may have been too high for some, as in four subjects pharmacodynamic assessments were delayed or omitted as a result of adverse events associated with subjective effects and nausea. However, on a group level only a reasonable reduction in subjective alertness was reported. Furthermore, a post-hoc analysis excluding the measurements that may have been affected by AEs of moderate intensity did not lead to a different interpretation of the results compared to the Intention-To-Treat (ITT) analysis. Therefore the ITT analysis was maintained and reported here. On the other hand, it is known that chronic and even acute exposure to Δ9-THC can induce a "transient amotivational state" (Lawn et al. 2016), which may be misinterpreted as an apparent hyperalgesic state. This hyperalgesic state is in fact the result of the psychotropic effect profile of Δ9-THC, as subjects become less motivated to complete the pain tasks. Despite our efforts, human evoked pain tasks remain also sensitive to the affective components of pain sensation, and thus susceptible to detect changes in motivation as well as pure analgesia.

Over the recent years some evidence has gathered for the effectiveness of antihistaminergic drugs as adjuvant in the treatment of various pain states. (Behrbalk et al. 2014; Friedman et al. 2016; Friedman et al. 2013) However, there is no evidence for any acute analgesic effect in humans. As such, promethazine (50 mg) was selected as a negative control for Δ9-THC and to investigate the effects of sedation on the battery of evoked pain tasks. In addition to an increased sensitivity for electrical and pressure pain, a decreased pain detection threshold for inflammatory pain was observed. Even though histamine is involved in the initial phase of erythema development, this role is not prominent in the delayed erythemic response (Woodward and Owen 1982) and as such administration 24 hours after UVB exposure is not likely to have influenced the pathophysiology of the UVB induced erythema. Thus, the results of promethazine treatment may indicate a reduction of pain endurance, which could result from reduced motivation associated with sedative effects (expressed as a reduction in subjective alertness), rather than suppositious analgesia resulting from delayed or impaired responsiveness.

The present study adds to a body of research studies in which this exact battery of evoked pain tasks was used to investigate various analgesic compounds alone (Okkerse, Hay, Sitsen, et al. 2016; Okkerse, Hay, Versage, et al. 2016; Okkerse, van Amerongen, et al. 2016) or combined (Okkerse, Alvarez-Jimenez, et al. 2016). As such, the battery of evoked pain tasks is pharmacologically validated for the effects of cannabinoids and sedatives. The battery of evoked pain tasks was not sensitive to detect analgesic effects of paracetamol, but that finding by itself provides information on the much debated and yet unrevealed pharmacological mechanism of action, as we are able to compare the results to other compounds with known mechanism of action. As recognized before (van Amerongen et al. 2016; Lotsch et al. 2016; Lotsch, Oertel, and Ultsch 2014), translatability of findings from human evoked pain models to clinical pain remains elusive. Nonetheless, if used prudently, this battery of pain tasks can provide invaluable information on pharmacodynamic and pharmacokinetic relationships in the early phases of drug development, especially when combined with other neurocognitive assessments.

REFERENCES

Abel, Ernest L. 1980. 'Cannabis in the Ancient world.' in, *Marijuana - The First Twelve Thousand Years* (Plenum Publishers: New York City).

Abrams, D.I. 2016. 'Integrating cannabis into clinical cancer care', *Curr Oncol*, 23: S8-S14.

Ahmed, A. I., G. A. van den Elsen, A. Colbers, M. A. van der Marck, D. M. Burger, T. B. Feuth, M. G. Rikkert, and C. Kramers. 2014. 'Safety and pharmacokinetics of oral delta-9-tetrahydrocannabinol in healthy older subjects: a randomized controlled trial', *Eur Neuropsychopharmacol*, 24: 1475-82.

Arendt-Nielsen, L., J.C. Nielsen, and P. Bjerring. 1991. 'Double-blind, placebo controlled comparison of paracetamol and paracetamol plus codeine – a quantitative evaluation by laser induced pain', *Eur J Clin Pharmacol*, 40: 241-47.

Bandschapp, O., J. Filitz, A. Urwyler, W. Koppert, and W. Ruppen. 2011. 'Tropisetron blocks analgesic action of acetaminophen: a human pain model study', *Pain*, 152: 1304-10.

Beaulieu, P. 2006. 'Effects of nabilone, a synthetic cannabinoid, on postoperative pain', *Can J Anaesth*, 53: 769-75.

Behrbalk, E., P. Halpern, B.M. Boszczyk, R.M. Parks, O. Chechik, N. Rosen, A. Shapira, O. Merose, and O. Uri. 2014. 'Anxiolytic medication as an adjunct to morphine analgesia for acute low back pain management in the emergency department: a prospective randomized trial', *Spine (Phila Pa 1976.)*, 39: 17-22.

Bertolini, A., A. Ferrari, A. Ottani, S. Guerzoni, R. Tacchi, and S. Leone. 2006. 'Paracetamol: new vistas of an old drug', *CNS Drug Rev*, 12: 250-75.

Bond, A., and M. Lader. 1974. 'The use of analogue scales in rating subjective feelings', *Br J Med Psychol*, 47: 211-18.

Bowdle, T.A., A.D. Radant, D.S. Cowley, E.D. Kharasch, R.J. Strassman, and R.P. Roy-Byrne. 1998. 'Psychedelic effects of ketamine in healthy volunteers. Relationship to steady-state plasma concentrations.', *Anesthesiology*: 82-88.

Boychuk, D.G., G. Goddard, G. Mauro, and M.F. Orellana. 2015. 'The effectiveness of cannabinoids in the management of chronic nonmalignant neuropathic pain: a systematic review', *J Oral Facial. Pain Headache*, 29: 7-14.

Cooper, Z. D., S. D. Comer, and M. Haney. 2013a. 'Comparison of the analgesic effects of dronabinol and smoked marijuana in daily marijuana smokers', *Neuropsychopharmacology*, 38: 1984-92.

Cooper, Z.D., S.D. Comer, and M. Haney. 2013b. 'Comparison of the analgesic effects of dronabinol and smoked marijuana in daily marijuana smokers', *Neuropsychopharmacology*, 38: 1984-92.

de Visser, S.J., J. van der Post, M.S. Pieters, A.F. Cohen, and J.M. van Gerven. 2001. 'Biomarkers for the effects of antipsychotic drugs in healthy volunteers', *Br J Clin Pharmacol*, 51: 119-32.

Derry, S., R.A. Moore, and H.J. McQuay. 2010. 'Paracetamol (acetaminophen) with or without an antiemetic for acute migraine headaches in adults', *Cochrane Database Syst Rev*: CD008040.

Filitz, J., H. Ihmsen, W. Gunther, A. Troster, H. Schwilden, J. Schuttler, and W. Koppert. 2008. 'Supra-additive effects of tramadol and acetaminophen in a human pain model', *Pain*, 136: 262-70.

Friedman, B.W., V. Adewunmi, C. Campbell, C. Solorzano, D. Esses, P.E. Bijur, and E.J. Gallagher. 2013. 'A randomized trial of intravenous ketorolac versus intravenous metoclopramide plus diphenhydramine for tension-type and all nonmigraine, noncluster recurrent headaches', *Ann Emerg Med*, 62: 311-18.

Friedman, B.W., L. Cabral, V. Adewunmi, C. Solorzano, D. Esses, P.E. Bijur, and E.J. Gallagher. 2016. 'Diphenhydramine as Adjuvant Therapy for Acute Migraine: An Emergency Department-Based Randomized Clinical Trial', *Ann Emerg Med*, 67: 32-39.

Graham, G.G., M.J. Davies, R.O. Day, A. Mohamudally, and K.F. Scott. 2013. 'The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings', *Inflammopharmacology*, 21: 201-32.

Greenwald, M.K., and M.L. Stitzer. 2000. 'Antinociceptive, subjective and behavioral effects of smoked marijuana in humans', *Drug Alcohol Depend*, 59: 261-75.

Hay, J.L., P. Okkerse, G. van Amerongen, and G.J. Groeneveld. 2016. 'Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery', *J Vis. Exp.*

Huestis, M.A. 2007. 'Human cannabinoid pharmacokinetics', *Chem Biodivers*, 4: 1770-804.

Klumpers, L. E., T. L. Beumer, J. G. van Hasselt, A. Lipplaa, L. B. Karger, H. D. Kleinloog, J. I. Freijer, M. L. de Kam, and J. M. van Gerven. 2012. 'Novel Delta(9) -tetrahydrocannabinol formulation Namisol(R) has beneficial pharmacokinetics and promising pharmacodynamic effects', *Br J Clin Pharmacol*, 74: 42-53.

Koppel, B.S., J.C. Brust, T. Fife, J. Bronstein, S. Youssof, G. Gronseth, and D. Gloss. 2014. 'Systematic review: efficacy and safety of medical marijuana in selected neurologic disorders: report of the Guideline Development Subcommittee of the American Academy of Neurology', *Neurology*, 82: 1556-63.

Kraft, B., N.A. Frickey, R.M. Kaufmann, M. Reif, R. Frey, B. Gustorff, and H.G. Kress. 2008. 'Lack of analgesia by oral standardized cannabis extract on acute inflammatory pain and hyperalgesia in volunteers', *Anesthesiology*, 109: 101-10.

Lawn, W., T.P. Freeman, R.A. Pope, A. Joye, L. Harvey, C. Hindocha, C. Mokrysz, A. Moss, M.B. Wall, M.A. Bloomfield, R.K. Das, C.J. Morgan, D.J. Nutt, and H.V. Curran. 2016. 'Acute and chronic effects of cannabinoids on effort-related decision-making and reward learning: an evaluation of the cannabis 'amotivational' hypotheses', *Psychopharmacology (Berl)*, 233: 3537-52.

Lotsch, J., V. Dimova, A. Ultsch, I. Lieb, M. Zimmermann, G. Geisslinger, and B.G. Oertel. 2016. 'A small yet comprehensive subset of human experimental pain models emerging from correlation analysis with a clinical quantitative sensory testing protocol in healthy subjects', *Eur J Pain*, 20: 777-89.

Lotsch, J., B.G. Oertel, and A. Ultsch. 2014. 'Human models of pain for the prediction of clinical analgesia', *Pain*, 155: 2014-21.

Machado, G.C., C.G. Maher, P.H. Ferreira, M.B. Pinheiro, C.W. Lin, R.O. Day, A.J. McLachlan, and M.L. Ferreira. 2015. 'Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomised placebo controlled trials', *BMJ*, 350: h1225.

McNicol, E.D., M.C. Ferguson, S. Haroutounian, D.B. Carr, and R. Schumann. 2016. 'Single dose intravenous paracetamol or intravenous propacetamol for postoperative pain', *Cochrane Database Syst Rev*: CD007126.

Miner, J.R. 2009. 'Randomized double-blind placebo controlled crossover study of acetaminophen, ibuprofen, acetaminophen/hydrocodone, and placebo for the relief of pain from a standard painful stimulus', *Acad Emerg Med*, 16: 911-14.

Munsterhjelm, E., N.M. Munsterhjelm, T.T. Niemi, O. Ylikorkala, P.J. Neuvonen, and P.H. Rosenberg. 2005. 'Dose-dependent inhibition of platelet function by acetaminophen in healthy volunteers', *Anesthesiology*, 103: 712-17.

Naef, M., M. Curatolo, S. Petersen-Felix, L. Arendt-Nielsen, A. Zbinden, and R. Brenneisen. 2003. 'The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions', *Pain*, 105: 79-88.

Nielsen, J.C., P. Bjerring, and L. Arendt-Nielsen. 1991. 'A comparison of the hypoalgesic effect of paracetamol in slow-release and plain tablets on laser-induced pain', *Br J Clin Pharmacol*, 31: 267-70.

Nielsen, J.C., P. Bjerring, L. Arendt-Nielsen, and K.J. Petterson. 1992. 'Analgesic efficacy of immediate and sustained release paracetamol and plasma concentration of paracetamol. Double blind, placebo-controlled evaluation using painful laser stimulation', *Eur J Clin Pharmacol*, 42: 261-64.

Norris, H. 1971. 'The action of sedatives on brain stem oculomotor systems in man', *Neuropharmacology*, 10: 181-91.

Okkerse, P., R. Alvarez-Jimenez, J.L. Hay, A. Tehim, R. Kumar, M.L. de Kam, and G.J. Groeneveld. 2016. 'No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery', *Eur J Pain*.

Okkerse, P., J. L. Hay, E. Sitsen, A. Dahan, E. Klaassen, W. Houghton, and G. J. Groeneveld. 2016. 'Pharmacokinetics and pharmacodynamics of intrathecally administered Xen2174, a synthetic conopeptide with norepinephrine reuptake inhibitor and analgesic properties', *Br J Clin Pharmacol*.

Okkerse, P., J. L. Hay, E. Versage, Y. Tang, G. Galluppi, B. Ravina, A. Verma, L. Williams, E. Aycardi, and G. J. Groeneveld. 2016. 'Pharmacokinetics and pharmacodynamics of multiple doses of BG00010, a neurotrophic factor with antihyperalgesic effects, in patients with sciatica', *Br J Clin Pharmacol*, 82: 108-17.

Okkerse, P., G. van Amerongen, M. L. de Kam, J. Stevens, R. P. Butt, R. Gurrell, A. Dahan, J. M. van Gerven, J. L. Hay, and G. J. Groeneveld. 2016. 'The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study', *Br J Clin Pharmacol*.

Olesen, A.E., C. Staahl, Z. Ali, A.M. Drewes, and L. Arendt-Nielsen. 2007. 'Effects of paracetamol combined with dextromethorphan in human experimental muscle and skin pain', *Basic Clin Pharmacol Toxicol*, 101: 172-76.

Ortner, C.M., I. Steiner, K. Margeta, M. Schulz, and B. Gustorff. 2012. 'Dose response of tramadol and its combination with paracetamol in UVB induced hyperalgesia', *Eur J Pain*, 16: 562-73.

Pickering, G., V. Esteve, M.A. Loriot, A. Eschalier, and C. Dubray. 2008. 'Acetaminophen reinforces descending inhibitory pain pathways', *Clin Pharmacol Ther*, 84: 47-51.

Raffa, R.B. 2001. 'Antihistamines as analgesics', *J Clin Pharm Ther*, 26: 81-85.

Redmond, W.J., P. Goffaux, S. Potvin, and S. Marchand. 2008. 'Analgesic and antihyperalgesic effects of nabilone on experimental heat pain', *Curr Med Res Opin*, 24: 1017-24.

Roberts, J.D., C. Gennings, and M. Shih. 2006. 'Synergistic affective analgesic interaction between delta-9-tetrahydrocannabinol and morphine', *Eur J Pharmacol*, 530: 54-58.

Romundstad, L., A. Stubhaug, G. Niemi, L.A. Rosseland, and H. Breivik. 2006. 'Adding propacetamol to ketorolac increases the tolerance to painful pressure', *Eur J Pain*, 10: 177-83.

Rumore, M.M., and D.A. Schlichting. 1985. 'Analgesic effects of antihistaminics', *Life Sci*, 36: 403-16.

Russo, M., A. Naro, A. Leo, E. Sessa, G. D'Aleo, P. Bramanti, and R.S. Calabro. 2016. 'Evaluating Sativex(R) in Neuropathic Pain Management: A Clinical and Neurophysiological Assessment in Multiple Sclerosis', *Pain Med*, 17: 1145-54.

Saragiotto, B.T., G.C. Machado, M.L. Ferreira, M.B. Pinheiro, Shaheed C. Abdel, and C.G. Maher. 2016. 'Paracetamol for low back pain', *Cochrane Database Syst Rev*: CD012230.

Stephens, G., S. Derry, and R.A. Moore. 2016. 'Paracetamol (acetaminophen) for acute treatment of episodic tension-type headache in adults', *Cochrane Database Syst Rev*: CD011889.

Tiippana, E., K. Hamunen, V. Kontinen, and E. Kalso. 2013. 'The effect of paracetamol and tropisetron on pain: experimental studies and a review of published data', *Basic Clin Pharmacol Toxicol*, 112: 124-31.

Turcotte, D., M. Doupe, M. Torabi, A. Gomori, K. Ethans, F. Esfahani, K. Galloway, and M. Namaka. 2015. 'Nabilone as an adjunctive to GABApentin for multiple sclerosis-induced neuropathic pain: a randomized controlled trial', *Pain Med*, 16: 149-59.

Utomo, W. K., M. de Vries, D. C. van Rijckevorsel, M. P. Peppelenbosch, H. van Goor, and G. M. Fuhler. 2015. 'Cannabinoid receptor agonist namisol does not affect cytokine levels in chronic pancreatitis patients', *Am J Gastroenterol*, 110: 1244-5.

Vadalouca, A., E. Raptis, E. Moka, P. Zis, P. Sykioti, and I. Siafaka. 2012. 'Pharmacological treatment of neuropathic cancer pain: a comprehensive review of the current literature', *Pain Pract*, 12: 219-51.

van Amerongen, G., M.W. de Boer, G.J. Groeneveld, and J.L. Hay. 2016. 'A literature review on the pharmacological sensitivity of human evoked hyperalgesia pain models', *Br J Clin Pharmacol*, 82: 903-22.

van Amerongen, G., K. Kanhai, A. C. Baakman, J. Heuberger, E. Klaassen, T. L. Beumer, R. L. Strijers, J. Killestein, J. van Gerven, A. Cohen, and G. J. Groeneveld. 2017. 'Effects on Spasticity and Neuropathic Pain of an Oral Formulation of Delta9-Tetrahydrocannabinol in Patients With Progressive Multiple Sclerosis', *Clin Ther*.

van Steveninck, A.L. 1993. 'Methods of assessment of central nervous system effects of drugs in man'.

Walter, C., B.G. Oertel, and J. Lotsch. 2015. 'THC may reproducibly induce electrical hyperalgesia in healthy volunteers', *Eur J Pain*, 19: 516-18.

Weil, K., L. Hooper, Z. Afzal, M. Esposito, H.V. Worthington, A.J. van Wijk, and P. Coulthard. 2007. 'Paracetamol for pain relief after surgical removal of lower wisdom teeth', *Cochrane Database Syst Rev*: CD004487.

Williams, C.M., C.G. Maher, J. Latimer, A.J. McLachlan, M.J. Hancock, R.O. Day, and C.W. Lin. 2014. 'Efficacy of paracetamol for acute low-back pain: a double-blind, randomised controlled trial', *Lancet*, 384: 1586-96.

Wilsey, B., T.D. Marcotte, R. Deutsch, H. Zhao, H. Prasad, and A. Phan. 2016. 'An Exploratory Human Laboratory Experiment Evaluating Vaporized Cannabis in the Treatment of Neuropathic Pain From Spinal Cord Injury and Disease', *J Pain*, 17: 982-1000.

Woodward, D.F., and D.A. Owen. 1982. 'Effect of H1-and H2-receptor antagonists on cutaneous inflammation evoked by histamine analogues and UV radiation', *Eur J Pharmacol*, 77: 103-12.

Yuan, C.S., T. Karrison, J.A. Wu, T.K. Lowell, J.P. Lynch, and J.F. Foss. 1998. 'Dose-related effects of oral acetaminophen on cold-induced pain: a double-blind, randomized, placebo-controlled trial', *Clin Pharmacol Ther*, 63: 379-83.

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

Age (years)	
Mean (SD)	24.0 (5.6)
Median	23
Min, Max	18, 45
BMI (kg/m²)	
Mean (SD)	23.5 (2.9)
Median	23.7
Min, Max	18.2, 29
Sex (n)	
Female (%)	12 (48%)
Male (%)	13 (52%)
Race	
Other	1 (4%)
White	24 (96%)
Fitzpatrick Skin Type	
II: Always burns & tans minimally	6 (24%)
III: Burns moderate & tan gradually	11 (44%)
IV: Burns minimally & tans well	8 (32%)
MED (mJ/cm)	
Mean (SD)	777 (249)
Median	702
Min, Max	351, 1321

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

TABLE 2 Summary of LSM analyses for battery of evoked pain tasks

Endpoint	Placebo (n=24)	Paracetamol (n=23)	Promethazine (n=23)	Δ9-THC (n=24)
	LSMean (95%CI)	Contrast vs. placebo* (95%CI)	Contrast vs.placebo (95%CI)	Contrast vs.placebo (95%CI)
COLD PRESSOR (S)				
PTT	13.4 (12.5-14.3)	5.2% (-4.6%, 16.0%) p=0.3090	**-11.1% (-19.3%, -1.9%) p=0.0189**	2.5% (-7.0%, 12.9%) p=0.6183
PDT	3.2 (2.5-4)	3.5% (-15.0%, 26.0%) p=0.7292	-17.0% (-31.8%, 1.2%) p=0.0648	-0.8% (-18.5%, 20.7%) p=0.9335
AAC	860 (803-922)	7.8% (-3.8%, 20.9%) p=0.1938	-10.6% (-20.2%, 0.2%) p=0.0548	4.1% (-7.0%, 16.6%) p=0.4806
VAS	56.9 (52.8-61)	2.59 (-0.45, 5.63) p=0.0940	1.48 (-1.56, 4.52) p=0.3366	0.11 (-2.93, 3.15) p=0.9444
ELECTRICAL STIMULATION (mA)				
PTT	22.1 (20.3-24.1)	-8.7% (-18.2%, 1.9%) p=0.1023	-7.7% (-17.2%, 3.0%) p=0.1495	**-12.7% (-21.5%, -2.8%) p=0.0134**
PDT	9.43 (8.44-10.53)	-9.9% (-22.8%, 5.3%) p=0.1896	0.8% (-13.7%, 17.8%) p=0.9211	-2.7% (-16.6%, 13.4%) p=0.7222
AUC	3244 (3112-3376)	171.37 (-21.12, 363.87) p=0.0805	76.86 (-115.81, 269.53) p=0.4311	**196.58 (6.98, 386.19) p=0.0423**
VAS	54.6 (51.8-57.4)	1.71 (-1.18, 4.61) p=0.2414	-1.38 (-4.28, 1.52) p=0.3467	-0.68 (-3.53, 2.17) p=0.6372
CPM: ELECTRICAL STIMULATION (mA)				
PTT	1.07 (0.64-1.51)	0.101 (-0.772, 0.973) p=0.8206	-0.186 (-1.050, 0.678) p=0.6716	0.099 (-0.785, 0.983) p=0.8254
PDT	1.24 (0.62-1.87)	0.678 (-0.566, 1.921) p=0.2841	0.052 (-1.186, 1.290) p=0.9339	-0.144 (-1.398, 1.109) p=0.8206
AUC	-147 (-192- -102)	-24.74 (-107.64, 58.17) p=0.5574	13.44 (-69.10, 95.98) p=0.7488	9.63 (-73.60, 92.87) p=0.8200
VAS	2.60 (1.95-3.47)	9.7% (-24.5%, 59.4%) p=0.6238	16.5% (-18.8%, 67.1%) p=0.4044	-1.7% (-32.8%, 44.0%) p=0.9311
PRESSURE STIMULATION (kPa)				
PTT	39.9 (36.3-43.9)	-5.1% (-11.9%, 2.2%) p=0.1653	**-8.9% (-15.4%, -1.8%) p=0.0149**	**-9.0% (-15.6%, -2.0%) p=0.0126**
PDT	16.7 (14.2-19.6)	-8.5% (-19.1%, 3.5%) p=0.1552	-7.1% (-17.8%, 5.1%) p=0.2392	-9.9% (-20.3%, 1.9%) p=0.0972
AUC	6761 (6457-7064)	**248.01 (22.33, 473.69) p=0.0314**	**341.93 (115.96, 567.89) p=0.0032**	**446.38 (221.09, 671.67) p=0.0001**
VAS	50.2 (44.2-56.2)	1.12 (-1.60, 3.85) p=0.4176	-0.20 (-2.92, 2.52) p=0.8841	0.35 (-2.35, 3.06) p=0.7964
NORMAL HEAT (°C)				
PDT	45.1 (44.7-45.6)	0.5% (-0.9%, 2.0%) p=0.4434	-0.6% (-2.0%, 0.8%) p=0.3830	0.3% (-1.1%, 1.7%) p=0.7229
UVB HEAT (°C)				
PDT	39.7 (39.1-40.2)	0.2% (-1.1%, 1.4%) p=0.8033	**-2.8% (-4.1%, -1.6%) p=<.0001**	-1.0% (-2.3%, 0.3%) p=0.1220

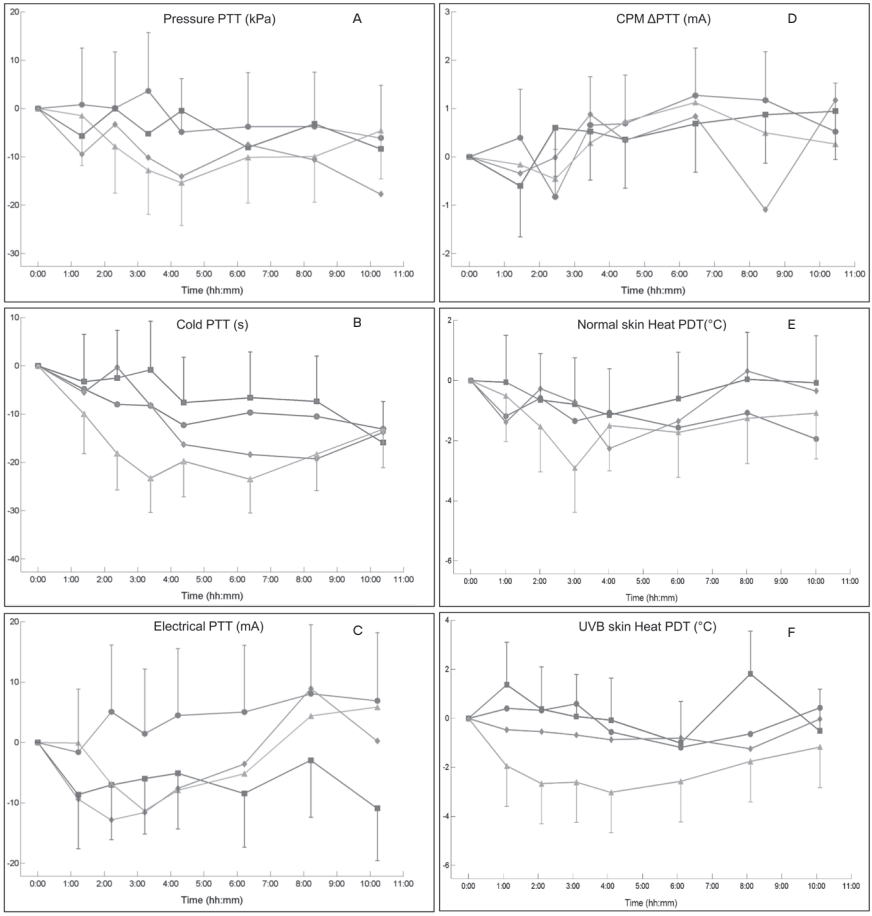
PTT = Pain Tolerance Threshold / PDT = Pain Detection Threshold / AAC = Area Above the Curve / AUC = Area Under the Curve / VAS = Visual Analogue Scale / CPM = Conditioned Pain Modulation /
** Contrasts over 0-4 hours post dose.*

TABLE 3 Summary of LSM analyses for subjective cognitive functioning and psychotomimetic symptoms

	Placebo (n=24)	Paracetamol (n=23)		Promethazine (n=23)		Δ9-THC (n=24)	
	LSMean (95%CI)	LSMean (95%CI)	Contrast vs. placebo* (95%CI)	LSMean (95%CI)	Contrast vs. placebo (95%CI)	LSMean (95%CI)	Contrast vs. placebo (95%CI)
VAS BOND & LADER							
Alertness (mm)	49.7 (48.7-50.7)	49.8 (48.8-50.8)	0.07 (-1.55, 1.68) p=0.9339	47.2 (46.2-48.2)	**-3.11 (-4.75, -1.48) p=0.0002**	45.7 (44.7-46.7)	**-5.83 (-7.45, -4.21) p=<.0001**
Calmness (mm)	51.6 (50.6-52.6)	51.1 (50-52.1)	-0.73 (-2.57, 1.12) p=0.4369	51.9 (50.9-53)	0.46 (-1.38, 2.30) p=0.6236	53.6 (52.6-54.7)	**3.97 (2.11, 5.82) p=<.0001**
Mood (mm)	50.4 (49.7-51.1)	50.2 (49.5-50.9)	-0.42 (-1.39, 0.55) p=0.3914	50.7 (50-51.4)	0.10 (-0.88, 1.07) p=0.8408	51 (50.3-51.6)	0.76 (-0.21, 1.74) p=0.1244
VAS BOWDLE							
Feeling High (LOGmm)	0.33 (0.28-0.39)	0.31 (0.26-0.37)	-0.0295 (-0.1347, 0.0757) p=0.5804	0.35 (0.29-0.4)	0.0189 (-0.0859, 0.1237) p=0.7225	0.71 (0.65-0.77)	**0.7232 (0.6164, 0.8300) p=<.0001**
Internal perception (LOGmm)	0.32 (0.29-0.34)	0.31 (0.29-0.34)	-0.0061 (-0.0470, 0.0348) p=0.7677	0.33 (0.31-0.35)	0.0269 (-0.0139, 0.0678) p=0.1951	0.4 (0.38-0.42)	**0.1705 (0.1292, 0.2117) p=<.0001**
External perception (LOGmm)	0.33 (0.28-0.37)	0.32 (0.27-0.36)	-0.0143 (-0.0957, 0.0670) p=0.7279	0.34 (0.29-0.39)	0.0148 (-0.0664, 0.0960) p=0.7189	0.55 (0.5-0.59)	**0.4289 (0.3466, 0.5112) p=<.0001**

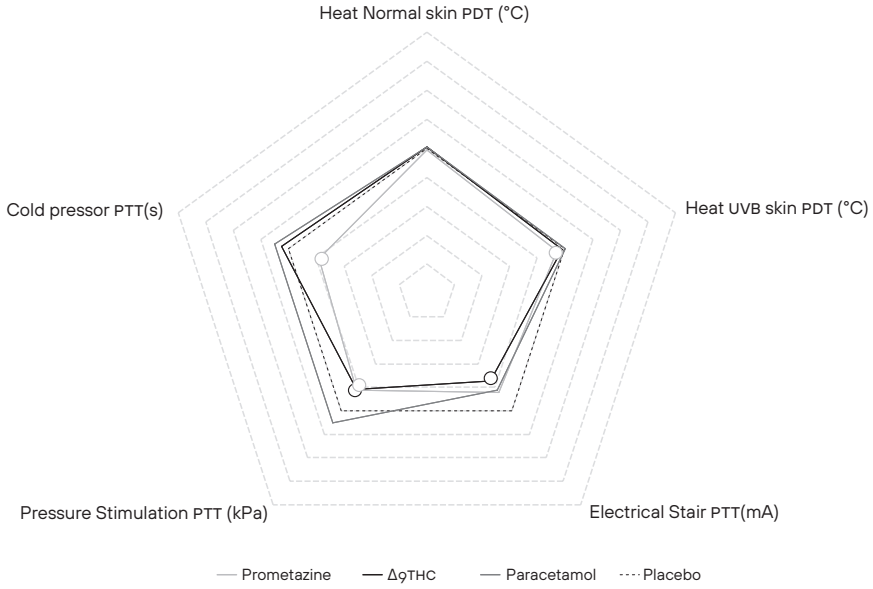
*VAS = Visual Analogue Scale / * Contrasts over 0-4 hours post dose.*

FIGURE 1 Overview of Change from Baseline time profiles for battery of evoked pain tasks



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

FIGURE 2 Spider plot overview of Pharmacodynamic response profile for battery of evoked pain tasks normalized to placebo (0-4 hours)



Dashed placebo line represents the value to which other treatment effects are normalized. Distal from placebo indicates Least Square Mean PTT greater than placebo, proximal indicates Least Square mean PTT lower than placebo. Actual values are described in Table 2. A circle indicates a statistically significant (P<0.05) difference compared to placebo for treatment on pain task.

CHAPTER 5

PF-06372865, A PARTIAL $GABA_A$ POSITIVE ALLOSTERIC MODULATOR EXHIBITS $\alpha2/\alpha3/\alpha5$ SUBTYPE-SELECTIVE CENTRAL NERVOUS SYSTEM EFFECTS IN HUMANS COMPARED TO PLACEBO AND LORAZEPAM

G van Amerongen,[1] RGJA Zuiker,[1] R Gurrell,[2] P Dua,[2] M. Whitlock,[2] D Gorman,[2] R Butt,[2] JMA van Gerven[1]

1 Centre for Human Drug Research (CHDR) / Leiden University Medical Centre (LUMC) Leiden, The Netherlands
2 Neuroscience and Pain Research Unit, Pfizer WRD, Cambridge, UK

ABSTRACT

AIMS This study investigated the pharmacodynamic (neurocognitive and neurophysiological) profile of a novel α2/α3/α5 $GABA_A$ subtype selective partial positive allosteric modulator (PAM), PF-06372865 with single dose levels ranging from 0.04 to 100 mg.

METHODS This was a two-part study in 45 healthy subjects (NCT01951144). Part A was a double-blind, randomised, placebo-controlled, ascending single oral dose, crossover study. Part B was performed after completion of Part A to further explore the pharmacodynamics of PF-06372865 alone and in combination with lorazepam (2 mg). Both parts used the NeuroCart®, a neurocognitive and neurophysiological test battery, which assessed pharmacodynamic measurements (saccadic peak velocity (SPV), smooth pursuit eye movements, body sway, adaptive tracking, Visual Verbal Learning Test (VVLT), Visual Analogue Scale (VAS) Bond & Lader and pharmaco-EEG) that were performed at baseline and up to 6 hours post-dose.

RESULTS The majority of the pharmacodynamic assessments were dose-dependently affected by PF-06372865, and plateaued at different dose levels between approximately 10 and 65 mg. In Part B, the combination of lorazepam (2 mg) with PF-06372865 65 mg demonstrated infra-additive effects or the lack thereof, depending on the functional domain, in line with the predicted functional selectivity for α2/α3/α5 $GABA_A$ receptor subtypes. In both parts, treatment with PF-06372865 alone or in combination with lorazepam is considered safe and well-tolerated.

CONCLUSION Pharmacodynamic neurocognitive and neurophysiological profiling, alone and in combination with lorazepam revealed the unique pharmacological characteristics of PF-06372865 that are suggestive of anxiolysis with fewer signs of sedation, compared to classic non-selective benzodiazepines.

INTRODUCTION

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS) and is involved in a vast number of functions and behaviours (Curtis and Johnston, 1974). $GABA_A$ receptors are heteropentameric ligand-gated chloride ion channels that mainly contain two α, two β, and one γsubunit (McKernan and Whiting, 1996). $GABA_A$ receptors are involved in the fast inhibitory function of GABA by providing rapid hyperpolarization of postsynaptic neurons. Conventional, non-selective benzodiazepines are positive allosteric modulators (PAMS) of the $GABA_A$ receptors (Curtis and Johnston, 1974).

Since the serendipitous discovery of chlordiazepoxide in 1960 (Frommele et al., 1960), benzodiazepines are among the most widely prescribed drugs for the treatment of a wide range of diseases, including generalised anxiety disorder (GAD) (Reinhold and Rickels, 2015), panic disorder (Starcevic, 2014), insomnia (Neubauer, 2014) and (neuropathic) pain (Chou and Huffman, 2007). Although conventional non-selective benzodiazepines are considered safe and well-tolerated, clinical use is dose-limited by adverse effects (AEs) including sedation, postural instability and memory disturbance (Mets et al., 2010; Davidson et al., 2010). Animal studies have shown that $GABA_A$ α1 activity is related to the sedative side effects (Rudolph et al., 1999), and studies in healthy human subjects with α1-sparing, α2/α3 PAMS have confirmed these findings (Atack, 2005; Atack, 2010). In addition, $GABA_A$ α2 and α3 subunits have been related to anxiolytic (Smith et al., 2012), analgesic (Knabl et al., 2008), and recently unexpectedly also suggested to be associated with a mild form of sedation in rhesus monkeys (Duke et al., 2018). Finally, the α5 subunits are believed to be involved in cognitive functioning and memory (Atack et al., 2006; Atack, 2011).

PF-06372865 is a novel $GABA_A$ subtype selective modulator which exhibits functional selectivity for receptors containing α2, α3 or α5 over those containing α1 subunits, a profile which was confirmed *in vivo* by assessing α2/α3 and α1 pharmacology through quantitative beta frequency and zolpidem drug discrimination in rats respectively (Nickolls et al., 2018). PF-06372865 is under development for the treatment of epilepsy.

The objective of this First-in-Human trial was to explore safety, tolerability, pharmacodynamics (PD) and pharmacokinetics (PK) of PF-06372865 after a single dose across a wide exposure range. It is possible that PF-06372865 will be administered as adjuvant therapy to a non-selective benzodiazepine, e.g. lorazepam. As such, an additional objective of the present study was to investigate the effects of PF-06372865 when co-administered with lorazepam (2 mg), compared to placebo and lorazepam alone. This also allows to benchmark the observed effects PF-06372865 to a non-selective benzodiazepine, as lorazepam has been shown to demonstrate no specificity for the $GABA_A$ receptor subtypes (Dämgen and Lüddens, 1999).

Pharmacodynamics were assessed using a validated CNS test battery, the NeuroCart®, which has been extensively used to investigate the effects of various CNS active compounds where it has been shown sensitive to detect the effects of subunit

selective positive allosteric $GABA_A$ receptor modulators (Zuiker et al., 2015; Chen et al., 2015; Chen et al., 2014) at the Centre for Human Drug Research (CHDR).

Previously, parts of the study results were presented in relation to preclinical findings for PF-06372865 (Nickolls et al., 2018). The current article focuses on the results of the neurocognitive test battery as a whole, and fully characterises the clinical pharmacodynamic effects in this First-in-Human study.

METHODS

This study was registered in ClinicalTrials.gov under registration number: NCT01951144. The final protocol, amendments and informed consent documentation were reviewed and approved by the Independent Ethics Committee (IEC) Stichting Beoordeling Ethiek Biomedisch Onderzoek (Stichting BEBO) in Assen, the Netherlands. This study was conducted at the Centre for Human Drug Research (Leiden, the Netherlands) in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH- GCP) Guidelines.

This was a two-part study. Part A was a double-blind, randomised, placebo-controlled, ascending single oral dose, placebo-substitution, 3-cohort crossover study. Part B (Cohort 4) was performed after completion of the first three cohorts to further explore the pharmacodynamics of PF-06372865 alone and in combination with lorazepam (2 mg). A total of 45 subjects were included: Cohorts 1, 2 and 3 each comprised 10 subjects and 15 subjects were included in Cohort 4. Cohorts 1 and 2 received five dose levels each in an alternating fashion, thus allowing to explore 10 ascending dose levels in a two-cohort crossover design. In cohort 3, another five ascending dose levels were investigated. For each cohort there was a washout period of at least seven days. The findings of Cohorts 1, 2 and 3 (Part A) were incorporated to decide on appropriate doses for Cohort 4 (Part B), in which the effects of two dose levels of PF-06372865 alone and one dose level in combination with lorazepam (2 mg) were compared to placebo and lorazepam (2 mg) as active control in a complete 5-period crossover design using a single Latin square. The investigator assigned subject numbers sequentially to the subjects as they were screened for the study. The sponsor provided a randomization schedule to the investigator and, in accordance with the randomization numbers, the subject received the study treatment regimen assigned to the corresponding randomization number. Cohort 4 included a block size of 5 to ensure all sequences were evenly allocated.

Sample size

A sample size of 10 subjects in each of Cohorts 1, 2 and 3 (with 8 active, 2 placebo in each period), was based on the need to minimise first exposure to humans of a new chemical entity and the requirement to provide adequate safety and pharmacodynamic

information at each dose level. A sample size of 15 subjects in Cohort 4 was selected to ensure a balanced design and to provide sufficient power to demonstrate a pharmacodynamic effect. This was based on assumed within subject standard deviations of 37 deg/second (sec) for SPV and 0.39 ln (mm) for body sway as observed in Cohorts 1, 2 and 3 of the study.

Subjects

Forty-five healthy subjects were recruited from CHDR's database. Subjects were male or postmenopausal female, aged 18-55 years, with a BMI of 17.5 to 30.5 kg/m^2. Subjects were to refrain from smoking, caffeine and alcohol from 24 hours prior to each investigational period. Subjects were instructed to use a highly effective method of contraception. The use of medication other than study drug was not allowed during study participation.

Treatments

In Part A subjects were randomised to receive 5 ascending single doses of PF-06372865 or placebo. The starting dose was determined based on preclinical modelling at 0.04 mg, which was expected to yield a C_{max} of 0.31ng/mL, corresponding to an unbound C_{max} of 0.04ng/mL, which in turn corresponds to a predicted total RO of 18%, and an α2 subunit RO of ~3%.

The findings of Part A were used to determine the dose levels of PF-06372865 for Part B. In this randomised 5-period placebo and active control crossover design, 15 subjects received PF-06372865 (15 mg), PF-06372865 (65 mg), lorazepam (2 mg), PF-06372865 (65 mg) + lorazepam (2 mg) and placebo.

Following an overnight fast of least 10 hours, subjects received study treatment in the morning. PF-06372865 was administered as an oral suspension and one dose level (25 mg) was administered as oral tablets as well. Lorazepam (2 mg) was administered as an oral tablet. To ensure blinding of treatment allocation, matching placebo was administered in a double-dummy fashion (Part B only).

Safety

Adverse events, clinical laboratory, electrocardiogram (ECG), blood pressure and heart rate measurements were collected throughout the study.

Pharmacokinetics

Plasma samples were analysed for PF-06372865 concentrations at York Bioanalytical Solutions (York, UK) using a validated, sensitive and specific high-performance liquid chromatography tandem mass spectrometric method (HPLC-MS/MS). PK samples were collected prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16 24, 36, 48 and 72 hours post-dose.

Pharmacodynamics

Pharmacodynamic assessments were performed using an integrated test battery, the NeuroCart®. Measurements were conducted by a trained operator in accordance with the Standard Operating Procedures of CHDR. The assessments to be performed as part of the NeuroCart® battery were: Saccadic Eye Movement, Smooth Pursuit, Adaptive Tracking, Body Sway, Bond and Lader Visual Analogue Scales (VAS) and Pharmaco-electroencephalography (p-EEG). An overview of the different tasks and associated functional domains can be found in *Table 1*. Pharmacodynamic measurements were performed prior to dosing (twice in order to record double baseline values, thus reducing variability) and at 0.5, 1, 1.5, 2, 4, 6, 12 hours post-dose. In addition, the Visual Verbal Learning Test (VVLT) was performed at 1 hour and 6 hours (Part A) or 2.5 hours (Part B) post-dose. Subjects were tested individually in a quiet room with ambient illumination.

Saccadic Eye Movements

Measurements of saccadic eye movements were recorded as previously described (de Haas et al., 2007; de Haas et al., 2009). Average values of Saccadic Peak Velocity (SPV) were calculated for all artefact free saccades. SPV is closely related to the anxiolytic properties of benzodiazepines (Chen et al., 2012) and this parameter has been validated as the most sensitive biomarker for their effects (de Visser et al., 2003; van Steveninck et al., 1994; van Steveninck et al., 1996; van Steveninck et al., 1999).

Smooth pursuit

For smooth pursuit eye movements, the target moved at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponded to 22.5 degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The method is validated at CHDR (van Steveninck et al., 1999) based on the work of Bittencourt et al (Bittencourt et al., 1983) and the original description of Baloh et al (Baloh et al., 1976). The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was the target parameter.

Adaptive Tracking

The adaptive tracking test was performed as originally described by Borland and Nicholson (Borland and Nicholson, 1975; van Steveninck et al., 1999) using customised equipment and software. The average performance over a 3.5-min period were used for analysis. Adaptive tracking is a pursuit-tracking task, where a circle moves randomly on a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. The test is adaptive in nature, namely 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.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxiameter (Wright, 1971), which integrates the amplitude of unidirectional body movements transferred through a string attached to the subject's waist. Two-minute measurements were made in the anteroposterior direction with eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart. Body sway is a measure of postural stability that has previously been shown to be sensitive to sleep deprivation (van Steveninck et al., 1999), ethanol (van Steveninck et al., 1993) and benzodiazepines (van Steveninck et al., 1993).

Bond and Lader Visual Analogue Scales (VAS)

Visual analogue scales as originally described by Norris (Norris, 1971) were previously used to quantify subjective effects of benzodiazepines (van Steveninck et al., 1991). From the set of 16 scales, three composite factors were derived as described by Bond and Lader (Bond and Lader, 1974), corresponding to alertness, mood, and calmness. These factors were used to quantify subjective drug effects.

Pharmaco-electroencephalography

EEG recordings were made using gold chloride electrodes with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the very low (0.5-2 Hz), delta (2-4 Hz), theta (4-7.5 Hz), alpha (7.5-13.5 Hz), beta (13.5-35 Hz) and gamma (35-48.9 Hz) frequency ranges. The duration of EEG measurements was 64 s per session. Change in amplitudes in the beta frequency band of the EEG was found to be a relevant measure of the pharmacological effect intensity of benzodiazepines (Mandema et al., 1992).

Visual Verbal Learning Test (VVLT)

Measurement of memory comprises different components of learning behaviour, i.e., acquisition, consolidation, storage, and retrieval. The VVLT (Schmitt et al., 2000) contained 3 different subtests that covered a wide scope of learning behaviour. The test started with sequential presentation of 30 common monosyllabic nouns. Each word was shown for 2 seconds. When the series ended, the subject was required to verbally recall as many words as possible. The same list was presented in the same way in 3 successive trials. The highest individual trial score was the Immediate Recall score. After a delay, subjects were asked again to recall as many words as possible without prompting. The number correctly recalled was the Delayed Recall score. Finally, old/new recognition was measured by showing the subjects a series of 30 words on the computer display that included words from the original set and 15 new words. Subjects responded to each presentation by indicating as quickly as possible whether the given word was one of the original set (Delayed recognition).

Statistical analysis

Data from cohorts 1, 2 and 3 (Part A) were analysed separately to data from cohort 4 (Part B). Body Sway was $\log_e$-transformed prior to analyses. A mixed effects model was fitted for each endpoint separately, using data collected during the first 6 hours post-dose (except VVLT endpoints which only had a single associated post-dose measurement). The randomisation code was generated by an independent team. Randomisation numbers were sequentially allocated by the study physician, and blinded study treatments were prepared and dispensed by an independent operating pharmacy.

For Cohorts 1, 2 and 3 (Part A), the fixed effects included in the model were baseline, time, treatment and treatment by time interaction. Time was included as a repeated effect within each subject*period. Baseline was included as 2 separate variables, the average baseline for the subject, and the deviation of each treatment period baseline from the average baseline for each subject. The interaction of the latter baseline and time was also included as a fixed effect. For VVLT, the fixed effect in the model was treatment, with subject fitted as a random effect. For Cohort 4 (Part B), the models were the same as above, except an additional fixed effect term for period was included (all endpoints, including VVLT).

For all cohorts, the Least Square Means (LSM) together with 95%CIs were obtained for each treatment averaged across all post-dose time points, and also for each treatment at each separate time point (if applicable). Differences in LSM between treatments (all PF doses, lorazepam 2 mg and the combination of PF and lorazepam) and placebo, together with 95%CIS were obtained. For the analysis of Cohort 4 (Part B), the differences in LSM between treatments (both PF doses and the combination) and lorazepam (2 mg), together with 95%CIS, were also obtained.

Pharmacodynamic interaction was investigated post-hoc for the neurocognitive and neurophysiological outcome measures by calculating the contrasts of the sum of the effects of individual treatments (PF-06372865 (65 mg) and lorazepam (2 mg)) versus the effects of co-administration of PF-06372865 (65 mg) + lorazepam (2 mg). Supra- or infra-additive pharmacodynamic interactions are signified by a statistically significant difference ($p < 0.1$). No difference indicates that the co-administration of both treatments results in the addition of both individual treatments. PF-06372865 (65 mg) co-administered with lorazepam (2 mg) vs. PF-06372865 (65 mg) + lorazepam (2 mg)

RESULTS

Subjects

Each subject provided written informed consent before any screening procedures were performed. A total 45 healthy subjects were enrolled in this study. CONSORT flow chart is available as supplemental. There were no discontinuations or withdrawals,

so all randomised subjects completed the study per protocol. All 30 subjects in part A (cohorts 1, 2 and 3) were healthy male subjects. The majority of subjects (26 out of 30) were caucasian. In part B (cohort 4, n = 15) all subjects except one were male. There were no notable differences between mean weight, height or BMI across all cohorts (*Table 2*).

Safety

A detailed overview of the observed adverse events in Part A is described by Nickolls et al. (Nickolls et al., 2018). No serious adverse events were observed during the study. No clinically significant changes in vital signs, ECGs or other safety parameters were observed. The most frequently reported AEs were dizziness, somnolence, bradyphrenia, elevated mood, fatigue, headache and orthostatic hypotension, all of which were mild in severity.

In Part B lorazepam (2 mg) treatment alone resulted in similar type of AEs compared to PF-06372865 15 mg treatment, but with a lower incidence of total AEs compared with 65 mg of PF-06372865 treatment group. Reported adverse events are summarised according to system organ class (SOC) in *Table 3*, where the most commonly observed AEs (dizziness and somnolence) are presented individually as well. All observed treatment emergent AEs were mild in severity. Co-administration of PF-06372865 65 mg and lorazepam 2 mg did not result in an additive effect in terms of number or intensity of adverse events. There was no clear differentiation or pattern observed in terms of distribution of AEs, in terms of SOC or intensity.

Pharmacokinetics

The plasma PK parameter estimates for 14 ascending doses of PF-06372865 (Part A) are described by Nickolls (2018). Summarising, PF-06372865 was well absorbed with median T_{max} of 1 to 4 hours. Average half-life ranged from 6.0 to 8.9 hours. In general, plasma PF-06372865 AUC_{inf} and C_{max} increased apparently linear across the dose range. However, no formal test was performed to confirm this. In cohort 3 (Part A) a comparison between the oral suspension formulation and an oral tablet formulation was performed for a dose of 25 mg. The observed median T_{max} for the tablet formulation was 2.0 hours, compared to a median T_{max} of 4.0 hours when administered as suspension at the same dose level. $T_{1/2}$ was similar with the mean value of 8.2 hours for both treatments. Inter-subject variability for PF-06372865 exposure based on geometric CV% was low to moderate, ranging between 13% and 39% for C_{max}. In cohort 4 (Part B), analogous to the observations in Part A, PF-06372865 was absorbed rapidly and demonstrated a similar half-life compared with the observations of the first part of study. When PF-06372865 65 mg was administered in combination with lorazepam 2 mg, a slight increase was seen for PF-06372865 AUC_{inf} and C_{max} by approximately 12% and 13% respectively, compared with PF-06372865 65 mg administered alone. Median T_{max} (2.0 hours) and mean half-life (approximately 8 hours) values were similar for PF-06372865 alone and co-administration with lorazepam 2 mg.

Pharmacodynamics – Part A

An overview of the results of the pharmacodynamic differences in overall Least Square Means (presented with 95% Confidence Interval) for the complete dose range (0.04 mg to 100 mg) compared with placebo is shown in Figures 1-3 and Supplement 1.

From a dose of 4 mg PF-06372865 onwards up to the highest dose (100 mg), Saccadic Peak Velocity (degrees/second) was statistically significantly decreased compared to placebo, in a dose-response manner. Smooth pursuit eye movements were only statistically significantly different from placebo at 10, 15 and 100 mg. Percentage correct in adaptive tracking was statistically significantly reduced compared to placebo for all dose levels above 10 mg PF-06372865, except for the 25 mg PF-06372865 tablet formulation. Between the dose levels of 10 and 25 mg a dose-related response was observed, from 25 mg onwards the effect demonstrated a plateau. Postural instability, measured using body sway, increased in a dose-related fashion for each cohort with statistically significantly increases in the higher dose levels. The maximum effect was observed at a dose level of 10 mg, indicating a plateau. VAS alertness showed a seemingly dose-related reduction from 4 mg of PF-06372865 onwards, except for the dose level of 6 mg of PF-06372865. (Supplement 1) Different parameters were measured using the VVLT (*Figure 2*). Immediate recall was significantly impaired during the first treatment visits for Cohort 1 (0.04 mg of PF-06372865). Then, from the dose level of 4 mg PF-06372865 onwards, immediate recall was statistically significantly reduced. Delayed recall demonstrated a similar pattern. Delayed recognition however, showed a more diffuse pattern. Pharmaco-EEG (*Figure 3*) demonstrated a dose-dependent power increase in the low frequency bands δ and θ, which appeared to reach a plateau between 6 and 25 mg. Beta (β) frequency band activity however demonstrated an apparent dose-response over all dose levels. No clear changes were was observed in the α frequency band.

Pharmacodynamics – Part B

In cohort 4, a placebo-controlled head-to-head comparison between 15 mg PF-06372865, 65 mg PF-06372865 (alone and in combination with 2 mg lorazepam) and lorazepam (2 mg) was performed. The results are presented in *Table 4* as LSM with contrasts to placebo and contrasts to lorazepam (2 mg). The results of the most relevant outcome measures are graphically summarised in *Figure 4*. Pharmacodynamic interaction analysis was performed on the primary endpoints and is described in *Table 5*.

Saccadic eye movements

All treatments statistically significantly reduced SPV compared to placebo. Additionally, both doses of PF-06372865 (alone or in combination with lorazepam (2 mg)) demonstrated a significant greater reduction in SPV than lorazepam (2 mg) alone. Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) did not result in an infra- or supra-additive pharmacodynamic interaction effect (p=0.25).

Smooth pursuit

None of the treatments were statistically significantly different from placebo. Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) did not result in an infra- or supra-additive pharmacodynamic interaction effect (p=0.37).

Adaptive tracking

All treatments statistically significantly reduced performance on adaptive tracking compared with placebo. The reduction in performance was greatest when lorazepam (2 mg) was administered alone. Contrasts to lorazepam (2 mg) showed that the difference in magnitude in adaptive tracking performance reduction was statistically significantly lower for both 15 and 65 mg of PF-06372865 (alone or in combination with lorazepam (2 mg)), compared to lorazepam (2 mg) alone. Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) induced a statistically significant (p<0.001) infra-additive effect.

Body Sway

All treatments statistically significantly increased postural instability expressed as body sway. Treatment with lorazepam (2 mg) alone resulted also in a statistically significant different compared with PF-06372865 (15 mg). Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) induced a statistically significant (p<0.001) infra-additive effect.

VAS Alertness

Subjective alertness was only statistically significantly reduced compared to placebo after treatment with a combination of PF-06372865 (65 mg) and lorazepam (2 mg). In addition, 65 mg PF-06372865 statistically significantly affected the subscales of Bond & Lader for mood and calmness. (*Results not presented)* Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) did not result in an infra- or supra-additive pharmacodynamic interaction effect (P = 0.98).

VVLT

Three outcome measures of the VVLT are presented here. Immediate recall, delayed recall and delayed recognition. For immediate recall, the number of correct words was statistically significant reduced for all treatments compared to placebo. Total number of correct words in delayed recall was statistically significantly reduced for all treatments and there was no difference between the treatments compared to lorazepam (2 mg). Delayed recognition showed a slightly different profile: all treatments, except for 15 mg PF-06372865, statistically significantly the reduced number of correct words compared to placebo. Consequently, treatment with lorazepam (2 mg) caused a statistically significantly larger impairment of delayed recall than 15 mg PF-06372865 treatment. The other treatments did not differ significantly from lorazepam (2 mg).

Co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg) induced a statistically significant ($p<0.001$) pharmacodynamic interaction on Immediate recall ($p=0.004$) and Delayed recall ($p=0.0057$), resulting in infra-additive effects. However, the observations for Delayed recognition showed a reverse profile: the largest deterioration in performance was induced by treatment of lorazepam (2 mg) alone, which was statistically significantly lower after co-administration of PF-06372865 (65 mg) in combination with lorazepam (2 mg).

p-EEG

All treatments resulted in statistically significantly lower α-power activity compared to placebo. β-power band activity was statistically significantly increased for all treatments compared with placebo. What is more, the increase observed for 65 mg PF-06372865 alone and in combination with lorazepam (2 mg) was statistically significantly higher compared to lorazepam (2 mg) alone. Treatment with 15 mg PF-06372865, 65 mg PF-06372865 alone or in combination with lorazepam (2 mg) statistically significantly decreased δ-power band activity compared with placebo and with lorazepam (2 mg) alone. Lorazepam (2 mg) alone was not different from placebo. In contrast, θ-power band activity did not show any differentiation between PF-06372865 or lorazepam (2 mg).

DISCUSSION

This First-in-Human (FIH) study investigated safety and tolerability as well as the pharmacodynamic and pharmacokinetic properties of PF-06372865, a novel α2/α3/α5 $GABA_A$ subtype selective partial positive allosteric modulator. Single doses of PF-06372865 were safe and well-tolerated. The observed adverse events were in line with what was expected based on its predicted pharmacology. The severity of the observed adverse events remained mild, irrespective of dose or co-administration with lorazepam.

In this study, a clear, dose-dependent, pharmacodynamic effect profile for PF-06372865 was observed. In Part A, saccadic peak velocity (SPV), adaptive tracking, body sway, EEG, and VAS alertness were affected in a dose-related fashion, up to certain dose levels where the effects appeared to plateau. Visual verbal learning (VVLT) demonstrated similar dose related impairments. The dose level where the plateau was reached differed per individual test. For adaptive tracking the maximum effect occurred around the dose level of 25 mg, whereas the maximum effect for SPV was observed at 65 mg. In contrast with the relatively large effects on SPV, the Smooth pursuit eye movements only demonstrated a significant reduction in performance at the highest dose levels for each cohort. Pharmaco-EEG revealed dose-related effects on the β-, δ-, and θ power bands.

The lowest dose at which a statistical significant difference was observed after treatment with PF-06372865 reflected the interplay between the relative affinity for

the different $GABA_A$ receptor subtypes in combination with the functional activity as demonstrated using the QPatch automated electrophysiology assay (Nickolls et al., 2018). For instance, higher relative binding affinity for $\alpha1\beta3\gamma2$ receptor, coupled with its low functional activity results in earlier onset of the $\alpha1$-mediated pharmacology and a small magnitude of effect. Conversely, the lower relative binding affinity and higher intrinsic activity at $\alpha2\beta2\gamma2$ and $\alpha3\beta3\gamma2$ results in a clear dose-response response, which becomes increasing apparent at higher dose levels.

When comparing the pharmacodynamic effects of two doses PF-06372865 (15 mg and 65 mg) to lorazepam (2 mg) alone, and in combination with lorazepam (2 mg) in Part B, distinct pharmacodynamic effect profiles were observed between the partial subtype selective and the non-selective $GABA_A$ agonists. This is illustrated in *Figure 1*, in which the effect profiles on the different tasks of the different treatments are visualised. It clearly illustrates the subtype selective effects of $\alpha2/\alpha3/\alpha5$ modulation over $\alpha1$ modulation, compared to non-selective $GABA_A$ modulation. A dose of 15 mg or 65 mg PF-06372865 induced a significantly greater reduction in SPV compared to lorazepam (2 mg), which by itself compared to placebo already significantly reduces SPV. A similar pattern was observed for VAS Alertness and Delayed recall (VVLT). On the other hand, administration of lorazepam (2 mg) alone showed a greater decline in performance on adaptive tracking and body sway compared to administration of PF-06372865. Overall, the observed effect profile of 65 mg PF-06372865 combined with lorazepam (2 mg) resembled the effect profile of treatment with 65 mg PF-06372865 alone more closely than the effect profile observed after treatment with lorazepam (2 mg) alone.

A reduction in SPV has previously been linked to modulation of the $\alpha2/\alpha3$ subunits of the $GABA_A$ receptor (Atack, 2010), whereas performance on adaptive tracking has been related to $\alpha1$ modulation (de Haas et al., 2010). As such, large effects on SPV, and relatively small effects on adaptive tracking of PF-06372865, compared to lorazepam (2 mg) are indicative of functional subtype selectivity for the $\alpha2$ and $\alpha3$ subunits (Chen et al., 2012). Cognition and memory impairment as measured with the VVLT have been shown to be associated with $\alpha5$ modulation (Collinson et al., 2002; Crestani et al., 2002). The observed increase in activity in the β power band and decrease in activity in the θ power bands are in line with what is previously reported for the non-selective benzodiazepine, diazepam (Jobert and Wilson, 2015; Yamadera et al., 1993). In contrast, the observed decrease in activity in the δ power band after treatment with lorazepam differentiates from the expected increase in this power band as previously reported (Chen et al., 2015), which was not seen in the present study.

Co-administration of PF-06372865 (65 mg) and lorazepam (2 mg) further underlines the subtype selective effect profile for PF-06372865. Combination of both treatments caused a slight increase in pharmacokinetic exposure of PF-06372865, which is not considered to have a significant impact on the observed pharmacodynamics. Post-hoc statistical testing was performed to assess pharmacodynamic interactions. No evidence for interaction of the pharmacodynamic effects was observed for SPV, smooth pursuit and VAS alertness, which can be explained by non-selective receptor activation

by lorazepam (2 mg) in addition to selective α2/α3 activation by PF-06372865. What is more, infra-additive effects were observed for adaptive tracking (p=0.0001) and body sway (p=0.0002). A reduction in effect for these psychomotor functions compared to administration of lorazepam (2 mg) alone, may indicate that due to competitive activity at the $GABA_A$ receptor, PF-06372865 acts as an antagonist, resulting from partial agonism, at α1 subunits, which diminishes lorazepam's high intrinsic activity as a non-selective full agonist. This is in line with the earlier observations that partial agonists are expected to antagonise the effects of full agonists that may require higher fractional receptor occupancy to exert their effects (Patat et al., 1995).

An interesting trend in the effect profile was observed for the VVLT outcome measures. Both Immediate recall (p=0.004) and Delayed recall (p=0.0057) demonstrated statistically significant antagonistic interaction. Here, the deteriorating effects of PF-06372865 65 mg appear to be diminished by co-administration of lorazepam 2 mg. Delayed recognition demonstrated the opposite: the magnitude of decline in performance by lorazepam 2 mg was reduced by co-administration of PF-06372865 (p=0.0029). These slightly divergent observations on the memory domain indicate that recall and recognition are potentially affected differently and as such can be considered pharmacologically distinct subdomains. A similar pattern was observed in the study by Patat et al. (1995). Here however, co-administration of lorazepam (2 mg) and a partial benzodiazepine agonist, alpidem (50 mg BID (*Bis In Diem)* for eight days), did produce an additive effect in terms of effect size, unlike the antagonistic interaction observed in the present study. More importantly, the apparent discrepant observations between (short-term and delayed) recall and delayed recognition were also observed here. Differential memory performance in (word) recognition and recall has been described before (Tulving, 1973). In experiments designed to investigate the generation-recognition theory, it was shown that under certain conditions subjects consistently failed to recognise words that they were in fact able to recall. This was the foundation for the "Encoding specificity principle", according to which the memory trace of an event and the properties of an effective retrieval cue are determined by the specific encoding operations, e.g. physical environment or semantics (Hannon and Craik, 2001), performed by the system during the encoding phase of input stimuli. Divergent effects of different benzodiazepines on these distinct subdomains have been reported subsequently (Curran, 1986). Hypothesising, as treatment with lorazepam alone in the current study resulted in the largest reduction in delayed recognition performance, this may be associated with lorazepam's sedative properties, mediated via α1 $GABA_A$ receptor subtype activation, rather than α5 $GABA_A$ receptor subtype activation inducing memory impairment. Thereby it effectively hampers the ability to store and retrieve information from the episodic memory (Patat et al., 1995). This hypothesis has not been confirmed by statistical testing, as the study was not powered to detect this effect, but remains an interesting trend in the observations.

The present study is a First-in-Human, hypothesis-generating clinical study designed to guide decision-making and explore the pharmacodynamics and pharmacokinetics, as

well as safety, of PF-06372865 in healthy subjects using a test battery of neurocognitive tasks (NeuroCart®). Therefore, the study was powered to detect a potential pharmacodynamic effect on the primary endpoints. However, given the novelty of the pharmacological mechanism of action, secondary parameters for which no formal power calculation was performed are taken into account when reviewing the generated results. Consequently, interpretation of these findings requires more caution and is potentially more prone to type I or type II error, since no correction for multiple testing was performed. These statistical limitations are partially offset however when the changes are corroborated by the dose-effect relationship described in the Results section and shown in Figures 1 to 3. However, in this two-phase study, two dose levels (15 mg and 65 mg) from Part A were investigated in more detail in a full crossover study using the positive control lorazepam (2 mg) in Part B. This approach allowed for an internal replication and the congruent findings on the different endpoints confirm the validity of the study findings and indicate the risk of bias due to chance findings is low.

In the current study, a similar reduction in SPV compared with lorazepam (2 mg) was observed at the dose level of 4 mg PF-06372865. This could reflect similar α2/α3 $GABA_A$ agonistic activity, which for a number of similar compound has been found to be closely associated with SPV-effects (Chen et al., 2012). On the other hand, performance on adaptive tracking, smooth pursuit eye movements and body sway was considerably less affected by PF-06372865 than by lorazepam (2 mg) alone, which would suggest a lower propensity for adverse events related to CNS depression. The effect profile appears to direct towards a favourable effect profile in terms of anxiolysis, with fewer signs of sedation, compared to classic non-selective benzodiazepines. Based on the findings in the present study an estimation of a potentially anxiolytic dose first takes into consideration that a typical dose of lorazepam for the treatment of anxiety is 2-3 mg one to three times daily. Second, there may be neurophysiological differences in the sensitivity to α2/α3 $GABA_A$ modulation between healthy subjects included in this investigation and the target population. This may be due to changes in the level of allosteric endogenous modulators, or changes in the subunit composition of the $GABA_A$ receptor. A single dose of 4 mg PF-06372865 resulted in a similar reduction in SPV as lorazepam (2 mg). Taking the abovementioned factors into consideration, the projected dose for a clinically relevant anxiolytic effect would be a two- or threefold of this dose.

The anxiolytic efficacy of PF-06372865 was investigated in 2016 in a 4-week clinical trial in GAD patients, who were currently treated with but partial non-responders to standard GAD treatment (Simen et al., 2019). Here, adjuvant therapy of dose levels of 2.5 mg and 7.5 mg BID did not result in a statistically significant improvement in anxiety symptoms measured with the Hamilton Anxiety Inventory. Different explanations have been proposed for the lack of clinically meaningful anxiolytic effect. Apart from the fact that the study was underpowered due to early termination (not for safety or efficacy reasons), pharmacokinetic exposure may have been too low. This could be attributable to administration of low dose levels in general. The translation of 15 mg single dose, as administered in the present study, to 7.5 mg BID repeated dose has not been confirmed

in terms of pharmacodynamic effect profile. As the present study is performed in a highly controlled environment in healthy subjects, higher receptor occupancy may need to be achieved to exert the desired clinical effects in an outpatient setting than the receptor occupancy that was achieved in the GAD patient study that has been performed. (Simen et al., 2019)

The functional selectivity for α2/α3 subunits of the $GABA_A$ receptor has also been identified as a potential target for the treatment of chronic (neuropathic) pain. After a clinical pharmacology study in healthy subjects was performed in which doses of 15 and 65 mg PF-06372865 demonstrated an analgesic effect on pressure pain and the cold pressure task (van Amerongen et al., 2019) a study in chronic low back pain patients was performed. (Gurrell et al., 2018) In this randomised, placebo and active-controlled clinical trial, the parallel treatment group trial consisted of a one-week single-blind placebo run-in phase, followed by a four-week double-blind treatment phase. Patients were randomised to receive either PF-06372865, naproxen or placebo BID for four weeks. The primary endpoint was the numerical rating score (NRS) of low back pain intensity (LBPI) after 4 weeks of active treatment. The study was stopped prematurely for futility, and whilst the reason for the lack of analgesic effect is not completely clear, it has been suggested to be a result of not achieving sufficient receptor occupancy to drive efficacy.

Both patient studies included secondary endpoints to assess pharmacodynamic response, for example the Digit Symbol Substitution Test (DSST) and the Hopkins Verbal Learning Test Revised (HVLT-R), measures for general cognitive functioning and memory performance. The results on these tasks indicate that pharmacologically active doses were administered, which unfortunately did not translate to clinical efficacy.

Overall, it can be concluded that PF-06372865 was safe and well-tolerated by the healthy subjects participating in this study. The pharmacodynamic profile that was characterised using a test battery of neurocognitive tests corresponds well with its predicted functional selectivity for the α2/α3/α5 subunits of the $GABA_A$ receptor, and was in line with preclinical observations (Nickolls et al., 2018). The head-to-head comparison to lorazepam (2 mg) alone and in combination with 65 mg PF-06372865 demonstrated that the combination was safe and well-tolerated. The treatment combination produced a distinct pharmacological interaction profile. On the endpoints representative for α1 $GABA_A$ subtype receptor activation, a competitive interaction diminished the effects of lorazepam 2 mg alone, due to PF-06372865's low intrinsic efficacy for this receptor subtype. On the endpoints representative for α2/α3/α5 $GABA_A$ subtype receptor activation an additive effect was observed, due to non-competitive binding activity at these receptor subtypes. Compared to lorazepam (2 mg) less sedation was observed at dose levels of PF-06372865 corresponding to a potentially anxiolytic dose.

REFERENCES

Atack JR. (2005) The benzodiazepine binding site of GABA(A) receptors as a target for the development of novel anxiolytics. *Expert Opin Investig Drugs* 14: 601-618.

Atack JR. (2010) $GABA_A$ receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. *Curr Top. Behav Neurosci* 2: 331-360.

Atack JR. (2011) $GABA_A$ receptor subtype-selective modulators. II. alpha5-selective inverse agonists for cognition enhancement. *Curr Top. Med Chem* 11: 1203-1214.

Atack JR, Bayley PJ, Seabrook GR, et al. (2006) L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing $GABA_A$ receptors. *Neuropharmacology* 51: 1023-1029.

Baloh RW, Kumley WE, Sills AW, et al. (1976) Quantitative measurement of smooth pursuit eye movements. *Ann Otol. Rhinol. Laryngol* 85: 111-119.

Bittencourt PR, Wade P, Smith AT, et al. (1983) Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol* 15: 259-262.

Bond A and Lader M. (1974) The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47: 211-218.

Borland RG and Nicholson AN. (1975) Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. *Br J Clin Pharmacol* 2: 9-17.

Chen X, de HS, de KM, et al. (2012) An Overview of the CNS-Pharmacodynamic Profiles of Nonselective and Selective GABA Agonists. *Adv Pharmacol Sci* 2012: 134523.

Chen X, Jacobs G, de Kam ML, et al. (2015) AZD6280, a novel partial gamma-aminobutyric acid A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers. *J Clin Psychopharmacol* 35: 22-33.

Chen X, Jacobs G, de KM, et al. (2014) The central nervous system effects of the partial GABA-Aalpha2,3 -selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. *Br J Clin Pharmacol* 78: 1298-1314.

Chou R and Huffman LH. (2007) Medications for acute and chronic low back pain: a review of the evidence for an American Pain Society/American College of Physicians clinical practice guideline. *Ann Intern Med* 147: 505-514.

Collinson N, Kuenzi FM, Jarolimek W, et al. (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci* 22: 5572-5580.

Crestani F, Keist R, Fritschy JM, et al. (2002) Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. *Proc Natl Acad Sci USA* 99: 8980-8985.

Curran H. (1986) Tranquillising memories: A review of the effects of benzodiazepines on human memory. *Biological Psychology* 23: 179-213.

Curtis DR and Johnston GA. (1974) Amino acid transmitters in the mammalian central nervous system. *Ergeb. Physiol* 69: 97-188.

Dämgen K and Lüddens H. (1999) Zaleplon displays a selectivity to recombinant $GABA_A$ receptors different from zolipdem, zopiclone and benzodiazepines. *Neuroscience Research Communications* 25: 139-148.

Davidson JR, Feltner DE and Dugar A. (2010) Management of generalized anxiety disorder in primary care: identifying the challenges and unmet needs. *Prim. Care Companion. J Clin Psychiatry* 12.

de Haas SL, de Visser SJ, van der Post JP, et al. (2007) Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA(A) alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol* 21: 374-383.

de Haas SL, Franson KL, Schmitt JA, et al. (2009) The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. *J Psychopharmacol* 23: 625-632.

de Haas SL, Schoemaker RC, van Gerven JM, et al. (2010) Pharmacokinetics, pharmacodynamics and the pharmacokinetic/ pharmacodynamic relationship of zolpidem in healthy subjects. *J Psychopharmacol* 24: 1619-1629.

de Visser SJ, van der Post JP, de Waal PP, et al. (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol* 55: 39-50.

Duke AN, Meng Z, Platt DM, et al. (2018) Evidence That Sedative Effects of Benzodiazepines Involve Unexpected $GABA_A$ Receptor Subtypes: Quantitative Observation Studies in Rhesus Monkeys. *J Pharmacol Exp Ther* 366: 145-157.

Frommele E, Fleury C, Schmidt-Ginskey J, et al. (1960) On the pharmacodynamic action of new tranquilizing agent: methaminodiazepoxide or Librium. Experimental study. *Therapie* 15: 1233-1244.

Gurrell R, Dua P, Feng G, et al. (2018) A randomised, placebo-controlled clinical trial with the alpha2/3/5 subunit selective $GABA_A$ positive allosteric modulator PF-06372865 in patients with chronic low back pain. *Pain* 159: 1742-1751.

Hannon B and Craik FIM. (2001) Encoding specificity revisited: The role of semantics. *Canadian Journal of Experimental Psychology/Revue canadienne de psychologie expérimentale* 55: 231-243.

Jobert M and Wilson FJ. (2015) Advanced Analysis of Pharmaco-EEG Data in Humans. *Neuropsychobiology* 72: 165-177.

Knabl J, Witschi R, Hosl K, et al. (2008) Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. *Nature* 451: 330-334.

Mandema JW, Tuk B, van Steveninck AL, et al. (1992) Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite alpha-hydroxymidazolam in healthy volunteers. *Clin Pharmacol Ther* 51: 715-728.

McKernan RM and Whiting PJ. (1996) Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci* 19: 139-143.

Mets MA, Volkerts ER, Olivier B, et al. (2010) Effect of hypnotic drugs on body balance and standing steadiness. *Sleep Med Rev* 14: 259-267.

Neubauer DN. (2014) New and emerging pharmacotherapeutic approaches for insomnia. *Int Rev Psychiatry* 26: 214-224.

Nickolls SA, Gurrell R, van Amerongen G, et al. (2018) Pharmacology in translation: the preclinical and early clinical profile of the novel alpha2/3 functionally selective $GABA_A$ receptor positive allosteric modulator PF-06372865. *Br J Pharmacol* 175: 708-725.

Norris H. (1971) The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology* 10: 181-191.

Patat A, Perault MC, Vandel B, et al. (1995) Assessment of the interaction between a partial agonist and a full agonist of benzodiazepine receptors, based on psychomotor performance and memory, in healthy volunteers. *Journal of Psychopharmacology* 9: 91-101.

Reinhold JA and Rickels K. (2015) Pharmacological treatment for generalized anxiety disorder in adults: an update. *Expert Opin Pharmacother* 16: 1669-1681.

Rudolph U, Crestani F, Benke D, et al. (1999) Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* 401: 796-800.

Schmitt JA, Jorissen BL, Sobczak S, et al. (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *J Psychopharmacol* 14: 21-29.

Simen A, Whitlock M, Qiu R, et al. (2019) An 8-Week, Randomized, Phase 2, Double-Blind, Sequential Parallel-Group Comparison Study of Two Dose Levels of the $GABA_A$ Positive Allosteric Modulator PF-06372865 Compared With Placebo as an Adjunctive Treatment in Outpatients With Inadequate Response to Standard of Care for Generalized Anxiety Disorder. *J Clin Psychopharmacol* 39: 20-27.

Smith KS, Engin E, Meloni EG, et al. (2012) Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct $GABA_A$ receptor subtypes in mice. *Neuropharmacology* 63: 250-258.

Starcevic V. (2014) The reappraisal of benzodiazepines in the treatment of anxiety and related disorders. *Expert Rev Neurother* 14: 1275-1286.

Tulving ET, M. (1973) Encoding specificity and retrieval processes in episodic memory. *Psychological reviews* 80: 352-373.

van Amerongen G, Siebenga PS, Gurrell R, et al. (2019) Analgesic potential of PF-06372865, an α2/α3/α5 subtype-selective $GABA_A$ partial agonist, in humans. *British Journal of Anaesthesia*.

van Steveninck AL, Gieschke R, Schoemaker HC, et al. (1993) Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. *Psychopharmacology (Berl)* 110: 471-478.

van Steveninck AL, Gieschke R, Schoemaker RC, et al. (1996) Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. *Br J Clin Pharmacol* 41: 565-573.

van Steveninck AL, Schoemaker HC, den HJ, et al. (1994) Effects of intravenous temazepam. I. Saccadic eye movements and electroencephalogram after fast and slow infusion to pseudo steady state. *Clin Pharmacol Ther* 55: 535-545.

van Steveninck AL, Schoemaker HC, Pieters MS, et al. (1991) A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* 50: 172-180.

van Steveninck AL, van Berckel BN, Schoemaker RC, et al. (1999) The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol* 13: 10-17.

Wright BM. (1971) A simple mechanical ataxia-meter. *J Physiol* 218 Suppl: 27P-28P.

Yamadera H, Kato M, Ueno T, et al. (1993) Pharmaco-EEG mapping of diazepam effects using different references and absolute and relative power. *Pharmacopsychiatry* 26: 254-258.

Zuiker RG, Chen X, Osterberg O, et al. (2015) NS11821, a partial subtype-selective $GABA_A$ agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. *J Psychopharmacol*.

TABLE 1 Overview of neurocognitive tasks included in the NeuroCart® and associated functional domains

Functional domain	NeuroCart Test	Endpoints
Neurophysiological functioning	Saccadic eye movement	Saccadic Peak Velocity (deg/sec) Saccadic reaction time (msec)* Saccadic inaccuracy (%)*
Neurophysiological functioning	Smooth pursuit	Proportion in smooth pursuit (%)
Visuomotor coordination	Adaptive tracking	Average performance (%) SD of average performance (%)*
Balance	Body sway	Anteroposterior movement (mm)
Subjective Alertness, mood, calmness	VAS Bond and Lader	VAS Alertness (mm) VAS Mood (mm)* VAS Calmness (mm)*
Memory	Visual Verbal Learning Test (VVLT)	Immediate recall: Number correct Immediate recall: Number incorrect* Delayed recall: Number correct Delayed recall: Number incorrect* Delayed recognition: Number correct Delayed recognition: Number incorrect* Average reaction time for Correct words (msec)* SD of average reaction time for Correct words (msec)*
Brain activity	Pharmaco-EEG	Alpha Fz-Cz Alpha Pz-Oz * Beta Fz-Cz Beta Pz-Oz * Delta Fz-Cz Delta Pz-Oz * Theta Fz-Cz Theta Pz-Oz*

** Results not presented in manuscript.*

TABLE 2 Summary of subject characteristics

		Part A		Part B	Total
Number of Subjects	**N=10**	**N=10**	**N=10**	**N=15**	**N=45**
Gender: n (%)					
Male	10 (100)	10 (100)	10 (100)	14 (93)	44 (98)
Female	0 (0)	0 (0)	0 (0)	1 (7)	1 (2)
Age (years)					
Mean (SD)	33.0 (10.3)	28.0 (8.0)	27.6 (7.6)	28.2 (8.8)	29.1 (8.6)
Range	22-48	20-47	18-43	18-53	18-53
Weight (kg)					
Mean (SD)	77.6 (11.0)	76.1 (9.7)	77.0 (9.5)	73.1 (9.2)	75.5 (9.3)
Range	59.0-92.6	57.8-93.2	62.7-93.8	62.5-98.1	57.3-98.1
Height (cm)					
Mean (SD)	179.4 (6.9)	183.6 (4.8)	183.7 (5.8)	179.6 (5.6)	181.3 (6.0)
Range	167.2-192.6	176.3-194.0	172.7-192.2	168.8-191.9	167.2-194.0
BMI (kg/m^2)					
Mean (SD)	24.1 (3.3)	22.6 (2.8)	22.8 (2.3)	22.7 (3.2)	23.0 (2.8)
Range	19.0-28.4	17.5-27.8	17.8-25.6	18.9-29.9	17.5-29.9

BMI = Body Mass Index / SD = standard deviation / Kg = kilogram

TABLE 3 Overview of All Causality Adverse Events reported in Part B

Number of Subjects with AEs by SOC MedDRA Preferred Term	Placebo (N=15)	PF-06372865 15 mg (N=15)	PF-06372865 65 mg (N=15)	Lorazepam 2 mg (N=15)	PF-06372865 65 mg + Lorazepam 2 mg (N=15)
Cardiac Disorders	0	0	0	1 (0)	0
Ear and Labyrinth Disorders	0	1 (1)	0	0	0
Eye Disorders	0	0	2 (2)	2 (2)	2 (2)
Gastrointestinal Disorders	1 (1)	2 (1)	2 (1)	0	1 (1)
General Disorders and Administration Site Conditions	2 (1)	5 (5)	6 (4)	5 (4)	7 (6)
Infections and Infestations	0	0	1 (0)	0	0
Metabolism and Nutrition Disorders	0	0	1 (1)	1 (1)	0
Nervous System Disorders	4 (4)	11 (10)	12 (12)	11 (11)	14 (13)
Dizziness	2 (2)	6 (6)	8 (8)	3 (3)	7 (7)
Somnolence	2 (2)	7 (7)	7 (7)	8 (8)	8 (8)
Psychiatric Disorders	0	0	5 (5)	2 (2)	0
Renal and Urinary Disorders	0	0	2 (2)	0	1 (1)
Respiratory, Thoracic and Mediastinal Disorders	1 (0)	0	0	0	0
Total preferred term events	**10 (8)**	**24 (22)**	**43 (38)**	**24 (22)**	**34 (31)**

SOC = System Organ Class / MEDDRA = Medical Dictionary for Regulatory Activities / Numbers shown in brackets represent the number of treatment-related adverse events.

TABLE 4 Pharmacodynamic profile of 2 doses of PF-06372865 presented as overall LSM (95% Confidence Interval) compared with placebo and lorazepam 2mg for pharmacodynamics tests

		PF-06372865 15 mg (N=15)	PF-06372865 65 mg (N=15)	PF-06372865 65 mg + Lorazepam 2 mg (N=15)	Lorazepam 2 mg (N=15)	Placebo (N=15)
Saccadic peak velocity (deg/sec)	LSMeans	391.0 (372.4, 409.6)	371.7 (352.7, 390.8)	355.8 (336.7, 374.9)	425.1 (405.6, 444.6)	463.7 (444.8, 482.6)
	LSM vs placebo	**-72.7 (-99.1, -46.2)***	**-91.9 (-119.0, -64.9)***	**-107.9 (-134.7, -81.0)***	**-38.6 (-66.2, -11.0)***	
	LSM vs Lorazepam	**-34 (-61.0, -7.1)***	**-53.3 (-80.4, -26.3)***	**-69.3 (-96.5, -42.0)***		
Smooth pursuit (%)[a]	LSMeans	44.5 (40.8, 48.2)	47.1 (43.3, 50.8)	45.8 (42.0, 49.5)	42.7 (39.0, 46.5)	47.4 (43.7, 51.2)
	LSM vs placebo	-2.9 (-8.2, 2.3)	-0.4 (-5.7, 4.9)	-1.7 (-7.0, 3.6)	-4.7 (-10.0, 0.6)	
	LSM vs Lorazepam	1.8 (-3.5, 7.0)	4.3 (-0.9, 9.6)	3.0 (-2.3, 8.3)		
Adaptive tracking (%)[b]	LSMeans	29.03 (26.83, 31.23)	28.09 (25.89, 30.28)	26.99 (24.78, 29.21)	23.77 (21.57, 25.97)	34.20 (31.99, 36.41)
	LSM vs placebo	**-5.17 (-8.28, -2.06)***	**-6.11 (-9.23, -2.99)***	**-7.20 (-10.36, -4.05)***	**-10.43 (-13.55, -7.31)***	
	LSM vs Lorazepam	**5.26 (2.15, 8.37)***	**4.32 (1.21, 7.42)***	**3.23 (0.11, 6.34)***		
Body Sway (ln mm)	LSMeans	5.54 (5.39, 5.69)	5.66 (5.51, 5.81)	5.72 (5.58, 5.87)	5.85 (5.70, 6.00)	5.16 (5.01, 5.31)
	LSM vs placebo	**0.38 (0.17, 0.59)***	**0.50 (0.29, 0.71)***	**0.56 (0.35, 0.77)***	**0.68 (0.47, 0.90)***	
	LSM vs Lorazepam	**-0.31 (-0.52, -0.09)***	-0.19 (-0.40, 0.03)	-0.12 (-0.33, 0.09)		
VVLT Immediate Recall (number correct)	LSMeans	14.1 (11.5, 16.7))	11.8 (9.2, 14.4)	12.4 (9.7, 15.0)	13.1 (10.5, 15.7)	16.8 (14.2, 19.4)
	LSM vs placebo	**-2.7 (-4.7, -0.8)***	**-5.0 (-7.0, -3.0)***	**-4.4 (-6.4, -2.4)***	**-3.7 (-5.6, -1.7)***	
	LSM vs Lorazepam	0.9 (-1.0, 2.9)	-1.3 (-3.3, 0.6)	-0.8 (-2.8, 1.3)		
VVLT Delayed Recall (number correct)	LSMeans	9.6 (6.8, 12.4)	6.5 (3.6, 9.3)	6.6 (3.7, 9.4)	8.3 (5.5, 11.2)	13.2 (10.4, 16.0)
	LSM vs placebo	**-3.6 (-6.0, -1.2)***	**-6.7 (-9.1, -4.4)***	**-6.6 (-9.1, -4.2)***	**-4.9 (-7.3, -2.4)***	
	LSM vs Lorazepam	1.3 (-1.2, 3.7)	-1.9 (-4.3, 0.6)	-1.8 (-4.3, 0.7)		
VVLT Delayed Recognition (number correct)	LSMeans	24.3 (21.7, 26.9)	22.6 (20.0, 25.2)	22.2 (19.5, 24.8)	20.2 (17.5, 22.8)	26.1 (23.5, 28.7)
	LSM vs placebo	-1.9 (-4.3, 0.6)	**-3.5 (-6.0, -1.1)***	**-4.0 (-6.5, -1.5)***	**-5.9 (-8.4, -3.4)***	
	LSM vs Lorazepam	**4.1 (1.6, 6.6)***	2.4 (-0.1, 4.9)	2 (-0.6, 4.5)		
VAS Alertness (mm)	LSMeans	48.3 (45.7, 50.9)	48.9 (46.3, 51.5)	47.1 (44.5, 49.7)	50.1 (47.5, 52.6)	51.8 (49.2, 54.5)
	LSM vs placebo	-3.6 (-7.3, 0.1)	-2.9 (-6.6, 0.8)	**-4.8 (-8.5, -1.0)***	-1.8 (-5.5, 1.9)	
	LSM vs Lorazepam	-1.8 (-5.4, 1.9)	-1.1 (-4.8, 2.5)	-3.0 (-6.7, 0.7)		

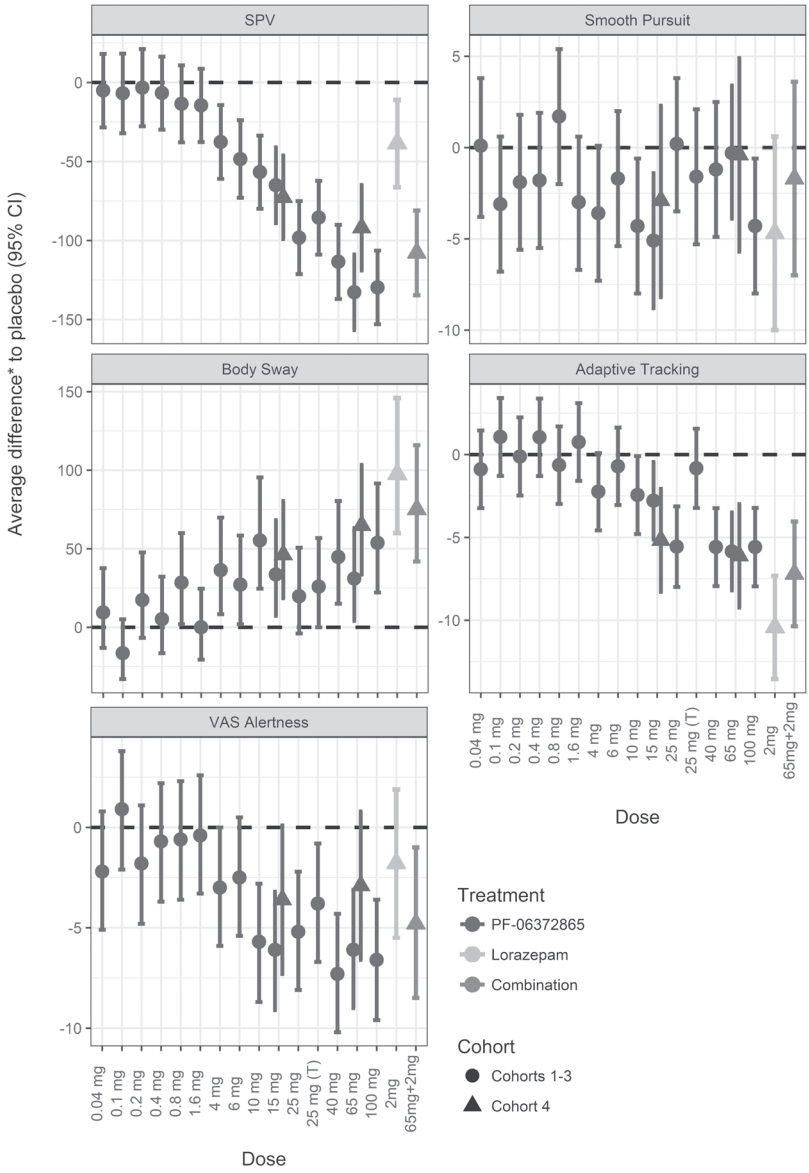
*a = Percentage correct in smooth pursuit / b = Percentage correct / * (p<0.05) / deg = degrees / sec = seconds*

TABLE 5 Pharmacodynamic Interaction profile of PF-06372865 (65 mg) and lorazepam (2 mg) presented as overall LSMeans (95% Confidence Interval)

		Calculated summation of (PF-06372865 65 mg) + (Lorazepam 2 mg)	Co-administration of PF-06372865 65 mg + Lorazepam 2 mg
Saccadic peak velocity (deg/sec)	LSMeans v. Placebo	-130.6	-107.9
	Estimate of difference	-22.7 (-61.5, 16.1)	
	P value	0.25	
Smooth pursuit (%)[a]	LSMeans v. Placebo	-5.1	-1.7
	Estimate of difference	-3.4 (-10.9, 4.1)	
	P value	0.37	
Adaptive tracking (%)[b]	LSMeans v. Placebo	-16.5	-7.2
	Estimate of difference	-9.3 (-13.7, -4.9)	
	P value	0.0001	
Body Sway (ln mm)	LSMeans v. Placebo (%)	+226%	+76%
	Estimate of %difference	+86% (38, 150%)	
	P value	0.0001	
VVLT Immediate Recall (number correct)	LSMeans v. Placebo	-8.7	-4.4
	Estimate of difference	-4.2 (-7.1, -1.4)	
	P value	0.004	
VVLT Delayed Recall (number correct)	LSMeans v. Placebo	-11.6	-6.6
	Estimate of difference	-5.0 (-8.4, -1.5)	
	P value	0.0057	
VVLT Delayed Recognition (number correct)	LSMeans v. Placebo	-9.5	-4.0
	Estimate of difference	-5.5 (-9.0, -2.0)	
	P value	0.0029	
VAS Alertness (mm)	LSMeans v. Placebo	-4.7	-4.8
	Estimate of difference	0.058 (-5.1, 5.2)	
	P value	0.98	

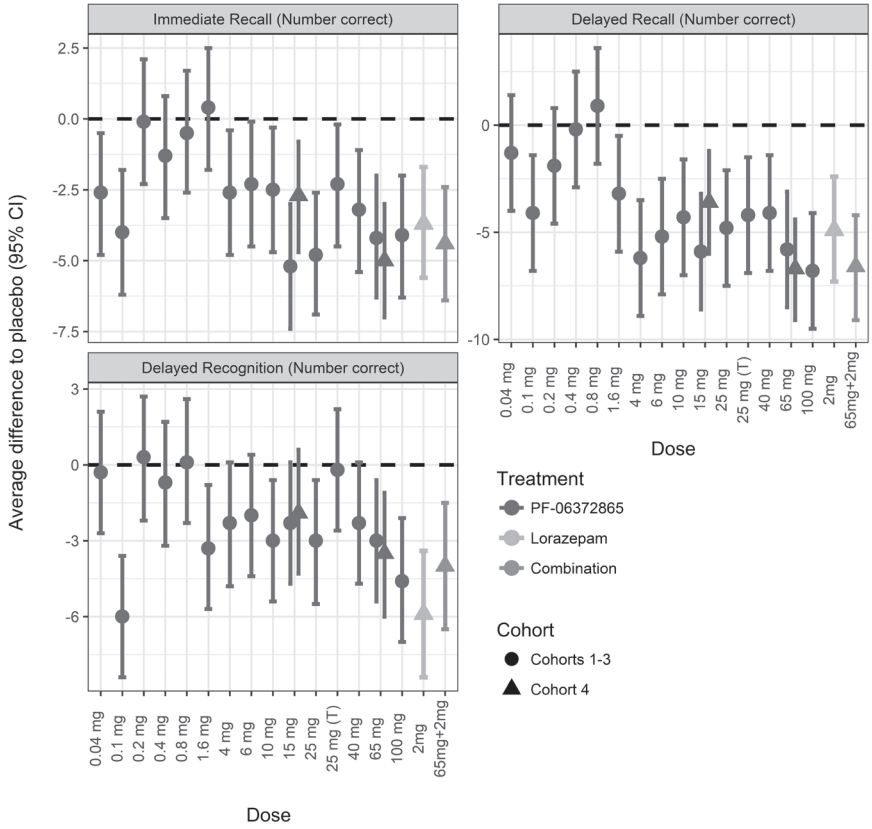
VAS = Visual Analogue Scale / VVLT = Visual Verbal Learning Test

FIGURE 1 Pharmacodynamic effects on Saccadic Peak Velocity (SPV), Smooth Pursuit, Adaptive tracking, Visual Analogue Scale (VAS) Alertness and Body sway of PF-06372865 and lorazepam presented as overall LSM (95% Confidence Interval) compared with placebo.



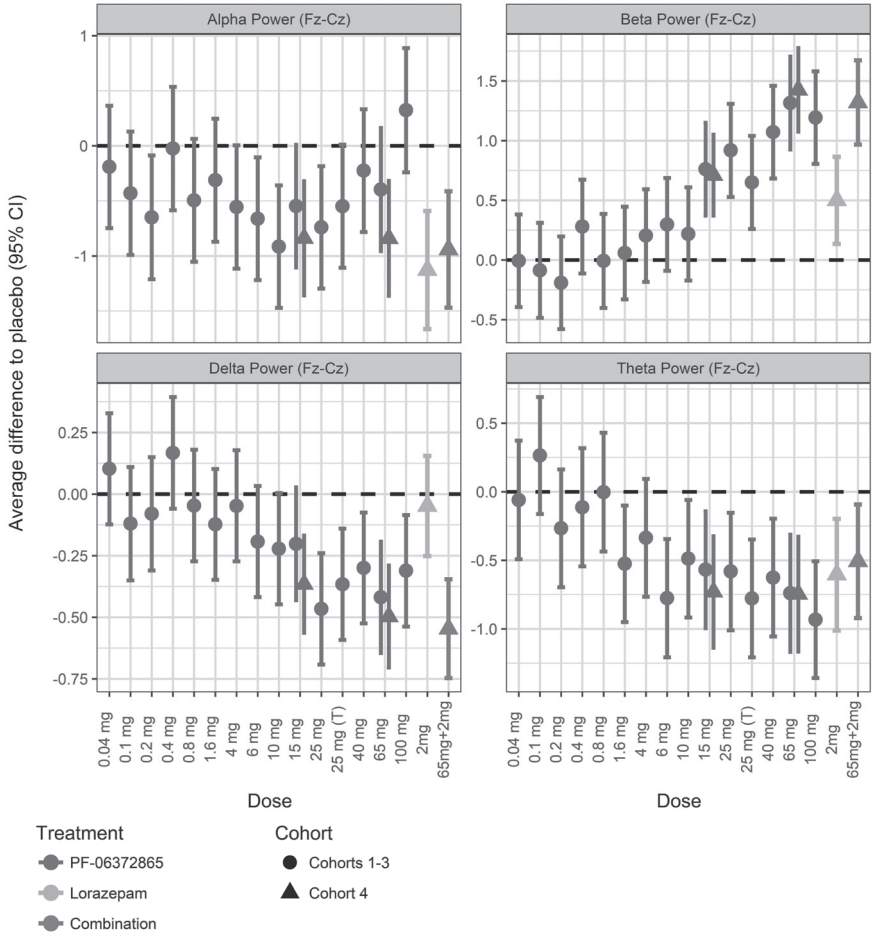
*mg = milligram / (T) =tablet formulation / SPV = Saccadic Peak Velocity (deg/sec) / VAS = Visual Analogue Scale / *Differences to placebo for body sway are represented as %differences, otherwise represent absolute differences.*

FIGURE 2 Pharmacodynamic effects on VVLT of PF-06372865 and lorazepam presented as overall LSM (95% Confidence Interval) compared with placebo.



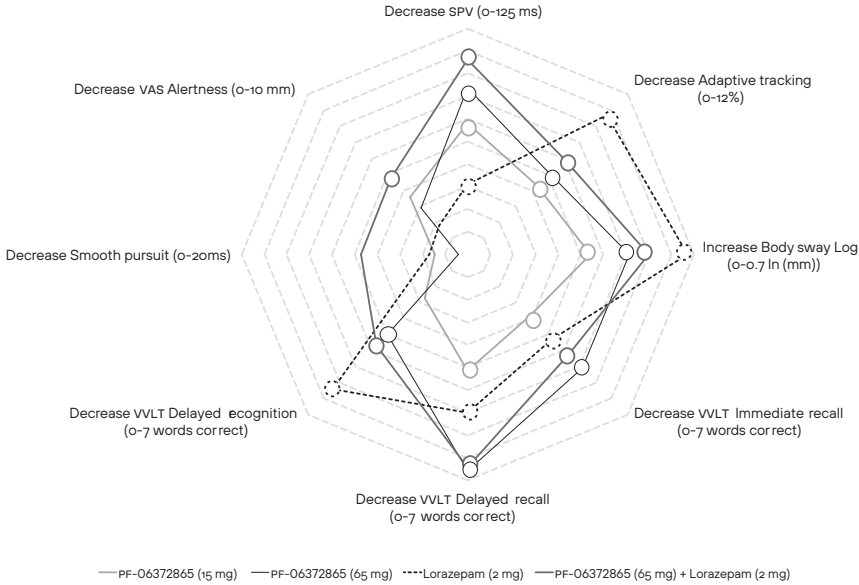
mg = milligram / (T) = tablet formulation

FIGURE 3 Pharmacodynamic effects on EEG of PF-06372865 and lorazepam presented as overall LSM (95% Confidence Interval) compared with placebo.



mg = milligram / (T) = tablet formulation

FIGURE 4 Spiderplot overview of LSM for saccadic eye movements, smooth pursuit eye movements, adaptive tracking, body sway, immediate recall memory, subjective alertness



Graph represents contrasts per treatment per functional domain compared with placebo. Distal from centre indicates Least Square Mean estimation greater than placebo. An open circle (❍) indicates a statistically significant (P<0.05) difference.

CHAPTER 6

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

G. van Amerongen[1], P. Siebenga[1], R. Gurrell[2], P. Dua[2], M. Whitlock[2], D. Gorman[2], P. Okkerse[1], J.L. Hay[1], R. Butt[2], GJ Groeneveld[1]

1 Centre for Human Drug Research (CHDR) / Leiden University Medical Centre (LUMC) Leiden, The Netherlands
2 Early Clinical Development, Pfizer WRD, Cambridge, UK

ABSTRACT

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

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

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

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

INTRODUCTION

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

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

The role for GABAergic pain modulation in humans is supported by two clinical studies in healthy subjects using evoked acute and hyperalgesic pain models to

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

PF-06372865 (IUPAC name: 7-ethyl-4-(4'- ethylsulfonyl)-6-flouro- 2'methoxybiphenyl-3- yl)-7H-imidazo4,5-2-pyridazine) is a potent ligand of the allosteric benzodiazepine site of the $GABA_A$ receptor, which exhibits functional selectivity for receptors containing α2, α3 or α5 over those containing α1.[26] Therefore, PF-06372865 has the potential to provide analgesia but with less sedation than non-selective benzodiazepines.

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

METHODS

Subjects and study design

The study was a double-blind, double dummy, single dose, randomised, placebo-controlled, 4-period crossover study in which the effects of two dose levels of PF-06372865 were compared to placebo and pregabalin (300 mg) was included as a positive control. The study was conducted at the Centre for Human Drug Research in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. This study was registered in the public registry of ClinicalTrials.gov under registration number: NCT02238717.

Each subject provided written informed consent before any screening procedures were performed. A total of 20 healthy male subjects between 18 and 55 years of age

with a body mass index of 17.5 to 30.5 kg m² were enrolled. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and haematology, urinalysis, electrocardiogram (ECG) and assessment of the minimal erythema dose (MED) for UVB light to assess eligibility. Subjects with a clinically significant known medical condition, in particular any existing condition that would affect sensitivity to cold or pain were excluded. Subjects with Fitzpatrick skin type V or VI, widespread acne, tattoos or scarring on the back were excluded due to the inability to accurately assess MED. Also, any subject who was a regular user of any illicit drugs, had a history of drug abuse or a positive drug screen at screening was excluded. Smoking and the use of xanthine-containing products was not allowed during dosing days. Alcohol was not allowed at least 24 hours before each scheduled visit and during the stay in the research unit.

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

Safety

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

Pharmacodynamic assessments

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

were responsible for starting and ending each pain task. To eliminate the risk of tissue damage, all pain tasks had a maximum safety cut-off.

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

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

Pharmacokinetic assessments

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

Statistics

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

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

RESULTS

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

Pharmacodynamics

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

A detailed overview of the results of the analyses for the pharmacodynamic output variables (PTT, PDT, AUC and VAS) is provided in *Table 2*. 15 mg PF-06372865 significantly increased Pressure PTT at a ratio of 1.11 (90%CI: 1.02, 1.22) compared to placebo. A dose of 65 mg PF-06372865 significantly increased Cold pressor PTT at a ratio of 1.17 (90%CI: 1.03, 1.32), as well as VAS for Electrical stimulation compared to placebo. Over the 6-hour period, a statistically significant increase at a ratio of 1.11 (90%CI: 1.01, 1.21) on Pressure PTT was also observed for PF-06372865 65 mg versus placebo. However, Pressure PDT was statistically significant decreased, indicating an increased sensitivity in pain detection. The positive control, pregabalin (300 mg) significantly affected pain sensation for Cold pressor PTT by 1.31 (90%CI: 1.16, 1.48) compared to placebo as well as Cold pressor AUC. Additionally, Pressure PTT was significantly increased at a ratio of 1.15 (95%CI: 1.06,

1.26), as well as Pressure AUC. No statistically significant effects were detected for any of the three treatments versus placebo for any of the endpoints related to the thermal pain tasks (normal skin and UVB skin). As a result of the conditioning stimulus, the PTT for electrical pain was on average decreased by 1.16 mA in the placebo group. None of the CPM parameters were statistically significant different between the three active treatments and placebo.

Pharmacokinetics

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

Safety

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

DISCUSSION

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

PF-06372865 demonstrated an analgesic effect on Pressure pain and the Cold pressor task. The magnitude of effect was greater for a dose of 65 mg compared with a

dose of 15 mg for the Cold pressor but the effects were similar for the Pressure pain task. Pregabalin 300 mg attenuated pain induced using the Cold pressor and the Pressure pain task.

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

Absence of analgesia against UV induced inflammatory pain of PF-06372865 is perhaps surprising, as spinal $GABA_A$ α2 receptors have been identified as potential targets to exert antihyperalgesic effects in preclinical research.[13,16,18,38] $GABA_A$ α2 receptors are located and act at a spinal level.[39] Hyperalgesia resulting from cutaneous (UVB induced) inflammation, is thought (albeit debatable) to be of peripheral origin[40], resulting from decreased activation thresholds for local nociceptive and non-nociceptive neurons alike. This is a response to damaged DNA as a result of exposure to UVB radiation, which results in the induction of NFκB that leads to local production of cytokines IL-1, IL-6 and TNF-α.[41] This specific type of inflammation and associated inflammatory hyperalgesia is distinct from other types of experimentally induced inflammation typically used in preclinical research in its origin and underlying pathophysiology.[42,43] UVB-induced hyperalgesia is most effectively counteracted by inhibition of cyclooxygenase (COX), thus preventing the formation of prostaglandins and thromboxane, which are responsible for the lowered activation thresholds.[32,44,45] PF-06372865 exerts its effects via GABAergic modulation of the central nociceptive system, which is known to demonstrate mixed effects on UVB induced inflammatory pain in human evoked pain studies.[46] The absence of antihyperalgesic effects is at odds with previously reported antihyperalgesic effects of clobazam on UVB induced heat perception thresholds.[47] A potential explanation could be the less profound $GABA_A$ receptor subtype selectivity of clobazam compared to PF-06372865, as its metabolite *N*-desmethyl clobazam is proposed to demonstrate more selectivity for α2 $GABA_A$ receptors over α1 $GABA_A$ receptors, compared to the parent.[48] Similar to the findings in the present study, it was shown that CPM was not affected by treatment with the nonselective benzodiazepine lorazepam.[49] The role for GABAergic interneurons in descending modulation and their role in chronification of pain has been recognised previously.[50–52] The underlying mechanism has been described as the GABA disinhibition hypothesis of analgesia.[53] This hypothesis describes a particular

descending periaqueductal gray - rostroventricular medulla (PAG-RVM) pathway that mediates the phenomenon of stress-induced analgesia. This pathway exerts analgesia via suppression of inhibitory GABAergic inputs onto output neurons that constitute the descending analgesic pathway. However, the CPM paradigm based on electrical pain, which was used in the current study, may not be the most suitable paradigm to determine the potential effects of GABAergic intervention, as it has been shown that spinal enkephalins and GABA presynaptically modulate mechanonociception via Aδ fibres, whereas electrical stimulation is generally thought to activate both Aδ and C fibres.[54,55] Finally, as also suggested previously[49] the lack of a modulating effect on CPM by $GABA_A$ agonism in the present study, may be explained by the fact that in the healthy state the role of GABAergic interneurons in CPM is optimised for endogenous $GABA_A$ agonism and thus not susceptible for external influence in the absence of true stressors. In contrast, patients suffering from chronic pain, where it is known that GABAergic dysregulation may be a large contributing factor[16], may be susceptible for pain alleviation through $GABA_A$ modulation. This hypothesis is moderately substantiated by the overlap observed in effect profiles of pregabalin and PF-06372865. This overlap in clinical application may suggest that PF-06372865 has a potential role in the treatment of different types of neuropathic pain, similar to pregabalin.[56] Alternatively, variability of CPM response is higher than the variability of the other pain tasks and perhaps the study was underpowered to detect an effect on CPM response.

Even though the clinical application of PF-06372865 and pregabalin may demonstrate a slight overlap, their mechanism of action is different. Pregabalin is a calcium channel antagonist that shows specific binding affinity for the α_2-δ auxiliary subunits of voltage-gated calcium channels (VGCC). There have been a number of studies showing an up-regulation of VGCCS in dorsal root ganglion and dorsal horn in neuropathic pain.[57] Administration of pregabalin is shown to partially reverse the up-regulated calcium α_2-δ subunits at the pre-synaptic nerve terminals in the dorsal horn.[58] Even though there is a structural resemblance with GABA, different studies have shown that pregabalin does not appear to mimic GABA or pharmacologically enhance its actions.[59] As such, pregabalin is not considered a positive control from a mechanistic perspective, but rather from a clinical perspective. More importantly its analgesic effects and the reproducibility thereof on the pain test battery that was used in the current study have been demonstrated multiple times before. (Siebenga et al., 2018).

The population included in the study was considered to be very homogeneous, which improves the internal validity but may impact generalisability of the study results to other populations. The study included only male subjects, of which 90% was white. The existence of sex differences in terms of sensitivity to clinical and experimental pain is widely known.[60,61] It has been suggested that sex-specific differences in $GABA_A$ receptors may play a role in this differentiation.[62] However, it is also known that the menstrual cycle influences pain perception[63,64], which would interfere significantly with the crossover design of the study, therefore it was decided to include only male subjects were in the present study. Furthermore, since the UVB model was a primary endpoint of the study,

subjects with Fitzpatrick skin type V or VI were excluded due to the inability to accurately assess MED and to safely induce UVB induced inflammation, leading to a predominantly white study population. Research has identified differences in pain sensitivity between different ethnic or racial populations in experimental pain research.[65,66] Whether those findings can be extrapolated to the Dutch population and what is the exact cause of the differences in pain perception is unknown.

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

The findings in the present study are indicative of the analgesic potential for PF-06372865 at the doses tested, in addition to the neurocognitive anxiolytic potential that has been identified previously.[26] The observed effect profile does not appear to result from sedation alone for various reasons. In both this study and a previous study, PF-06372865 was shown to exert only mild sedative effects. Second, in a recently performed study[67], we showed that the test battery of evoked pain tasks was not sensitive to the effects of sedation, by investigating the effects of a sedative H1 antihistaminergic agent, promethazine.

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

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

REFERENCES

1 Curtis DR, Johnston GA. Amino acid transmitters in the mammalian central nervous system. *Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie* 1974; **69**: 97-188

2 McKernan RM, Whiting PJ. Which $GABA_A$-receptor subtypes really occur in the brain? *Trends in neurosciences* 1996; **19**: 139-43

3 Rudolph U, Knoflach F. Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. *Nature reviews Drug discovery* 2011; **10**: 685-97

4 Rudolph U, Crestani F, Benke D, et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* 1999; **401**: 796-800

5 Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing $GABA_A$ receptors. *Neuropharmacology* 2006; **51**: 1023-9

6 Atack JR, Hallett DJ, Tye S, et al. Preclinical and clinical pharmacology of TPA023B, a $GABA_A$ receptor α2/α3 subtype-selective partial agonist. *Journal of psychopharmacology (Oxford, England)* 2011; **25**: 329-44

7 Zuiker RG, Chen X, Osterberg O, et al. NS11821, a partial subtype-selective $GABA_A$ agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. *Journal of psychopharmacology (Oxford, England)* 2016; **30**: 253-62

8 Smith KS, Engin E, Meloni EG, Rudolph U. Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct $GABA_A$ receptor subtypes in mice. *Neuropharmacology* 2012; **63**: 250-8

9 Crestani F, Keist R, Fritschy JM, et al. Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. *Proceedings of the National Academy of Sciences of the United States of America* 2002; **99**: 8980-5

10 Wainwright A, Sirinathsinghji DJ, Oliver KR. Expression of GABA(A) receptor alpha5 subunit-like immunoreactivity in human hippocampus. *Brain research Molecular brain research* 2000; **80**: 228-32

11 Cheng VY, Martin LJ, Elliott EM, et al. Alpha5$GABA_A$ receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. *J Neurosci* 2006; **26**: 3713-20

12 12 Melzack R, Wall PD. Pain mechanisms: a new theory. *Science* 1965; **150**: 971-9

13 Paul J, Zeilhofer HU, Fritschy JM. Selective distribution of GABA(A) receptor subtypes in mouse spinal dorsal horn neurons and primary afferents. *J Comp Neurol* 2012; **520**: 3895-911

14 Vuilleumier PH, Fritsche R, Schliessbach J, et al. Mutations affecting glycinergic neurotransmission in hyperekplexia increase pain sensitivity. *Brain : a journal of neurology* 2018; **141**: 63-71

15 Zeilhofer HU, Acuna MA, Gingras J, Yevenes GE. Glycine receptors and glycine transporters: targets for novel analgesics? *Cellular and molecular life sciences : CMLS* 2018; **75**: 447-65

16 Knabl J, Witschi R, Hosl K, et al. Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. *Nature* 2008; **451**: 330-4

17 Munro G, Lopez-Garcia JA, Rivera-Arconada I, et al. Comparison of the novel subtype-selective GAAA receptor-positive allosteric modulator NS11394 [3'-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitrile] with diazepam, zolpidem, bretazenil, and gaboxadol in rat models of inflammatory and neuropathic pain. *The Journal of pharmacology and experimental therapeutics* 2008; **327**: 969-81

18 Di Lio A, Benke D, Besson M, et al. HZ166, a novel $GABA_A$ receptor subtype-selective benzodiazepine site ligand, is antihyperalgesic in mouse models of inflammatory and neuropathic pain. *Neuropharmacology* 2011; **60**: 626-32

19 Ralvenius WT, Benke D, Acuna MA, Rudolph U, Zeilhofer HU. Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive $GABA_A$ receptor subtype. *Nature communications* 2015; **6**: 6803

20 Besson M, Daali Y, Di Lio A, Dayer P, Zeilhofer HU, Desmeules J. Antihyperalgesic effect of the GABA(A) ligand clobazam in a neuropathic pain model in mice: a pharmacokinetic-pharmacodynamic study. *Basic & clinical pharmacology & toxicology* 2013; **112**: 192-7

21 Vuilleumier PH, Besson M, Desmeules J, Arendt-Nielsen L, Curatolo M. Evaluation of anti-hyperalgesic and analgesic effects of two benzodiazepines in human experimental pain: a randomized placebo-controlled study. *PLoS One* 2013; **8**: e43896

22 Chou R, Deyo R, Friedly J, et al. Systemic Pharmacologic Therapies for Low Back Pain: A Systematic Review for an American College of Physicians Clinical Practice Guideline. *Annals of internal medicine* 2017; **166**: 480-92

23 Abdel Shaheed C, Maher CG, Williams KA, McLachlan AJ. Efficacy and tolerability of muscle relaxants for low back pain: Systematic review and meta-analysis. *Eur J Pain* 2017; **21**: 228-37

24 Davidson JR, Feltner DE, Dugar A. Management of generalized anxiety disorder in primary care: identifying the challenges and unmet needs. *Primary care companion to the Journal of clinical psychiatry* 2010; **12**

25 Mets MA, Volkerts ER, Olivier B, Verster JC. Effect of hypnotic drugs on body balance and standing steadiness. *Sleep medicine reviews* 2010; **14**: 259-67

26 Nickolls SA, Gurrell R, van Amerongen G, et al. Pharmacology in translation: the preclinical and early clinical profile of the novel alpha2/3 functionally selective $GABA_A$ receptor positive allosteric modulator PF-06372865. *British journal of pharmacology* 2018; **175**: 708-25

27 Hay JL, Okkerse P, van Amerongen G, Groeneveld GJ. Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery. *J Vis Exp* 2016

28 Moore RA, Straube S, Wiffen PJ, Derry S, McQuay HJ. PreGABAlin for acute and chronic pain in adults. *Cochrane Database Syst Rev* 2009: CD007076

29 Dong J, Li W, Wang Y. The effect of preGABAlin on acute postoperative pain in patients undergoing total knee arthroplasty: A meta-analysis. *Int J Surg* 2016; **34**: 148-60

30 Cheng GS, Ilfeld BM. An Evidence-Based Review of the Efficacy of Perioperative Analgesic Techniques for Breast Cancer-Related Surgery. *Pain Med* 2016

31 Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 2015; **14**: 162-73

32 Okkerse P, van Amerongen G, de Kam ML, et al. The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study. *Br J Clin Pharmacol* 2016

33 Loudon P, Siebenga P, Gorman D, et al. Demonstration of an anti-hyperalgesic effect of a novel pan-Trk inhibitor PF-06273340 in a battery of human evoked pain models. *Br J Clin Pharmacol* 2018; **84**: 301-9

34 Okkerse P, Hay JL, Sitsen E, et al. Pharmacokinetics and pharmacodynamics of intrathecally administered Xen2174, a synthetic conopeptide with norepinephrine reuptake inhibitor and analgesic properties. *Br J Clin Pharmacol* 2016

35 Okkerse P, Alvarez-Jimenez R, Hay JL, et al. No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery. *Eur J Pain* 2017; **21**: 494-506

36 Kenward MG, Roger JH. The use of baseline covariates in crossover studies. *Biostatistics (Oxford, England)* 2010; **11**: 1-17

37 Baidya DK, Agarwal A, Khanna P, Arora MK. PreGABAlin in acute and chronic pain. *Journal of Anaesthesiology, Clinical Pharmacology* 2011; **27**: 307-14

38 Knabl J, Zeilhofer UB, Crestani F, Rudolph U, Zeilhofer HU. Genuine antihyperalgesia by systemic diazepam revealed by experiments in $GABA_A$ receptor point-mutated mice. *Pain* 2009; **141**: 233-8

39 Paul J, Yevenes GE, Benke D, et al. Antihyperalgesia by alpha2-$GABA_A$ receptors occurs via a genuine spinal action and does not involve supraspinal sites. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2014; **39**: 477-87

40 Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog Neurobiol* 1992; **38**: 397-421

41 Abeyama K, Eng W, Jester JV, et al. A role for NF-kappaB-dependent gene transactivation in sunburn. *The Journal of clinical investigation* 2000; **105**: 1751-9

42 Muley MM, Krustev E, McDougall JJ. Preclinical Assessment of Inflammatory Pain. *CNS Neuroscience & Therapeutics* 2015; **22**: 88-101

43 Sisignano M, Angioni C, Ferreiros N, et al. Synthesis of Lipid Mediators during UVB-Induced Inflammatory Hyperalgesia in Rats and Mice. *PLOS ONE* 2013; **8**: e81228

44 Lopes DM, McMahon SB. Ultraviolet Radiation on the Skin: A Painful Experience? *CNS Neuroscience & Therapeutics* 2016; **22**: 118-26

45 Ferreira SH, Moncada S, Vane JR. Further experiments to establish that the analgesic action of aspirin-like drugs depends on the inhibition of prostaglandin biosynthesis. *British Journal of Pharmacology* 1973; **47**: 629P-30P

46 van Amerongen G, de Boer MW, Groeneveld GJ, Hay JL. A literature review on the pharmacological sensitivity of human evoked hyperalgesia pain models. *Br J Clin Pharmacol* 2016; **82**: 903-22

47 Besson M, Matthey A, Daali Y, et al. GABAergic modulation in central sensitization in humans: a randomized placebo-controlled pharmacokinetic-pharmacodynamic study comparing clobazam with clonazepam in healthy volunteers. *Pain* 2015; **156**: 397-404

48 Ralvenius WT, Acuña MA, Benke D, et al. The clobazam metabolite N-desmethyl clobazam is an α2 preferring benzodiazepine with an improved therapeutic window for antihyperalgesia. *Neuropharmacology* 2016; **109**: 366-75
49 Kunz M, Scholl KE, Schu U, Lautenbacher S. GABAergic modulation of diffuse noxious inhibitory controls (DNIC): a test by use of lorazepam. *Exp Brain Res* 2006; **175**: 363-71
50 Bravo-Hernandez M, Corleto JA, Barragan-Iglesias P, et al. The alpha5 subunit containing $GABA_A$ receptors contribute to chronic pain. *Pain* 2016; **157**: 613-26
51 Jasmin L, Wu MV, Ohara PT. GABA puts a stop to pain. *Curr Drug Targets CNS Neurol Disord* 2004; **3**: 487-505
52 Kirkpatrick DR, McEntire DM, Hambsch ZJ, et al. Therapeutic Basis of Clinical Pain Modulation. *Clin Transl Sci* 2015; **8**: 848-56
53 Lau BK, Vaughan CW. Descending modulation of pain: the GABA disinhibition hypothesis of analgesia. *Current opinion in neurobiology* 2014; **29**: 159-64
54 François A, Low SA, Sypek EI, et al. A Brainstem-Spinal Cord Inhibitory Circuit for Mechanical Pain Modulation by GABA and Enkephalins. *Neuron* 2017; **93**: 822-39.e6
55 Handwerker HO, Kobal G. Psychophysiology of experimentally induced pain. *Physiological reviews* 1993; **73**: 639-71
56 Dosenovic S, Jelicic Kadic A, Miljanovic M, et al. Interventions for Neuropathic Pain: An Overview of Systematic Reviews. *Anesthesia and analgesia* 2017; **125**: 643-52
57 Catterall WA. Structure and regulation of voltage-gated Ca2+ channels. *Annual review of cell and developmental biology* 2000; **16**: 521-55
58 Li CY, Song YH, Higuera ES, Luo ZD. Spinal dorsal horn calcium channel alpha2delta-1 subunit upregulation contributes to peripheral nerve injury-induced tactile allodynia. *J Neurosci* 2004; **24**: 8494-9
59 Verma V, Singh N, Singh Jaggi A. PreGABAlin in Neuropathic Pain: Evidences and Possible Mechanisms. *Current Neuropharmacology* 2014; **12**: 44-56
60 Bartley EJ, Fillingim RB. Sex differences in pain: a brief review of clinical and experimental findings. *BJA: British Journal of Anaesthesia* 2013; **111**: 52-8
61 Paller CJ, Campbell CM, Edwards RR, Dobs AS. Sex-Based Differences in Pain Perception and Treatment. *Pain Med* 2009; **10**: 289-99
62 Esterlis I, McKee SA, Kirk K, et al. Sex-specific differences in GABA(A)-benzodiazepine receptor availability: Relationship with sensitivity to pain and tobacco smoking craving. *Addiction biology* 2013; **18**: 370-8
63 Payne LA, Rapkin AJ, Seidman LC, Zeltzer LK, Tsao JCI. Experimental and Procedural Pain Responses in Primary Dysmenorrhea: A Systematic Review. *Journal of Pain Research* 2017; **10**: 2233-46
64 Veldhuijzen DS, Keaser ML, Traub DS, Zhuo J, Gullapalli RP, Greenspan JD. The role of circulating sex hormones in menstrual cycle dependent modulation of pain-related brain activation. *Pain* 2013; **154**: 548-59
65 Kim HJ, Yang GS, Greenspan JD, et al. Racial and ethnic differences in experimental pain sensitivity: systematic review and meta-analysis. *Pain* 2017; **158**: 194-211
66 Mechlin B, Heymen S, Edwards CL, Girdler SS. Ethnic differences in cardiovascular-somatosensory interactions and in the central processing of noxious stimuli. *Psychophysiology* 2011; **48**: 762-73
67 van Amerongen G, Siebenga P, de Kam ML, Hay JL, Groeneveld GJ. Effect profile of paracetamol, Delta9-THC and promethazine using an evoked pain test battery in healthy subjects. *Eur J Pain* 2018
68 Gurrell R, Dua P, Feng G, et al. A randomised, placebo-controlled clinical trial with the alpha2/3/5 subunit selective $GABA_A$ positive allosteric modulator PF-06372865 in patients with chronic low back pain. *Pain* 2018

TABLE 1 Summary of subject characteristics

Age (years)	
Mean (SD)	30.3 (10.8)
Range	18-50
Race (N)	
White	18
Other	2
Weight (kg)	
Mean (SD)	78.7 (10.5)
Range	60.1-100.7
BMI (kg/m²)	
Mean (SD)	23.3 (2.9)
Range	18.5- 27.2

BMI = Body Mass Index / SD = standard deviation / Kg = kilogram

TABLE 2 Summary of LSM analyses for pain test battery

Endpoint	Placebo	PF-06372865 15mg			PF-06372865 65mg			Pregabalin 300mg		
	LSMean (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)
COLD PRESSOR (S)										
PTT	18.4 (16.8, 20.1)	19.6 (17.9, 21.4)		1.06 (0.94, 1.21)	21.4 (19.6, 23.4)		**1.17 (1.03, 1.32)***	24.0 (22.0, 26.2)		**1.31 (1.16, 1.48)***
PDT	4.5 (3.9, 5.4)	4.2 (3.6, 5.0)		0.94 (0.74, 1.19)	4.2 (3.6, 4.9)		0.92 (0.73, 1.16)	5.4 (4.6, 6.3)		1.18 (0.94, 1.49)
AUC	10.54 (10.32, 10.76)	10.34 (10.12, 10.56)	-0.199 (-0.507, 0.110),		10.33 (10.12, 0.55)	-0.211 (-0.515, 0.093)		10.03 (9.82, 10.23)	**-0.516 (-0.816, -0.216)***	
VAS	76.1 (73.8, 78.5)	78.2 (75.7, 80.7)	2.1 (-1.3, 5.4)		74.9 (72.7, 77.2)	-1.2 (-4.5, 2.1)		74.1 (71.6, 76.5)	-2.1 (-5.5, 1.3)	
ELECTRICAL STIMULATION (mA)										
PTT	19.3 (18.1, 20.6)	20.3 (19.0, 21.7)		1.05 (0.96, 1.15)	21.1 (19.7, 22.5)		1.09 (1.00, 1.20)	20.3 (19.0, 21.6)		1.05 (0.96, 1.15)
PDT	6.1 (5.3, 7.1)	6.2 (5.4, 7.2)		1.02 (0.83, 1.25)	6.9 (5.9, 7.9)		1.12 (0.92, 1.38)	6.6 (5.8, 7.6)		1.09 (0.89, 1.33)
AUC	3.360 (3.214, 3.506)	3.331 (3.181, 3.480)	-0.029 (-0.239, 0.180)		3.196 (3.049, 3.344)	-0.164 (-0.372, 0.045)		3.278 (3.134, 3.422)	-0.082 (-0.286, 0.122)	
VAS	75.0 (72.5, 77.5)	74.3 (71.6, 77.0)	-0.7 (-4.5, 3.1)		70.3 (67.8, 72.9)	**-4.7 (-8.3, -1.0)***		72.2 (69.6, 74.8)	-2.8 (-6.3, 0.7)	

Endpoint	Placebo	PF-06372865 15mg			PF-06372865 65mg			Pregabalin 300mg		
	LSMean (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)	LSMean (90%CI)	Difference vs. placebo (90%CI)	LSMean Ratio to placebo (90%CI)
CPM: ELECTRICAL STIMULATION (DIFFERENCE PRE-POST COLD PRESSOR) (mA)										
PTT	1.2 (0.8, 1.6)	1.2 (0.8, 1.6)	0.00 (-0.6, 0.6)		1.4 (1.0, 1.7)	0.2 (-0.3, 0.8)		1.0 (0.7, 1.4)	-0.1 (-0.7, 0.4)	
PDT	0.5 (-0.2, 1.3)	0.3 (-0.5, 1.0)	-0.2 (-1.3, 0.8)		-0.1 (-0.8, 0.7)	-0.6 (-1.6, 0.5)		0.4 (-0.4, 1.1)	-0.2 (-1.2, 0.9)	
AUC	-0.088 (-0.145, -0.0315)	-0.089 (-0.147, -0.032)	-0.001 (-0.812, 0.080)		-0.079 (-0.135, -0.023)	0.010 (-0.071, 0.090)		-0.080 (-0.134, -0.025)	0.009 (-0.070, 0.088)	
VAS	0.4 (-0.8, 1.6)	1.1 (-0.1, 2.3)	0.7 (-1.0, 2.4)		1.6 (0.5, 2.7)	1.2 (-0.5, 2.8)		0.5 (-0.6, 1.6)	0.1 (-1.5, 1.7)	
PRESSURE STIMULATION (kPa)										
PTT	40.2 (37.8, 42.8)	44.7 (42.0, 47.7)		**1.11 (1.02, 1.22)***	44.6 (41.9, 47.5)		**1.11 (1.01, 1.21)***	46.4 (43.6, 49.3)		**1.2 (1.1, 1.3)***
PDT	11.2 (8.8, 14.2)	9.0 (7.0, 11.6)		0.81 (0.57, 1.15)	7.1 (5.6, 9.1)		**0.64 (0.46, 0.90)***	9.3 (7.4, 11.8)		0.8 (0.6, 1.2)
AUC	6.982 (6.633, 7.332)	6.841 (6.483, 7.198)	-0.141 (-0.647, 0.364)		6.699 (6.352, 7.046)	-0.283 (-0.781, 0.215)		6.439 (6.102, 6.775)	**-0.543 (-1.026, -0.061)**	
VAS	68.8 (66.2, 71.4)	70.1 (67.4, 72.8)	1.3 (-2.5, 5.0)		64.8 (65.8, 71.0)	-0.4 (-4.1, 3.2)		66.5 (64.0, 69.0)	-2.3 (-6.0, 1.3)	
NORMAL HEAT (°C)										
PDT	47.1 (46.6, 47.5)	46.9 (46.5, 47.4)	-0.1 (-0.8, 0.5)		46.8 (46.3, 47.2)	-0.3 (-0.9, 0.3)		47.3 (46.9, 47.7)	0.2 (-0.4, 0.8)	
UVB HEAT (°C)										
PDT	41.0 (40.5, 41.7)	41.2 (40.6, 41.8)	0.1 (-0.7, 1.0)		41.4 (40.8, 42.0)	0.3 (-0.5, 1.2)		41.4 (40.9, 42.0)	0.4 (-0.5, 1.2)	

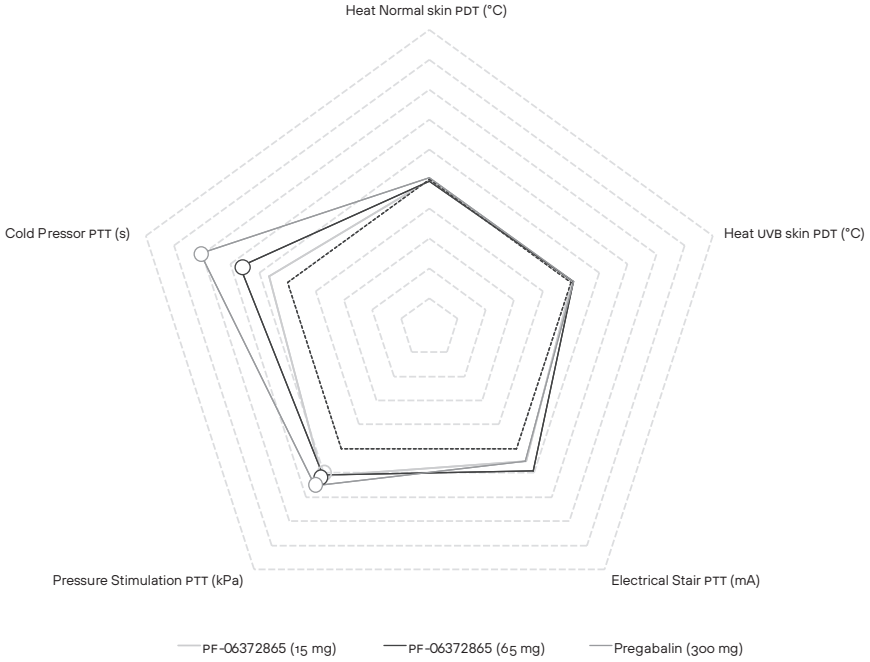
PTT = Pain Tolerance Threshold / PDT = Pain Detection Threshold / AUC = Area Under the Curve / VAS = Visual Analogue Scale / CPM = Conditioned Pain Modulation / Contrasts over 0-6 hours post dose.*

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

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

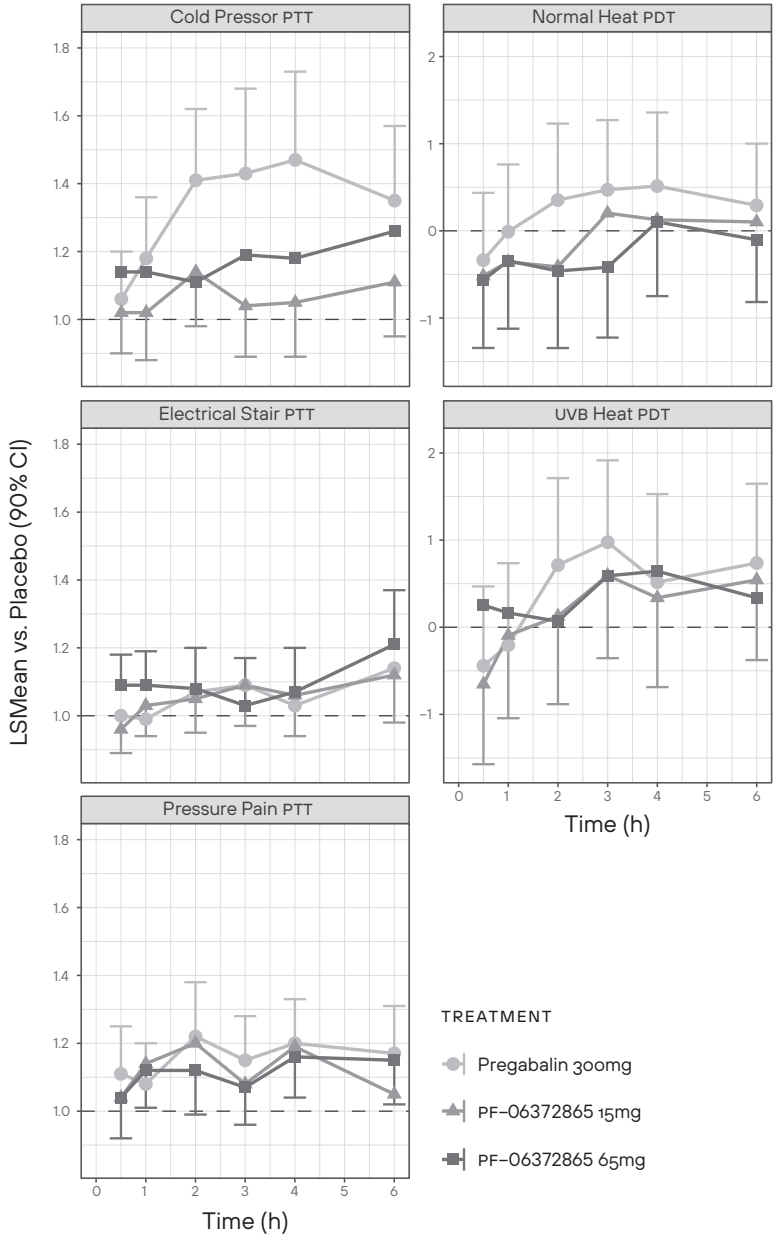
a =Geometric mean (%Geometric CV) / b Median (range)

FIGURE 1 Spiderplot summary of Pharmacodynamic response profile for pain test battery normalised to placebo



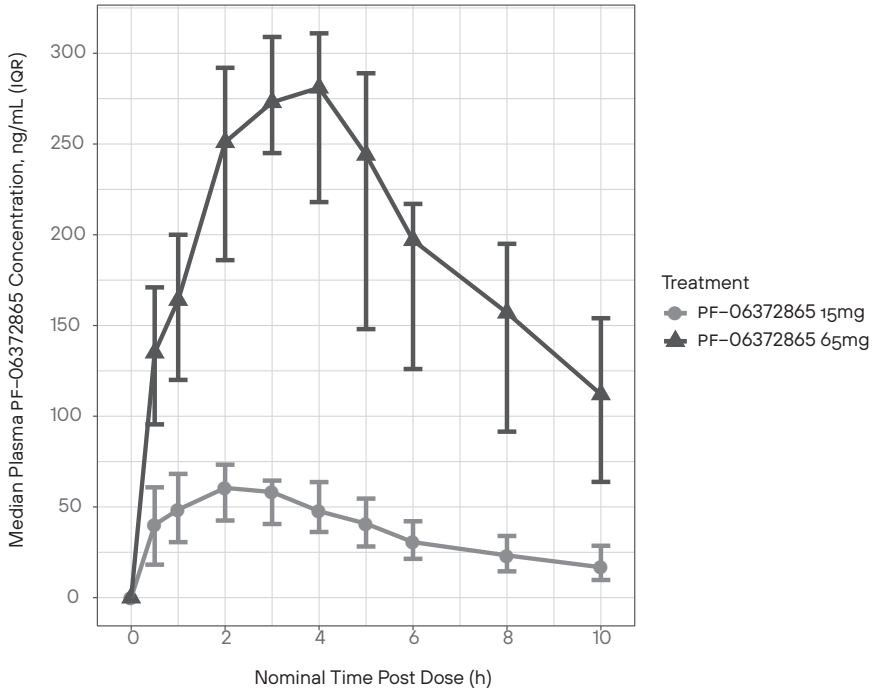
Dashed placebo line (green) represents a value of 1 to which other treatment effects are normalised. Distal from the centre beyond the placebo line indicates Least Square Mean PTT/PDT greater than placebo, towards the centre and within the placebo line indicates Least Square mean PTT/PDT lower than placebo.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

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



CI = confidence interval / h = hours / PTT = Pain Tolerance Threshold / PDT = Pain Detection Threshold

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



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

CHAPTER 7

SUMMARY, DISCUSSION AND CONCLUSIONS

The present thesis describes the use of broad pharmacodynamic effect profiling to characterise the clinical pharmacology of classic and non-classical analgesia. Analgesic drugs that modulate widespread targets in the nervous system can be expected to affect numerous CNS functions, which requires multimodal characterisation of pain processing and neurocognition. This is illustrated on the basis of two case studies of pharmacological agents that target cannabinoid CB1 and GABAergic $GABA_A$ receptors: two of the most widely distributed systems of receptors and neurotransmitters that are involved in a myriad of physiological functions. The distribution of receptors throughout the central nervous system render ECP002A, an oral formulation of Δ9-THC, and PF-06372865, a positive allosteric modulator of α2/3/5 subunit-containing $GABA_A$ receptors, ideal candidates for extensive neurophysiological and analgesic effect profiling in early phase clinical research. Profiling human pharmacology with a strong focus on pharmacodynamics may help to better understand the therapeutic potential and safety limitations of a compound before selection of doses and patient populations for phase II proof-of-concept studies.

SUMMARY OF STUDIES

Over the past two decades, two multimodal test batteries for CNS drug profiling have been developed and validated at CHDR: the NeuroCart® as a compilation of drug sensitive neurocognitive assessments, and the PainCart® for the quantification of different aspects of pain processing. Originally, the PainCart addressed various major types of evoked pain responses, but it did not contain measurements for hyperalgesia, which is a hallmark of inflammatory or neuropathic pain. The second chapter describes a literature study into the pharmacological sensitivity of hyperalgesia pain models. Due to variability in utilised methodology, the report focused on three models: the UVB model, the capsaicin model and the thermode burn model. Although capsaicin has generally been regarded as a model for neuropathic pain, the model appeared to be insensitive to the classes of pharmacological compounds clinically prescribed in the first-line treatment of neuropathic pain (e.g. tricyclic antidepressants, calcium channel α_2-δ ligands). The thermode burn model is used as a translational model for both neuropathic and inflammatory pain but is only moderately sensitive to the attenuating effects of NMDA receptor antagonists. However, the inherent risk of tissue damage, in combination with the limited pharmacological sensitivity, result in an inappropriate model for either inflammatory or neuropathic pain. The UVB model for inflammatory pain demonstrated high pharmacological sensitivity to NSAIDS, in line with their proposed anti-inflammatory mode of action. Based on this literature review, the test battery of evoked pain tasks, PainCart, was expanded with the UVB model as a biomarker for inflammatory hyperalgesia, while it was decided to continue the search for a reproducible and predictive model of neuropathic pain, either in healthy subjects or in patients.

An oral formulation of Δ9-THC was investigated in a four-week clinical trial in patients with (primary or secondary) progressive Multiple Sclerosis (MS) and moderate spasticity, which was described in Chapter 3. Efficacy and secondary effects were expressed in both objective (e.g. electrophysiology, postural stability) and subjective Numerical Rating Scales (e.g. NRS spasticity / NRS Pain, VAS Bowdle and VAS Bond and Lader) endpoints.

To overcome inter-individual variability in pharmacokinetics and pharmacodynamic response, a crossover challenge phase was built in the study design, to enable individualised dosing. The challenge phase consisted of an up-titration of three consecutive doses, 3, 5 and 8 mg, accompanied by intensive PK sampling, NeuroCart measurements of functional CNS effects and monitoring of adverse events (AEs). PK/PD modelling was included to identify the appropriate dose for each individual at which the desired effects were observed in the absence of adverse events. However, due to the lack of a robust PD response during the challenge phase, a reliable model to predict individual doses could not be established. Since there was also no effect on pain during the challenge phase, dosing for the treatment phase was based on tolerability.

During the treatment phase, the selected dose levels were generally well-tolerated, although some limited dose adjustments were needed. No significant treatment effect was observed on the objective endpoints for spasticity: H/M ratio and Ashworth score. Subjective spasticity measured with an NRS repeatedly during the treatment visits on weeks 0, 2, and 4 improved after 2 and 4 weeks of treatment, which was significant at 2 weeks of treatment. The same pattern was observed in a more pronounced way for the NRS for pain. This outcome, measured as an NRS repeatedly during each treatment visit, revealed a significant overall improvement in favour of treatment compared with placebo. The psychoactive effects observed after short-term administration of Δ9-THC during the challenge phase were not observed during the treatment phase.

Similar to the observed variability in PD outcomes observed in this trial, moderate variability in PK properties was observed during both the challenge and treatment phases. This is most likely attributable to the more heterogeneous patient population compared with healthy volunteers that were investigated before. PK modelling revealed a relatively high typical apparent clearance and typical apparent volume of distribution compared with previous findings, which is most likely related to slightly lower bioavailability of the oral formulation. In addition, a slower absorption rate was observed, which we assumed to be resulting from reduced gastrointestinal motility, which has previously been reported in patients with MS.

The clinical trial described in Chapter 4 was performed to expand earlier pharmacological validation studies of the original PainCart and further elucidate the findings described in the third chapter of this thesis. The aim was to investigate the analgesic effects of classic and non-classic analgesics compared to a sedating negative control in a randomized placebo-controlled crossover study in 24 healthy volunteers, using the battery of evoked pain tasks that was previously validated for other classes of analgesics. The biomarker battery consists of pain tasks eliciting electrical, pressure, heat, cold and inflammatory

pain. For each pain task, the Pain Detection Threshold (PDT), Pain Tolerance Threshold (PTT) and subjective score (VAS) were recorded. Subjective scales for alertness, mood and psychotomimetic effects were included. Subjects were administered each of the following oral treatments: paracetamol (1000 mg), Δ9-THC (ECP002A) (10 mg), promethazine (50 mg) or matching placebo. Contrary to our expectations, we found that paracetamol was not effective at reducing any of the pain modalities measured using the battery of evoked pain tasks. Furthermore, Δ9-THC did not show any acute analgesic effect, and even showed a hyperalgesic effect on two of five pain tasks, namely electrical and pressure pain. Finally, the negative control promethazine showed an increase in pain sensitivity for cold, pressure and inflammatory pain. In addition to the pain tasks, cognitive tests were performed to assess subjective alertness, mood and psychotomimetic symptoms, which were moderately impaired by treatment with Δ9-THC (alertness, calmness, internal and external perception) or promethazine (alertness).

Paracetamol has been shown to be effective in the treatment of different types of clinical pain, although not in all. However, when looking at available literature on human evoked pain tasks in healthy volunteers, the image becomes more diffuse. For each of the pain tasks that were investigated in more than one clinical trial, positive as well as negative results have been reported. The effects of paracetamol on acute evoked pain processing remain elusive, which may reflect its uncertain mechanism of action.

The lack of acute effects of Δ9-THC on the PainCart was not entirely unexpected. Based on its interaction with the endocannabinoid system, Δ9-THC cannot be considered an antinociceptive analgesic, even if it may have analgesic effects in some conditions. This is reflected in the results of clinical studies using human evoked pain models to investigate pharmacology and mechanism of action. The finding of Δ9-THC induced hyperalgesia has also been observed in the clinic. This may be the result from a narrow therapeutic window in combination with variable pharmacokinetics. Alternatively, this could have been a primary pharmacodynamic effect by which the participants were less motivated to complete the pain tasks, resulting in lower pain detection and pain tolerance thresholds. Another possibility is that CNS depression can reduce the cognitive control of predictably evoked pain. These last two hypotheses are supported by the observed lack of acute analgesia and mild hyperalgesia on some pain tasks after administration of promethazine. At any rate, these findings illustrate the importance of broad profiling to determine the optimal therapeutic window of centrally acting (CNS depressant) analgesics.

The results of the studies in Chapters 3 and 4 support the notion that repeated Δ9-THC administration exerts a gradual pharmacodynamic effect that may treat the perception of neuropathic pain or spasticity, rather than exerting pure acute nociceptive effects or affecting electrophysiologically measurable phenomena.

The chapters 5 and 6 describe a series of early human studies, which for the first time integrated the NeuroCart and PainCart profiling of a centrally acting drug, PF-06372865. This is a novel partial $GABA_A$ α2/α3/α5 subtype-selective positive allosteric modulator

that is under development for the treatment of different indications, including anxiety disorders, neuropathic pain and photosensitive epilepsy. The two-part study design allowed for the investigation of different objectives. In Part A, a wide dose range was investigated to determine safety, PK and PD response. Based on the results, two dose levels (15 and 65 mg) were selected for a direct comparison and interaction study with lorazepam (2 mg) in Part B.

The comparison with placebo and the combination of lorazepam (2 mg) with PF-06372865 65 mg demonstrated additive or infra-additive effects, depending on the functional domain, in line with the predicted functional selectivity for α2/α3/α5 $GABA_A$ receptor subtypes, but also revealing for which $GABA_A$ subtypes there is a competitive or non-competitive receptor interaction. No synergy was observed. For Saccadic peak velocity (SPV), Smooth pursuit and VAS Alertness no interaction, but addition was observed, which can be explained by non-selective receptor activation by lorazepam (2 mg) in addition to selective α2/α3 activation by PF-06372865. For the other functional domain tests, Adaptive tracking, Body Sway and VVLT, infra-additive effects were observed when lorazepam and PF-06372865 were administered simultaneously. A reduction in effect size for these psychomotor functions compared to administration of lorazepam (2 mg) alone, may indicate that due to competitive activity at the $GABA_A$ receptor subtypes, PF-06372865 acts as an antagonist, resulting from partial agonism, at α1 subunits, which diminishes lorazepam's high intrinsic activity as a non-selective full agonist.

Extensive pharmacodynamic and pharmacokinetic profiling as performed in Chapter 5 provided valuable insight in the receptor pharmacology of PF-06372865 specifically, but also of subtype-specific $GABA_A$ positive allosteric modulators in general. The subtle interplay between intrinsic efficacy and receptor subtype affinity was unveiled. In terms of its clinical development, a clear dose-response effect was established. Based on this study, two pharmacologically active doses were selected for further pharmacodynamic effect profiling in Chapter 6.

Based on the findings of the preceding chapter and the accumulating evidence that $GABA_A$ receptors may play a modulating role of nociceptive processing in various neuropathic pain states, a study was performed focusing on analgesic effect profiling of PF-06372865. This was performed as a double-blind, randomised, placebo-controlled, single dose, crossover study in which the effects of two doses of PF-06372865 (15 and 65 mg) were compared with placebo and pregabalin (300 mg) as a positive control. Pharmacodynamic assessments (pain thresholds) were executed using the battery of evoked pain tasks and intensive PK sampling was done. PF-06372865 had an analgesic effect on pressure pain and the cold pressor task. The magnitude of effect was greater for 65 mg compared with 15 mg for the cold pressor, but the effects were similar for the pressure pain task. Pregabalin 300 mg lowered pain thresholds induced by the cold pressor and the pressure pain tasks. From a mechanistic perspective, translation of findings from a human evoked pain model in healthy subjects to clinical pain remains uncertain, but the present study clearly shows that PF-06372865 has analgesic potential at doses that do not induce significant sedation or other intolerable AEs.

DISCUSSION

Pain is a complex multifaceted process that involves many peripheral and central pharmacological systems. This thesis centred on the early development of two centrally acting analgesics that affect the endocannabinoid CB1 system and subtypes of $GABA_A$ receptors. In both cases, extensive profiling of neurocognitive, neurophysiological and pain processing was performed to provide further insights into the complicated interplays between CNS depression, acute nociception and pain experience. Integration of this information can be useful in the determination of the anticipated therapeutic window, the expected adverse effect profile and potential indications for subsequent clinical trials.

The endocannabinoid system is a highly complex, not fully understood, biological system that exerts various subtle and less subtle effects when targeted. This context makes ECP002A (Δ9-THC), which specifically targets endocannabinoid receptors in the CNS, an ideal candidate for pharmacological characterisation of neurocognitive, neurophysiological and pain processing in early phase clinical research. The analgesic potential revealed in the study in MS patients was investigated in healthy volunteers using the PainCart, after earlier studies had identified pharmacologically active doses using the NeuroCart. Here it became clear that the analgesia against neuropathic pain observed in MS patients, is distinct from purely nociceptive analgesia. Instead Δ9-THC alters pain experience at a different level. In line with the effects observed on subjective pain in MS patients, the effects observed on spasticity suggest an analogous mode of action: there was a significant difference in subjective spasticity after two weeks of treatment, albeit not significant over the 4 week treatment period, but this was not reflected in the objective electrophysiological measurements of spasticity (EMG). Even though no clear change in objective endpoints (pain thresholds or EMG) was observed, the subjective experience of these unpleasant sensations was altered nonetheless.

It has been suggested that the efficacy Δ9-THC is attributable to the fact that patients are too "high" to accurately report the level of experienced pain. This is not confirmed in the study in healthy subjects where feeling high (changes in internal and external perception) was recorded, but not associated with analgesia or reports of reduced pain perception.

Δ9-THC demonstrates moderately variable PK and PD. In an effort to reach sufficient plasma concentrations to exert analgesia, the selected dose level of this oral formulation of Δ9-THC in the study in healthy subject measuring evoked pain thresholds may have been too high for some, as evidenced by reported adverse events and increased sensitivity to pain (hyperalgesia). This experimental model supports the earlier observations of a narrow therapeutic window for Δ9-THC and the interactions between neurocognitive control and pain processing. Metabolism of Δ9-THC is known to be complex, resulting in various active and inactive metabolites, with different and largely unknown PK and PD characteristics and interactions. Therefore it is difficult to directly compare acute and chronic effects, as these may be driven by different pharmacological constituents.

Even though the clinical benefits and analgesic potential of Δ9-THC are widely recognised, the complete mechanism of action remains incompletely explained. Nonetheless, the two chapters described in this thesis have contributed to the body of research by taking two different viewpoints: from the mechanistic approach of biomarkers for pain in healthy subjects to clinical endpoints for pain and spasticity in a target population of MS patients.

GABAergic neurons and interneurons are distributed throughout the CNS and are involved in a broad spectrum of functions. Targeting $GABA_A$ receptor subtypes instead of non-selective receptor modulation by conventional benzodiazepines has become increasingly interesting since the discovery that the various receptor subtypes show distinct distribution patterns which are associated with different functional and clinical effects. Treatment with α1 sparing positive allosteric modulators for $GABA_A$ will potentially achieve analgesia or anxiolysis in the absence of dose-limiting sedation, which is largely attributed to the α1 subunit. The extensive neurophysiological effect profiling of a wide dose range of PF-06372865 and subsequent comparison to lorazepam alone and in combination with PF-06372865, provided valuable insight in the receptor pharmacology of partial and full, or subtype specific and non-selective allosteric agonists. The interplay between intrinsic activity and relative affinity of PF-06372865 resulted in a comprehensive pharmacodynamic effect profile in man. Per task, or range of tasks, respective for a specific $GABA_A$ receptor subtype and individual dose-effect relationship was established. The findings in this study confirmed the predicted α2/α3/α5 $GABA_A$ receptor subtype selectivity for PF-06372865 and guided dose selection for subsequent investigations in evoked pain although a dose of 65 mg more convincingly demonstrated analgesic effects on different pain tasks.

Even though a dose of 65 mg PF-06372865 demonstrated substantial CNS effects, including a reduction in SPV, reduction in adaptive tracking, increase in postural instability and reduced memory performance, it is unlikely that these specific effects drive the observation in the absence of analgesia, as it has been demonstrated that the battery of evoked pain tasks is not overly sensitive to the effects of mere sedation, which may even reduce pain thresholds.

Before the results of the early human studies of PF-06372865 described in this thesis were fully integrated, a study in low back pain patients was performed. Mainly based on preclinical predictions and early human adverse effects, a loading dose of 2.5 mg BID for the first week was followed by 7.5 mg BID for the following three weeks. (Gurrell et al., 2018)*. The study did not show clinically relevant effects. Without the results reported in this thesis, the negative findings in the latest study could have been interpreted in such a way that the findings from preclinical research are not readily translatable to humans. However, reviewing the preclinical and clinical reports in their entirety, the results of the study described in Chapters 5 and 6, suggest that insufficient receptor occupancy was

* Gurrell R, Dua P, Feng G, et al. (2018) A randomised, placebo-controlled clinical trial with the alpha2/3/5 subunit selective GABAA positive allosteric modulator PF-06372865 in patients with chronic low back pain. *PAIN* 159: 1742–1751.

achieved in this clinical trial. The effects on the NeuroCart and the PainCart suggested that targeting α2/α3/α5 $GABA_A$ receptor subunits has the potential to result in detectable analgesic effects in humans at doses of around 65 mg. Therefore, integration of the detailed assessments that are described in this thesis could be used to redesign a study in patients suffering from neuropathic or other types of pain, perhaps also including low back pain at higher doses.

At first glance, translation of the results of the PainCart studies appears ambiguous. In chapter 4 the biomarker test battery did not demonstrate acute analgesia from Δ9-THC in healthy volunteers, even though this compound had shown analgesic effects in a phase IIa study in patients, reported in Chapter 3. Similarly (and conversely), PF-06372865 exerted different degrees of analgesia in Chapter 6, but this contrasted with the negative findings of a clinical study in chronic low-back pain patients PF-06372865.

However, the apparently incongruent findings essentially illustrate how test batteries should be composed and used and how the results need to be interpreted. Foremost, this thesis shows that translation of the pharmacodynamic characterisation of centrally acting analgesics in healthy volunteers using evoked pain biomarkers is not unequivocal, as has been recognised before. The findings of an early phase study using biomarkers should be interpreted in the correct context, acknowledging their merits and limitations. This includes two important aspects, which are both sensitive to CNS active drugs: the complexity of pain processing and the interactions with other neurocognitive and neurophysiological functions.

Pain is a complex phenomenon that is by definition a subjective experience. Quantifying this subjective experience can be performed using wide range of methods, signifying the multifaceted nature of the concept of pain. Rating scales, including a Numerical Rating Scale (NRS), Visual Analogue Scale (VAS) or pictorial scales are widely used, but their robustness and sensitivity to intervention has been questioned. More comprehensive scales measuring specific qualitative dimensions including McGill's Pain Questionnaire, the Brief Pain Inventory (BPI), or condition specific questionnaires are used to provide more insight in both affective and sensorial qualities of pain experience. To overcome to potential source of variability stemming from cognitive processing needed to complete such scales, more "objective" psychophysical methods can be used. The primary endpoints for the tasks in the multi-modal test battery are pain thresholds: the stimulus intensity increases linearly over time, at a certain stimulus intensity pain is first detected, the Pain Detection Threshold. The pain score is subsequently recorded using a VAS slider, which is moved to the other side until the Pain Tolerance Threshold is reached. This automatically ceases the stimulus. Since no visual or other feedback is provided to a subject at the point of reaching a threshold, no anchors are created. Apart from the experienced sensation of pain, the subject is not guided in a certain direction for subsequent tasks. Using this methodology provides a more robust and reliable pain recording, by eliminating part of cognitive processing. The test remains subject however to task vigilance and compliance.

Considering that pain is a subjective experience, different elements need to be integrated in a clinical study design. For example, using a crossover study design is imperative to reduce the impact of inter-individual variability. Additionally, typically a highly homogenous study population of Caucasian males in the same age range is selected during the early phases of research. These measures greatly improve the internal validity of a study, but may impact generalizability of study findings to other populations. This applies to other healthy population is general, but especially to patients suffering from pain. Evidently, in early phase clinical pharmacology research there are definite differences in the population under investigation and the patient suffering from neuropathic pain who is treated eventually. For obvious ethical reasons, it is impossible to induce chronic neuropathic pain in a controlled clinical setting in healthy volunteers. Apart from the ethical reasons, this is not even desirable, because different questions are answered at different stages of clinical research.

The questions that can be answered by using a multi-modal pain biomarker in early phase clinical research are abundant. By using standardised pain stimuli – compared with the fluctuating pain experienced by a patient – outcome measures are more robust and allow reliable comparison of multiple measurements over time. By executing assessments in a controlled environment, different measures can be taken to minimise bias stemming from affect, including anxiety, uncertainty on the duration and cause of the experienced pain. Different steps that are taken at CHDR to reduce this potential source of variability include the use of a standardised script for the measurement assistants, the subjects receive a training session before that actual measurements, and most importantly, the subjects themselves are responsible for starting and stopping the pain task. These elements are distinctively different from a clinical pain patient, but thereby greatly improve its use as a research tool.

Applying a multi-modal pain test battery allows to investigate different elements of the nociceptive system. The differentiation in underlying mechanisms results in a separation in pharmacodynamic response to a pharmacological agent. Comparing the pharmacodynamic effect profile of a novel potential analgesic to the effect profile of a known mechanism of action sheds new light on the (known) mechanism of action. Investigating the mechanism of action, or establishing a dose-response relationship is not only valuable for novel treatments, but clinical practice may also benefit from expanding knowledge on the mode of action of widely used treatments.

When this pain biomarker is combined with pharmacokinetic sampling, the relationship between pharmacodynamic effects and plasma concentration can be determined. A potential lag in onset of effect may indicate specific target tissue needs to be reached, or metabolites are formed that drive pharmacodynamic response. What is more, by determining these relationships as early as possible the design of a subsequent proof-of-concept study could be greatly improved.

The development of analgesic drugs is greatly facilitated by incorporation of multimodal evoked pain models as PD-measurements in methodologically sound early human

drug studies. But it should also be realised that centrally active agents generally affect other central nervous systems and functions. These functions in turn can impact on the results of pain tests and the predictability of therapeutic doses and indications, in various ways. First, performance of tasks is directly affected by motivation and attention, which may be reduced by drugs that cause sedation or interfere with reward processing. Pain awareness can be altered –enhanced, modified or reduced– by drug interactions with sensory information processing, including subcortical or insular projections and prefrontal control areas. Second, adverse CNS effects can limit the therapeutic window of potentially effective analgesics. For drug developers who are not an expert in pain pharmacology, adverse effects in healthy subjects have a larger 'face validity' than complicated artificially evoked pain models. Consequently, the selection of clinical doses for an early 'proof-of-clinical-efficacy' study is based less on analgesia models than on the intensity of adverse CNS effects, which are either used as recognisable 'surrogates of CNS penetration' or to optimise clinical tolerability and market competitiveness.

Early development of central analgesics requires a broad assessment and proper integration of all relevant pharmacological characteristics of the compounds, and the interactions with (and between) neurophysiological processes that are involved in pain processing and neurocognition. In a way, this thesis failed to provide empirical evidence for these points, since neither the early development program of the CB1-agonist or the partial subtype selective $GABA_A$ agonist showed clear predictive value of early multimodal characterization in healthy subjects, for clinical efficacy in a patient population. This lack of predictability may have been related to inevitable differences between healthy subjects and patients, which can only be solved by performing these types of multimodal studies in the target population. But before this can be concluded, other more feasible improvements in the early development program of central analgesics should be considered, particularly the proper integration of all preclinical and human data. This will help to carefully predict an (individualised) therapeutic dosing regimen that balances beneficial and potentially detrimental effects, and matches fluctuations of symptoms or adverse effects. The multimodal CNS effect profile and the complete pharmacological characterisation of the drug may also provide support for selection of the most eligible patient population, by attuning the drug's pharmacological properties and evoked pain response profile to the pathophysiological or symptomatic characteristics of the pain condition. In addition, secondary pharmacological properties and neurocognitive effects can be matched to comorbid conditions like sleep disturbance, mood disorders or spasticity. The predictability of early human studies is further increased by the use of well-characterised, pharmacologically comparable and therapeutically established analgesics as positive or historic controls. Several of these prerequisites were not sufficiently fulfilled in the early development programs in this thesis, which may well explain their limited clinical predictive value. At the same time, this illustrates how essential it is to rational early drug development to integrate all information, and how easily practical issues and complexity tend to interfere.

As with all models, the use of the PainCart or the NeuroCart have their intrinsic limitations. However, by understanding and acknowledging their strengths and weaknesses, a pharmacological characterisation performed using multi-modal test batteries of evoked pain and neurocognition can provide a deeper understanding of:

- A pharmacological agent's mechanistic mode of action;
- The relationship between pharmacokinetics and pharmacodynamics;
- The relationship between intended and secondary pharmacological actions.

These very basic attributes of a drug under development should be known as early as possible, in order to allow for an efficient and effective developmental path. Omitting these questions in the early phases of research may pose difficulties later on, as they will need to be answered eventually.

OVERALL CONCLUSIONS

The studies reported in this thesis add to a body of research in which this battery of evoked pain tasks (PainCart) has been used as a biomarker to characterise the analgesic potential of eight currently marketed drugs in the treatment of various types of pain and various novel compounds. This extensive experience with the battery of evoked pain tasks is beneficial in two ways: it provides a context that allows for comparison and benchmarking of the pharmacological agent under investigation against compounds with an established mode of action. The different pain modalities deployed in the battery of evoked pain tasks represent different elements of the nociceptive pathway and pain experience. Consequently each pain task demonstrates distinct sensitivity to pharmacological intervention. By integrating the results of the different pain tasks, a pharmacodynamic effect profile emerges, representative for its mode of action. Moreover, from a different perspective: the addition of six more treatments, all from different drug classes, thus with various modes of action, enriches the biomarker itself. To date, a database of approximately 25 pharmacological agents have been characterised. This includes both pharmacological agents that are marketed and agents that are still under development. As a result, the test battery of evoked pain tasks becomes a more comprehensive and informative biomarker.

Implementing biomarkers for pain and neurophysiological functioning in early phase clinical research provides a better understanding of a compound's characteristics, especially when used successively. Characterising a wide dose range of a novel compound using the neurophysiological test battery with tasks that have been shown to be representative for specific receptor pharmacology yields a neurophysiological pharmacodynamic effect profile that provides the insight in the relationship between intended and adverse effects. Selecting one or more dose levels that were shown to be pharmacologically active for further characterisation using the multi-modal pain

test battery confirms the initial findings of pharmacological activity. More importantly, it reveals valuable mechanistic information on the relationship between the concepts that are measured using the different biomarker test batteries, creating a comprehensive pharmacodynamic effect profile. This knowledge will help to effectively design the next steps in clinical development.

Obviously, measuring biomarkers for pain in early phase research will not provide all the answers. The complete cascade of pain perception is highly complex and involves potential pharmacological targets throughout the complete peripheral and central nervous system. Chronic pain is highly heterogeneous, which makes clinical research complex in itself. However, even though it may be impossible to capture this pathology within a single model, or even a set of models, the nociceptive system remains a fundamental element of the cascade. Therefore, implementing the multi-modal test battery of evoked pain tasks to challenge this particular element from multiple angles provides valuable pharmacological information of an intervention.

Some treatments are known not to exert direct nociceptive analgesia but are effective in the treatment of different neuropathic pain states regardless, for example anticonvulsants and tricyclic antidepressants. Previously, compounds of these classes (i.e. pregabalin and imipramine) have been investigated using the battery of evoked pain tasks as described in this thesis. These experiments have shown that these treatments, even though they would not be considered direct analgesics, did induce analgesia on different pain tasks, analogous to PF-06372865. As demonstrated in Chapter 4 of this thesis, the battery of evoked pain tasks in not sensitive to the effects of sedation alone, which indicates that these observations are not driven by secondary psychoactive pharmacodynamic effects. Therefore, even though the multi-modal battery of evoked pain tasks does not mimic a neuropathic pain patient to the complete extent, providing a nociceptive stimulus activates the cascade of pain perception nonetheless. This pharmacological sensitivity illustrates the congruence between the pathology under investigation and the model.

Notwithstanding the above, the absence of statistically significant acute analgesia does not necessarily demonstrate that a pharmacological agent will be ineffective in treating pain perception in a specific pain population. Apart from the statistical consideration that the lack of a statistically significant difference with placebo is not evidence that the effect of a treatment is equal to placebo, there are other factors that require careful consideration when interpreting "negative" results. The most obvious reason is an underpowered study: due to the novel nature of an early phase clinical investigation, the variability is unknown at the time of initiating a study. This may have contributed to the observed lack of analgesic effect after administration of paracetamol. Furthermore, when benchmarking against clinical effects of for example a gold standard treatment in a patient population, the magnitude of effects in healthy subjects may be diminished, leading to more subtle effects. These factors increase the risk of a type II error, thus failing to reject a false null hypothesis. In addition to the risk of inducing more subtle effects in healthy subjects compared with patients, there is also the possibility

that a specific element of the pain cascade that is dysregulated in the pathological state is not susceptible for improvement in the healthy state as it is optimised. This is where additional pharmacodynamic profiling may play a role: by demonstrating that a drug is pharmacologically active at a certain dose level, it may still have an analgesic potential, even in the absence of statistically significant differences with placebo when characterised using the test battery of evoked pain tasks. The results of a clinical study in which pharmacodynamic effect profiling is performed using biomarkers in the form of a multi-model test battery of pain tasks or neurophysiological tasks, provide a piece of the puzzle, thereby connecting the pharmacokinetic, preclinical, safety and further clinical pieces of the puzzle.

NEDERLANDSE SAMENVATTING

Het klinisch onderzoek naar nieuwe therapieën voor de behandeling van (chronische) pijn beweegt zich in twee richtingen. Enerzijds worden er volledig nieuwe moleculen ontwikkeld, ofwel door het optimaliseren van reeds bestaande farmaceutische concepten dan wel door het ontwikkelen van compleet nieuwe 'First-in-Class' geneesmiddelen. Anderzijds kunnen bestaande farmaceutische stoffen een bestemming krijgen voor een nieuwe indicatie. Bekende voorbeelden hiervan zijn het antihypertensivum clonidine, het anticonvulsivum pregabaline, en het tricyclische antidepressivum amitriptyline, die tegenwoordig een belangrijke plek innemen in de klinische praktijk van pijnbestrijding. Dat de meeste ontwikkelingen plaatsvinden in de tweede categorie illustreert de immense uitdagingen waar men voor staat in het ontwikkelen van een nieuw type geneesmiddel.

Nociceptie versus pijn

Pijnsensatie heeft een duidelijke fysiologische functie als waarschuwingssysteem voor (potentiele) fysieke schade. Aangezien de dreiging van schade mogelijk directe actie noodzakelijk maakt, is de sensatie van pijn onlosmakelijk verbonden met mentale processen zoals cognitie en affect. De pijncascade wordt geïnitieerd vanuit het nociceptieve systeem. Nociceptie bestaat uit vier processen. Een pijnprikkel wordt door een nociceptor omgezet in een actiepotentiaal zodra de prikkeldrempel is bereikt (*transductie*). Vervolgens wordt het signaal overgedragen via specifieke nociceptoren (Aδ zenuwvezels of C-zenuwvezels) van het perifere zenuwstelsel via het ruggenmerg naar de hersenen (*transmissie*). De zenuwbanen lopen uit naar specifieke gebieden in de hersenen die betrokken zijn bij ofwel het versterken dan wel het verzwakken van het signaal (*modulatie*): de hersenstam, het periaqueductaal grijs (PAG), de thalamus, maar ook de amygdala, hypothalamus en de cortex cingularis anterior. De totaliteit van het sensorisch-discriminatieve en het cognitief-affectieve signaal resulteert in de ervaring van pijn (*perceptie*). De overdracht, regulatie en modulatie van het oorspronkelijke nociceptieve signaal tot aan de daadwerkelijke perceptie vindt plaats in een cascade waarin verscheidene fysiologische (neurotransmitter)systemen zijn betrokken op alle betreffende niveaus. Pijnperceptie is de subjectieve ervaring van het gewaarworden van een signaal vanuit het nociceptieve systeem. Deze perceptie is niet alleen het resultaat van diverse fysieke en mentale factoren, maar staat ook onder invloed van externe factoren waaronder cultuur, opvoeding en situatie.

Het ontwikkelen van behandeling gericht op pijnperceptie

Om de perceptie van chronische pijn te behandelen is het cruciaal om het zenuwstelsel in zijn totaliteit te benaderen. Daarom richten de meest effectieve farmacologische therapieën zich niet op zeer specifieke receptoren, maar op het moduleren van pijnperceptie op verschillende niveaus in de pijncascade. Centrale pijnmodulatie is het thema van deze thesis, waarin twee zeer wijdverspreide neurotransmittersystemen

en farmacologische aangrijpingspunten in het centrale zenuwstelsel centraal staan. Het GABA-erge systeem en het endocannabinoïde systeem spelen niet alleen een rol in pijnperceptie, maar zijn ook betrokken bij diverse andere functies van het centrale zenuwstelsel. Om deze reden is een geïntegreerde benadering van complementaire biomarkers noodzakelijk om inzicht te krijgen in het werkingsmechanisme van een geneesmiddel dat aangrijpt op een van deze wijdverspreide neurotransmittersystemen.

Het gebruik van farmacodynamische biomarkers in geneesmiddelenontwikkeling

In het veranderende landschap van geneesmiddelenontwikkeling op het gebied van pijn, lijkt het 'blockbuster' model, waarbij *one size fits all* geneesmiddelen voor grote groepen patiënten worden ontwikkeld, op zijn einde te lopen. Er ligt een sterkere nadruk op het identificeren van subpopulaties van patiënten die ofwel meer kans hebben om goed te reageren op de behandeling, of juist een groter risico lopen op het ontwikkelen van bijwerkingen. In principe zou dit moeten leiden tot een effectiever ontwikkeltraject, met een kleiner risico op falen ten gevolge van gebrekkige werkzaamheid of onaanvaardbare nevenwerkingen. Een elementair onderdeel van effectieve geneesmiddelenontwikkeling, is het verkrijgen van meer kennis van het complete werkingsmechanisme van een nieuwe stof en van de invloed op fysiologie en pathofysiologie, in iedere stap van de ontwikkeling. Dit vergt een investering, maar zal uiteindelijk leiden tot efficiëntere geneesmiddelenontwikkeling en effectievere geneesmiddelen.

Om mechanistische kennis te genereren vanaf de vroege fase van het klinisch onderzoek, spelen biomarkers een cruciale rol. Een biomarker wordt gedefinieerd als een meetbare indicator van een normaal biologisch proces, een pathogeen proces of een farmacologische respons op een therapeutische interventie.

Aangezien chronische pijn vaak leidt tot wijdverspreide verstoringen binnen de pijncascade, is het waarschijnlijk dat de meest effectieve nieuwe geneesmiddelen zich zullen richten op receptoren binnen het perifere en centrale zenuwstelsel. In de ontwikkeling van dergelijke geneesmiddelen, is het geïntegreerd meten van zowel de analgetische effecten, als de effecten op het centrale zenuwstelsel van grote waarde om inzicht te krijgen in het profiel van gewenste en ongewenste farmacologische effecten. Met dit als doel heeft het Centre for Human Drug Research (CHDR) twee testbatterijen ontwikkeld, de PainCart en de NeuroCart.

Multimodale biomarker voor pijn: PainCart

In het licht van het bovenstaande kan de multimodale testbatterij van opgeroepen pijnprikkels, de PainCart, een belangrijke rol spelen in vroege fase klinisch onderzoek. Deze kan worden toegepast in zowel gezonde vrijwilligers als in specifieke patiëntenpopulaties. Ondanks dat de klinische presentatie van chronische pijn in geen enkele model kan worden nagebootst, kan de testbatterij van opgeroepen

pijnprikkels waardevolle informatie verschaffen over het werkingsmechanisme en de aangrijpingspunten van een geneesmiddel. Wanneer dit instrument wordt toegepast in een data-intensief Fase I of II onderzoek, in combinatie met frequente bepalingen van farmacokinetiek en veiligheid, is het mogelijk mechanistische kennis te vergaren op het gebied van humane farmacologie van een nieuw geneesmiddel. De waarde van de toepassing van dit instrument ligt in de handen van de onderzoeker en/of opdrachtgever van het onderzoek. Waar de beperkte overeenkomsten tussen chronische pijn, met zeer heterogene oorzaken en pathofysiologie, en het oproepen van pijnprikkels in gezonde vrijwilligers een tekortkoming lijkt, is dit eigenlijk een pluspunt. Enkele voordelen van het laatste zijn: een homogene populatie, gestandaardiseerde stimuli, gecontroleerde intensiteit en duur van de stimuli en de kwantitatieve uitkomsten die vergeleken kunnen worden over tijd en tussen proefpersonen. (Arendt-Nielsen, 2007) Daarom is dit instrument uitermate geschikt voor het kwantificeren van specifieke elementen van een systeem dat verstoord raakt wanneer er sprake is van pijn.

De PainCart is een multimodale biomarker, die bestaat uit verschillende complementaire pijntaken, die individueel uitgebreid zijn beschreven in eerder klinisch onderzoek. (Okkerse, 2018). Oorspronkelijk bestond de PainCart uit vier taken die ieder een verschillende modaliteit van pijn vertegenwoordigen: koudepijn, drukpijn, elektrische pijn en hittepijn. De PainCart bevatte echter geen pijntaak om hyperalgesie te kwantificeren, een kenmerkend symptoom van inflammatoire pijn en neuropathische pijn. Daarom beschrijft **hoofdstuk 2** van dit proefschrift een literatuurstudie naar de farmacologische sensitiviteit van hyperalgesiemodellen in gezonde vrijwilligers. Hierin zijn drie modellen geselecteerd op basis van frequentie van gebruik en toepasbaarheid. Alhoewel het capsaicine model beschreven wordt als een model voor neuropathische pijn, lijkt het niet gevoelig voor behandeling met geneesmiddelen uit de klasse die worden voorgeschreven in de behandeling van neuropathische pijn (tricyclische antidepressiva, calciumkanaal α2-δ liganden). Het UVB model voor inflammatoire pijn liet een sterke farmacologische sensitiviteit voor behandeling met NSAID's zien. Dit pleit voor een onderscheid tussen neuropathische hyperalgesie, die in de klinische praktijk nauwelijks op NSAID's reageert, en hyperalgesie bij ontstekingen die hiermee wel goed behandeld kunnen worden. Op basis van deze literatuurstudie is de PainCart uitgebreid met een modaliteit voor ontstekingspijn, het UVB model.

Wanneer de PainCart wordt toegepast in een klinisch onderzoek, worden de taken na elkaar uitgevoerd binnen een meetronde. Voorafgaande aan de toediening van een behandeling (actief of placebo) wordt een meetronde uitgevoerd, die wordt herhaald na de toediening op tijdstippen afhankelijk van het verwachte farmacokinetische profiel. Om de power ten gevolge van inter-individuele variabiliteit te vergroten, wordt zo mogelijk een crossover onderzoeksopzet toegepast. Daarnaast wordt variabiliteit als gevolg van de affectieve componenten van pijnperceptie geminimaliseerd door middel van een gestandaardiseerd protocol waarin de proefpersonen zelf verantwoordelijk zijn voor het opstarten en afbreken van de taak. Tevens heeft iedere pijntaak een maximale veiligheidsgrens waardoor er geen risico is op weefselschade.

Multimodale biomarker voor het functioneren van het centrale zenuwstelsel: NeuroCart

Analoog aan de PainCart wordt in dit proefschrift gebruik gemaakt van de NeuroCart, een multimodale cognitieve en neurofysiologische testbatterij. Deze testbatterij bevat diverse taken die uitgebreid zijn toegepast om specifieke, tijd- en dosisafhankelijke effecten van geneesmiddelen te kwantificeren. De volgende functionele domeinen worden gemeten met de NeuroCart: hand-oog coördinatie, alertheid, geheugen, subjectieve effecten, stemming en neurobiologische hersenactiviteit (elektro encefalografie). In deze thesis zijn de taken beperkt tot degene die in het verleden zijn aangetoond sensitief te zijn voor de effecten van de twee geneesmiddelen die centraal staan in de thesis: cannabinoïden en α2/α3 subtype selectieve $GABA_A$ receptor modulatoren.

In deze thesis zijn de beide testbatterijen gebruikt als biomarkers om de farmacodynamische effecten in relatie tot de farmacokinetiek en veiligheidsmetingen, te beschrijven van potentieel nieuwe en bekende analgetica. De nadruk ligt hierbij op twee omvangrijke neurotransmittersystemen, het endocannabinoïde systeem en het GABA-erge systeem. Beide systemen zijn betrokken bij een scala aan fysiologische functies, met receptoren verspreid over het gehele centrale en perifere zenuwstelsel. De uitdaging ligt in het ontwikkelen van therapieën die aangrijpen op dit systeem, dat bij chronische pijn mogelijk verstoord is als gevolg van pathologie, en de balans binnen het systeem herstellen. Doordat deze beide systeem zo wijdverspreid zijn binnen het zenuwstelsel is er niet alleen een kans dat de behandeling pijn vermindert, maar ook een risico op bijwerkingen. Om deze reden is het van grote waarde om binnen geneesmiddelenontwikkeling op dit gebied gebruik te maken van een gecombineerde benadering, waarbij zowel de farmacodynamische effecten op de pijn als op andere centraal zenuwstelsel functies, worden geïntegreerd.

Centrale modulatie van pijn: het endocannabinoïde systeem

De twee belangrijkste cannabinoïd (CB) receptoren zijn CB1 en CB2. De focus in dit proefschrift ligt op de CB1 receptor die zich bevindt in het gehele zenuwstelsel. CB1 receptoren worden gevonden in verschillende hersenstructuren waaronder de cortex, basale ganglia, hippocampi, cerebellum, thalamus, amygdala, PAG en medulla oblongata. Daarnaast worden de CB1 receptoren ook gevonden in het ruggenmerg, voornamelijk in de interneuronen van de dorsale hoorn. Bovendien zijn CB1 receptoren in het perifere zenuwstelsel betrokken bij signaaloverdracht van onder andere nociceptieve zenuwen. Als gevolg van deze verspreiding van receptoren over het gehele zenuwstelsel, is het endocannabinoïde-systeem betrokken bij de regulatie van zeer uiteenlopende fysiologische processen, van geheugen tot slaap en van gastrointestinale motiliteit tot pijnperceptie.

In **hoofdstuk 3** wordt een onderzoek beschreven naar een nieuwe orale formulering van Δ9-THC**,** een exogene ligand van de CB1 receptor. Hierin werden 24 patiënten

met (primaire of secundaire) progressieve Multiple Sclerosis (MS) met spasticiteit en pijn placebo-gecontroleerd behandeld gedurende een periode van 4 weken. De werkzaamheid en secundaire effecten werden uitgedrukt in zowel objectieve (o.a. elektrofysiologische) als subjectieve uitkomstmaten (alertheid, stemming). Om tot een geschikte dosis te komen, werd de behandelfase voorafgegaan door een 'challenge fase', waarin een individuele effectieve en tolereerbare dosis werd geselecteerd.

De objectieve uitkomstmaten lieten tijdens de behandelfase geen significant effect zien. Subjectieve spasticiteit daarentegen liet een verbetering zien, die statistisch significant verschillend was van placebo na twee weken behandeling. Pijnscores verbeterden eveneens en lieten een statistisch significante verbetering zien na 2 en 4 weken behandeling.

Het farmacokinetische model toonde aan dat er sprake was van een meer variabel profiel ten opzichte van eerder onderzoek dat werd uitgevoerd in gezonde vrijwilligers. De geobserveerde variabiliteit heeft mogelijk geleid tot meer variabiliteit in de farmacodynamische respons.

Dezelfde orale formulering van Δ9-THC werd onderzocht in **hoofdstuk 4**, ditmaal in gezonde vrijwilligers, gebruikmakend van de PainCart. Dit onderzoek werd uitgevoerd in het kader van de eerder uitgevoerde farmacologische validatie van de PainCart. Het doel was het in kaart brengen van het analgetische profiel van een klassiek analgeticum (paracetamol) en een centrale modulator van pijn (Δ9-THC) in vergelijking met een negatieve controle in de vorm van een sedativum (promethazine). Dit onderzoek werd placebo-gecontroleerd uitgevoerd in 24 proefpersonen gebruikmakend van een cross-over onderzoeksopzet.

Ogenschijnlijk tegen de verwachting in, liet paracetamol geen significant analgetisch effect zien op de pijntaken van de PainCart. Behandeling met Δ9-THC vertoonde eveneens geen acuut analgetisch effect en er leek zelfs sprake van hyperalgesie op twee van de vijf pijnmodaliteiten, elektrische pijn en drukpijn. Na de behandeling met promethazine werd eveneens een toename in pijnsensitiviteit geconstateerd: gevoeligheid voor koudepijn, drukpijn en inflammatoire pijn nam toe. Naast de PainCart, werden ook enkele taken van de NeuroCart uitgevoerd, alwaar behandeling met Δ9-THC en promethazine duidelijke subjectieve effecten lieten zien op alertheid en veranderde waarneming.

Dit onderzoek toonde opnieuw aan hoe complex het werkingsmechanisme van paracetamol is en dat het nog steeds niet volledig is ontrafeld, ondanks dat het behoort tot de meest gebruikte analgetica wereldwijd. Tevens toonde het onderzoek aan dat Δ9-THC geen acuut effect heeft op pijndrempels van nociceptieve pijndrempels, ofschoon het in verschillende situaties een analgetisch effect kan bewerkstelligen. In combinatie met de bevindingen uit **Hoofdstuk 3** onderschrijft dit onderzoek de notie dat herhaaldelijk toedienen van Δ9-THC leidt tot een geleidelijk farmacodynamisch effect dat de perceptie van pijn of spasticiteit behandelt, en niet een acuut effect bewerkstelligt op nociceptie of elektrofysiologisch meetbare uitkomstmaten. Bovendien toonde dit onderzoek de specificiteit van de PainCart aan op het gebied van

analgetische interventie, aangezien pijndrempels niet verhoogd werden door sedatie zoals geïnduceerd door promethazine.

Centrale modulatie van pijn: het GABA systeem

Gamma-aminoboterzuur (GABA) is de voornaamste remmende neurotransmitter in het centrale zenuwstelsel. Er zijn twee types GABA receptoren, en de focus in dit proefschrift ligt op het type $GABA_A$**.** Dit zijn pentamerische ionotrope receptoren, waarvan de meer dan 20 subtypes zijn opgebouwd uit verschillende combinaties van de subeenheden α1-6, β1-4, γ1-3, δ, ε, σ. Er is sprake van een grote heterogeniteit in functie van de diverse subtypes, wat gereflecteerd wordt in de distributie van de verschillende subtypes over diverse anatomische locaties binnen het centrale zenuwstelsel. Hierdoor zijn de verschillende subtypes geassocieerd met verschillende specifieke neurofysiologische functies. Het α1 subtype bijvoorbeeld, is geassocieerd met sedatie en amnesie, waar de subtypes α2 en α3 in grotere mate betrokken zijn bij anxiolyse en de modulatie van pijn, en het α5 subtype bij geheugenfuncties.

Hier volgt de beschrijving van twee humane onderzoeken waarin voor het eerst de beide biomarkers, NeuroCart en PainCart, werden geïntegreerd om het farmacodynamische effect van een centraal werkend geneesmiddel te beschrijven. Dit betreft PF-06372865**,** een nieuwe partiële $GABA_A$ α2/α3/α5 subtype-selectieve positieve allostere modulator die wordt ontwikkeld voor verschillende indicaties, waaronder angststoornissen, neuropathische pijn en epilepsie. In **Hoofdstuk 5** wordt een tweedelig First-in-Human onderzoek in gezonde vrijwilligers beschreven, dat werd uitgevoerd in een partiële cross-over onderzoeksopzet. Het doel van het eerste deel was het bestuderen van een groot bereik aan doseringen, om inzicht te krijgen in de veiligheid, de farmacodynamiek (PD) en farmacokinetiek (PK). Op basis hiervan werden twee doseringen geselecteerd (15 en 65 mg) voor een directe vergelijking met de non-selectieve positieve allostere modulator van de $GABA_A$ receptor, lorazepam (2mg), in het tweede deel van het onderzoek. De vergelijking met zowel placebo als lorazepam (2 mg), als de combinatie van PF-06372865 met lorazepam**,** toonde een differentieel profiel per receptor subtype van competitieve en non-competitieve receptorinteractie, zonder synergie. Afhankelijk van het functionele domein, werden er additieve of infra-additieve farmacodynamische effecten gevonden, in lijn met de voorspelde functionele selectiviteit voor de α2/α3/α5 subtypes. Zo werd er een additief effect gevonden op die taken die activiteit op α2/α3 receptor subtypes vertegenwoordigen. Daarnaast werd het effect van lorazepam (2mg) uitgedoofd op taken die $GABA_A$ α1 receptor subtypes vertegenwoordigen, mogelijk doordat PF-06372865 werkt als een antagonist ten opzichte van het PD effect van lorazepam, als gevolg van lage intrinsieke activiteit voor het $GABA_A$ α1 receptor subtype. De bevindingen over de wisselwerking tussen intrinsieke activiteit en receptoraffiniteit illustreren niet alleen de receptorfarmacologie van PF-06372865, maar ook die van $GABA_A$ α2/α3/α5 subtype-selectieve positieve allostere modulators in het algemeen.

Gebaseerd op bovenstaande uitvoerige farmacologische karakterisering, werden twee doseringen geselecteerd om het analgetische effectprofiel van PF-06372865 in kaart te brengen. **Hoofdstuk 6** beschrijft een dubbelblind, placebo-gecontroleerd, cross-over onderzoek in gezonde vrijwilligers naar de effecten van twee enkelvoudige doseringen (15 mg en 65 mg) van PF-06372865, in vergelijking met pregabaline (300 mg) als positieve controle. Farmacodynamische metingen werden uitgevoerd met de biomarker testbatterij van opgewekte pijnprikkels, de PainCart, en PK werd bepaald. De behandeling met PF-06372865 resulteerde in een analgetisch effect, waarbij een dosering van 65 mg een statistisch significant effect vertoonde op de taak voor koudepijn, en beide doseringen op de taak voor drukpijn. Behandeling met pregabaline produceerde eveneens een analgetisch effect op de taken voor koudepijn en drukpijn. Vanuit een mechanistisch perspectief is het lastig om de bevindingen op de pijntaken van dit onderzoek te vertalen naar een neuropathische pijnpatiënt. Het onderzoek illustreert echter eenduidig dat PF-06372865 het vermogen bezit om analgesie te induceren, in doseringen die geen ernstige sedatie of andere bijzondere bijwerkingen veroorzaken.

DISCUSSIE EN CONCLUSIES

De sensatie van pijn is een complex en veelzijdig proces waarbij verschillende centrale en perifere neurotransmittersystemen betrokken zijn. Deze thesis beschrijft de vroege klinische ontwikkeling van twee analgetica die werken op respectievelijk de endocannabinoïde CB1 receptoren en α2/α3/α5 subtypes van $GABA_A$. Voor beide zijn de neurofysiologische en analgetische effecten profielen in kaart gebracht om inzicht te bieden in de complexe wisselwerking tussen CNS demping, acute nociceptie en de ervaring van pijn. De integratie van deze bevindingen helpt bij het bepalen van de therapeutische index, mogelijke bijwerkingen en mogelijke indicatiegebieden voor toekomstige klinische onderzoeken.

Op het eerst gezicht lijkt de vertaling van de resultaten van de onderzoeken gebruikmakend van de PainCart niet ondubbelzinnig. De schijnbaar incongruente bevindingen illustreren echter exact op welke manier de biomarker testbatterij moet worden toegepast en geïnterpreteerd. Zoals al eerder beschreven: de vertaling van opgewekte pijnprikkels in gezonde vrijwilligers naar specifieke patiëntenpopulaties is niet eenvoudig of eenduidig. Bevindingen kunnen niet direct worden geëxtrapoleerd, maar dienen te worden geplaatst in de juiste context, met inachtneming van de waarde en de beperkingen van nociceptieve tests. Voor geneesmiddelen die werkzaam zijn in het centrale zenuwstelsel zijn twee aspecten hierin van belang: de complexiteit van pijn en mogelijke interacties met andere neurofysiologische functies.

De voordelen van het gebruik van biomarkers voor pijn om mechanistische kennis te vergaren ten opzichte van onderzoek in patiënten zijn uiteenlopend: gestandaardiseerde eindpunten, minder variabiliteit in pijn en daardoor meer statistische power, meer

robuust, betrouwbaar herhaaldelijk te meten, minder bias als gevolg van de affectieve componenten van de pijncascade en de mogelijkheid om specifieke elementen van het nociceptieve systeem te bestuderen. Daarnaast is in het verleden aangetoond dat de pijntestbatterij sensitief is om reductie van pijnperceptie te detecteren, door analgetica die niet bekend staan als directe nociceptieve pijnstillers, zoals pregabaline of imipramine. Het huidige proefschrift heeft bovendien aangetoond dat deze testbatterij geen analgetische effecten toont van pure sedativa, zoals promethazine.

Vanzelfsprekend zijn er ook beperkingen aan het gebruik van humane pijnmodellen. Allereerst lijkt het veelvormige klinisch beeld van neuropathische pijn niet na te bootsen in een gezonde vrijwilliger. Vanuit ethisch perspectief is het evident dat het opwekken van chronische pijn niet mogelijk is. Daarnaast creëert streng gecontroleerd onderzoek in een zeer homogene populatie mogelijk een hogere sensitiviteit, waardoor subtiele signalen kunnen worden gedetecteerd, die niet waarneembaar zijn in een patiëntenpopulatie. Anderzijds kunnen potentiele farmacodynamische effecten gemist worden, doordat de pathofysiologie samenhangt met dysregulatie in het systeem. Hierdoor reageert het gezonde zenuwstelsel niet op dezelfde wijze op farmacologische interventie. Mogelijk kunnen aanvullende biomarkers dan een rol spelen om inzicht te verschaffen in de farmacodynamiek van een geneesmiddel.

Wanneer de metingen van de multimodale biomarker voor pijn worden geïntegreerd met de karakterisering van neurocognitieve en neurofysiologische effecten, ontstaat een completer beeld van de farmacodynamiek van een pijngeneesmiddel. Dit inzicht in de humane farmacologie vormt het stuk van de puzzel dat nodig is om de andere stukken, zoals farmacokinetiek, veiligheid en preklinisch onderzoek, met elkaar te verbinden.

CURRICULUM VITAE

Guido van Amerongen was born on February 27th, 1986 in Leidschendam, The Netherlands. After completing secondary school at the *Stedelijk Gymnasium* in Leiden in 2005, he studied General Health Sciences Sciences at *Vrije Universiteit Amsterdam* (VU University). This was followed by a research master with a strong focus on epidemiology and clinical research methodology, named Lifestyle and Chronic Disorders at VU University. During this Master's he performed an internship at the Centre for Human Drug Research (CHDR) in Leiden and obtained his Master's degree in 2011. After graduating he started working at CHDR/LUMC in the position of clinical scientist in the field of neurology, pain and psychiatry. During this period the research described in this thesis was performed under the supervision of dr. G.J.Groeneveld, prof. J.M.A van Gerven and prof. A.F. Cohen. The research focused on an integrated assessment of biomarkers for pain and neurophysiological endpoints for centrally acting pain modulators in early phase clinical research. Since 2018 Guido van Amerongen is working in the role of Scientific Research and Clinical Specialist in the field of medical devices at Medical Brands, in Amsterdam. Guido lives together with his spouse, Sanderein.

LIST OF PUBLICATIONS

Siebenga, P., **van Amerongen**, **G.,** Hay, J. L., McDonnell, A., Gorman, D., Butt, R., & Groeneveld, G. J. (2020). Lack of Detection of the Analgesic Properties of PF-05089771, a Selective Nav 1.7 Inhibitor, Using a Battery of Pain Models in Healthy Subjects. *Clin Transl Sci, 13*(2), 318-324.

van Amerongen, **G.,** Siebenga, P. S., Gurrell, R., Dua, P., Whitlock, M., Gorman, D., Okkerse, P., Hay, J. L., Butt, R. P., & Groeneveld, G. J. (2019). Analgesic potential of PF-06372865, an α2/α3/α5 subtype-selective $GABA_A$ partial agonist, in humans. *British Journal of Anaesthesia, 123*(2), e194-e203.

Siebenga, P. S., **van Amerongen**, **G.,** Klaassen, E. S., de Kam, M. L., Rissmann, R., & Groeneveld, G. J. (2019). The ultraviolet B inflammation model: Postinflammatory hyperpigmentation and validation of a reduced UVB exposure paradigm for inducing hyperalgesia in healthy subjects. *Eur J Pain, 23*(5), 874-883.

Siebenga, P. S., **van Amerongen**, **G.,** Okkerse, P., Denney, W. S., Dua, P., Butt, R. P., Hay, J. L., & Groeneveld, G. J. (2019). Reproducibility of a battery of human evoked pain models to detect pharmacological effects of analgesic drugs. *Eur J Pain*.

Brooks, S., Jacobs, G. E., de Boer, P., Kent, J. M., Van Nueten, L., **van Amerongen**, **G.,** Zuiker, R., Kezic, I., Luthringer, R., van der Ark, P., van Gerven, J. M., & Drevets, W. (2019). The selective orexin-2 receptor antagonist seltorexant improves sleep: An exploratory double-blind, placebo controlled, crossover study in antidepressant-treated major depressive disorder patients with persistent insomnia. *J Psychopharmacology*

van Amerongen, **G.,** Siebenga, P., de Kam, M. L., Hay, J. L., & Groeneveld, G. J. (2018). Effect profile of paracetamol, Delta9-THC and promethazine using an evoked pain test battery in healthy subjects. *Eur J Pain, 22*(7), 1331-1342.

van Amerongen, **G.,** Kanhai, K., Baakman, A. C., Heuberger, J., Klaassen, E., Beumer, T. L., Strijers, R. L. M., Killestein, J., van Gerven, J., Cohen, A., & Groeneveld, G. J. (2018). Effects on Spasticity and Neuropathic Pain of an Oral Formulation of Delta9-tetrahydrocannabinol in Patients WithProgressive Multiple Sclerosis. *Clin Ther, 40*(9), 1467-1482.

Siebenga, P., Okkerse, P., **van Amerongen**, **G.,** Doll, R., Mentink, A., Hay, J., & Groeneveld, G. (2018). Pharmacodynamic Evaluation: Pain Methodologies (pp. 1-31).

Nickolls, S. A., Gurrell, R., **van Amerongen**, **G.,** Kammonen, J., Cao, L., Brown, A. R., Stead, C., Mead, A., Watson, C., Hsu, C., Owen, R. M., Pike, A., Fish, R. L., Chen, L., Qiu, R., Morris, E. D., Feng, G., Whitlock, M., Gorman, D., van Gerven, J., Reynolds, D. S., Dua, P., & Butt, R. P. (2018). Pharmacology in translation: the preclinical and early clinical profile of the novel alpha2/3 functionally selective $GABA_A$ receptor positive allosteric modulator PF-06372865. *Br J Pharmacol, 175*(4), 708-725.

Loudon, P., Siebenga, P., Gorman, D., Gore, K., Dua, P., **van Amerongen**, **G.,** Hay, J. L., Groeneveld, G. J., & Butt, R. P. (2018). Demonstration of an anti-hyperalgesic effect of a novel pan-Trk inhibitor PF-06273340 in a battery of human evoked pain models. *Br J Clin Pharmacol, 84*(2), 301-309.

van de Loo, A., Bervoets, A. C., Mooren, L., Bouwmeester, N. H., Garssen, J., Zuiker, R., **van Amerongen**, **G.,** van Gerven, J., Singh, J., der Ark, P. V., Fedgchin, M., Morrison, R., Wajs, E., & Verster, J. C. (2017). The effects of intranasal esketamine (84 mg) and oral mirtazapine (30 mg) on on-road driving performance: a double-blind, placebo-controlled study. *Psychopharmacology (Berl), 234*(21), 3175-3183.

Okkerse, P., **van Amerongen**, **G.,** de Kam, M. L., Stevens, J., Butt, R. P., Gurrell, R., Dahan, A., van Gerven, J. M., Hay, J. L., & Groeneveld, G. J. (2017). The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study. *Br J Clin Pharmacol, 83*(5), 976-990.

van Amerongen, **G.,** de Boer, M. W., Groeneveld, G. J., & Hay, J. L. (2016). A literature review on the pharmacological sensitivity of human evoked hyperalgesia pain models. *Br J Clin Pharmacol, 82*(4), 903-922.

Hay, J. L., Okkerse, P., **van Amerongen**, **G.,** & Groeneveld, G. J. (2016). Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery. *J Vis Exp*(110).

Doll, R. J., **van Amerongen**, **G.,** Hay, J. L., Groeneveld, G. J., Veltink, P. H., & Buitenweg, J. R. (2016). Responsiveness of electrical nociceptive detection thresholds to capsaicin (8 %)-induced changes in nociceptive processing. *Exp Brain Res, 234*(9), 2505-2514. doi:10.1007/s00221-016-4655-z