
CLEARING
THE HAZE
INVESTIGATING
THE CLINICAL
PHARMACOLOGY OF
CANNABINOIDS
Andriy A. Gorbenko

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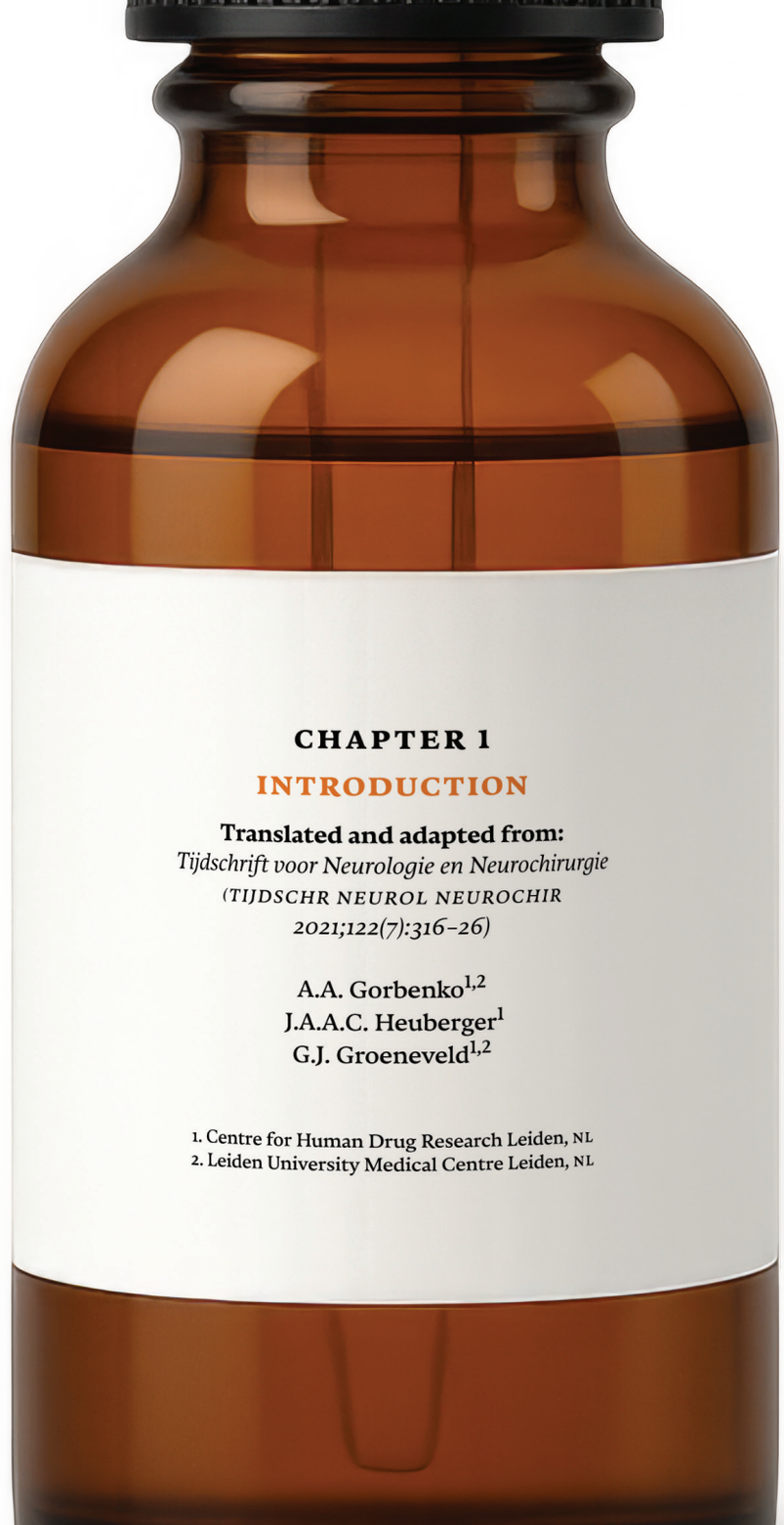

CHAPTER 1

INTRODUCTION

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The medicinal use of the *Cannabis sativa* plant has a long history. In China and India, cannabis was cultivated and used thousands of years ago for therapeutic purposes ranging from pain relief and anti-inflammatory effects to appetite stimulation and as an aphrodisiac. Since the 1960s, cannabis has enjoyed widespread popularity as a recreational substance. In recent decades, medical and scientific interest in cannabis has surged following the discovery and characterization of the receptors involved in cannabinoid signaling in the human nervous system. Cannabis has been investigated for treatment of glaucoma, reduced appetite and vomiting in oncology patients, epilepsy, pain, multiple sclerosis, and a host of other indications.[1] Starting from 2003, physicians have been legally allowed to prescribe medicinal cannabis in the Netherlands (and pharmacists to dispense it). Medicinal cannabis is dispensed approximately 39,000 times per year in the Netherlands.[2]

THE PHARMACOLOGY OF CANNABIS

The flowers of the female *Cannabis sativa* plant contain hundreds of different compounds, including 120 terpenes and 113 cannabinoids. Terpenes are responsible for the specific aroma and taste of a plant, and cannabinoids are responsible for the biological activity of cannabis. This thesis focuses on the two most prevalent and best-studied cannabinoids: Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[3]

THC and the Endocannabinoid System

THC is the most prevalent cannabinoid in cannabis and is responsible for its psychoactive effects. The pleasant effects of THC are euphoria ('feeling high') and altered sensory perception, while its adverse effects include reduced cognitive functioning, anxiety, delusions and hallucinations, and tachycardia. THC primarily exerts these effects via partial agonism of the CB_1 receptor. The CB_1 receptor is one of the most abundant G-protein-coupled receptors in the brain and is predominantly present in the neocortex, hippocampus, basal ganglia, cerebellum, and brainstem. Outside the nervous system, the CB_1 receptor is found in the myocardium. The CB_1 receptor is located presynaptically, and its activation results in a decrease in synaptic neurotransmitter release. Besides THC, the CB_1 receptor is also activated by the endogenous cannabinoids anandamide and 2-AG. The endocannabinoids are produced postsynaptically, and their release is triggered by sustained synaptic activity. Once released, endocannabinoids activate presynaptic CB_1 receptors, leading to a reduction in synaptic activity. In this way the endocannabinoid system (ECS) serves to reduce synaptic transmission by an activated neuron in a selective and controlled fashion.[4] The ECS modulates, among other things, the release of glutamate, GABA, serotonin, acetylcholine, dopamine, endorphins, and adrenaline, thereby influencing a multitude of processes involved in pain, immune response, appetite, thermoregulation, energy metabolism, and memory.[5]

Besides the CB_1 receptors, THC can also bind to CB_2 receptors, which are widely distributed on peripheral leukocytes and are involved in the regulation of inflammation and tissue damage. The expression of CB_2 receptors in the central nervous system is largely restricted to microglial cells.[6]

CBD

CBD is the second most prevalent cannabinoid in cannabis. In contrast to THC, CBD does not have (obvious) psychotropic effects and is commonly referred to as a 'non-intoxicating' cannabinoid. The pharmacology of CBD is complex – over 20 mechanisms of action have been identified to date, along with nearly as many potential therapeutic applications.[7] CBD is, among other effects, a negative allosteric modulator of the CB_1 receptor, meaning that when administered together with THC it can theoretically reduce the effects of THC. Furthermore, it is an agonist of the TRPV1 and TRPA1 receptors, which are involved in peripheral pain perception, a partial agonist of the 5-HT_{1A} receptor with a potential anxiolytic effect, and a partial agonist of the dopamine D_2^{high} receptor, suggestive of an antipsychotic effect.[7] Finally, CBD has a multitude of targets potentially leading to anti-epileptic (described below under *Epilepsy*) and anti-inflammatory effects.[8]

Pharmacokinetics and Pharmacodynamics

The most common routes of administration for cannabis are pulmonary (smoking or vaporizing the cannabis flower or extract), oromucosal (as oil or spray), and oral (as tea, incorporated into food, or in tablet or capsule form). The pharmacokinetics of cannabinoids depend on the route of administration. After inhalation, THC plasma concentrations peak almost immediately and decline within minutes, with the corresponding pharmacodynamic effects lasting up to several hours. For oral administration, both absorption and elimination are markedly slower. The onset of pharmacodynamic effects happens 30 to 60 minutes after administration, the maximum

effect may be reached 2-3 hours following administration, and duration of the effect can vary between 4 to 8 hours.[9] With oral administration, a substantial amount of the active metabolite 11-OH-THC is formed, which is assumed to be at least as potent as THC at CB_1 receptor agonism. Comparatively little 11-OH-THC is formed after THC inhalation.[10] This means that the scientific evidence for the effectiveness of inhaled cannabis cannot be directly extrapolated to orally administered cannabis formulations and vice versa. The absorption and elimination of (oral) CBD are somewhat slower compared to THC, with peak plasma concentration reached approximately after 2.5 to 5 hours after ingestion.[11]

The pharmacokinetics of THC and CBD is relatively variable for each route of administration. Inhalation technique varies among patients and between inhalations, leading to differences in the absorbed dose. For oral and oromucosal administration of THC and CBD, the low bioavailability (approx. 6% in a fasted state) is a source of variability. Additionally, since both THC and CBD are lipophilic molecules, their absorption is markedly increased after a meal, resulting in higher and longer-lasting exposure.

The pharmacodynamics of THC also exhibit considerable inter-individual variability: the subjective effects can differ markedly between individuals, even if plasma concentrations are similar. Consequently, the dosage of medicinal cannabis must be gradually titrated until an acceptable balance of beneficial and adverse effects is reached.[9]

MEDICINAL CANNABIS IN THE NETHERLANDS

In the Netherlands, the cultivation and delivery of medicinal cannabis fall under the responsibility of the Office for Medicinal Cannabis (OMC). The OMC supplies dried cannabis flower varieties that differ in their cannabinoid composition: e.g., the Bedrocan® variety is high in THC and contains almost no CBD, whereas the opposite is true for Bedrolite®. The medicinal cannabis is cultivated under standardized conditions and undergoes quality control, ensuring a constant composition for each variety.

Since 2015, several pharmacies have been preparing oil formulations from medicinal cannabis supplied by OMC, which is intended for oral or sublingual use.[9] Although the dried cannabis flowers and the oil formulations can be prescribed and dispensed legally, they are not formally approved as medicines by the Dutch regulator (the Medicines Evaluation Board) and are generally not reimbursed by health insurers.[9] The cost for cannabis flower is €33.40 per 5 grams (excluding VAT), as of 2025; the cost for cannabis oil varies depending on the type and the pharmacy. Based on an average use of 0.73 grams of flower per day, the average cost is approximately €120 per month per patient (excluding VAT).[12] Depending on usage, however, the costs for individual patients may run significantly higher.

In addition to the products supplied by the OMC and the oil formulations made from these, a multitude of over-the-counter (OTC) CBD products is available at drugstores, and recreational cannabis is available at coffeeshops. The quality and composition of recreational and OTC cannabis products are not guaranteed.[9]

Various pharmaceutical formulations of cannabinoids are also available, including nabiximols (Sativex®), a buccal spray containing THC and CBD in equal measure; Epidyolex®, an oral CBD formulation approved for treatment of seizures; various generic and branded formulations of dronabinol (which is the generic drug denomination for THC) of botanical or synthetic origin; and nabilone (Cesamet®), a synthetic CB_1 agonist with potent THC-like effects.

SCIENTIFIC EVIDENCE FOR CANNABINOIDS WITHIN NEUROLOGY

The involvement of the ECS in various physiological processes and the abundance of identified cannabinoid targets suggest a broad therapeutic applicability. Within the field of neurology, evidence exists for the use of cannabinoids for treatment of chronic neuropathic pain (CNP), of pain and spasticity in multiple sclerosis (MS) patients, and seizure frequency reduction in three rare epileptic syndromes. In a range of other disorders, clinical studies have not (yet) produced results supporting the use of cannabinoids.

Chronic Neuropathic Pain

The ECS plays a role in pain regulation through multiple mechanisms at peripheral, spinal, and cerebral levels. At the peripheral level, CB_1 receptors are present on the presynaptic nerve endings of peripheral nociceptors and neurons in the dorsal ganglion. Their activation leads to reduced neurotransmitter release and therefore, a modulation of nociceptive signal transmission. In addition, activation of the CB_2 receptors (which are predominantly expressed on leukocytes) reduced inflammatory hyperalgesia in animal models of neuropathic pain.[13,14] At the spinal level, the CB_1 receptor modulates the activation of the anterolateral system and suppresses nociception, windup

and central sensitization in the dorsal horn. At the cerebral level, the CB_1 receptors modulate pain via activation of descending inhibitory pathways in the periaqueductal gray and raphe nuclei, and by modulating the integration of the affective component of pain in the limbic system.[15]

A 2018 Cochrane review and meta-analysis reported an estimated number-needed-to-treat (NNT) for 30% reduction in pain of 11 (95% CI: 7, 33),[16] putting cannabinoids somewhat behind conventional therapies for neuropathic pain like tricyclic antidepressants (NNT of 3.6) and pregabalin (NNT of 7.7).[17] Studies administering oral, oromucosal and inhaled formulations were included in the meta-analysis; all interventions contained THC, either botanical or synthetic in origin, or nabilone, a synthetic CB_1 agonist; most also contained CBD. The quality of the evidence was assessed as 'moderate'.

The Dutch Neurology Association (Nederlandse Vereninging voor Neurologie (NVN)) guideline advises against the use of medicinal cannabis for diabetic neuropathy, based on the recommendation of the international Special Interest Group on Neuropathic Pain (NEUPSIG).[18] Internationally, there are differences in guidelines, and for instance, the Canadian Pain Society recommends cannabis as a third-line therapy for CNP.[19]

Multiple Sclerosis

Individual patients with MS and their clinicians have reported symptomatic relief after using cannabis. This anecdotal evidence has led to considerable medical-scientific interest in cannabinoids within this population. Recent reviews and meta-analyses concluded that the use of THC-containing formulations probably reduces spasticity in patients with MS, albeit with a modest effect size and only on patient-reported outcome measures.[20,21] The efficacy of cannabinoids is likely lower compared to conventional oral antispasmodics such as baclofen and tizanidine.[22] A review from 2014 concluded that THC-containing formulations are effective or probably effective against pain in MS.[20]

In the Netherlands, Sativex®, an oromucosal THC/CBD spray, is approved for the treatment of spasticity in adults with MS. Evidence for efficacy in improving bladder dysfunction is conflicting, and cannabinoids are probably not effective for the treatment of tremor in MS.[20] Pre-clinical research found cannabinoids to have a neuroprotective effect, with potential to modify MS disease progression, but a clinical trial failed to confirm this finding.[23]

Epilepsy

The anti-seizure effect of CBD has been demonstrated in a series of animal models of epilepsy, but the mechanism underlying this effect remains unclear. Proposed mechanisms include an effect on the Ca^{2+} flow (either via antagonism of the G protein-coupled receptor 55 (GPR55),[24,25] or via desensitization of the vanilloid receptor 1 (TRPV1)),[24] increased adenosine signaling, and interaction with GABA receptors.[26] At least in part, the antiepileptic effect of adjunctive CBD are thought to arise from a pharmacokinetic interaction with clobazam, another anti-seizure medication (ASM), via inhibition of CYP450 enzymes.[27]

Clinical phase 3 studies have shown that adjunctive treatment with CBD reduced seizure frequency in patients with Lennox-Gastaut syndrome (LGS)[28] and Dravet syndrome[29] (DS) with an effect size comparable to other adjunctive ASMs.[30] These results have led to the registration of Epidyolex® (purified CBD solution) for the treatment of seizures associated with these syndromes in the United States and European Union in 2018. In 2020, the approval was extended to include treatment of tuberous sclerosis complex (TSC), based on similarly positive trial results in this population.[31]

It is important to note that respectively 66% and 49% of patients with DS and LGS used clobazam during the trials. It is known that the plasma concentrations of the active metabolite of clobazam, N-desmethylclobazam, increase significantly due to the concomitant use of CBD. The subgroup of patients without clobazam use did no better than the placebo group.[27,32] This raises reasonable doubts regarding the extent to which anti-seizure efficacy of CBD is explained by its intrinsic pharmacodynamic actions, rather than the effects of increased clobazam and metabolite levels. The European Medicinal Agency only approved CBD for treatment of DS and LGS in conjunction with clobazam, reflecting the uncertainty regarding the origin of the anti-seizure effect. In the registration trial for TSC, a smaller proportion of participants (27%) used concomitant clobazam.[31] Seizure frequency reductions were observed both in patients using CBD with and without clobazam, although the reduction was greater in clobazam users. These results again implicated the interaction with clobazam as a central, although perhaps not the only, mode of action. We evaluated the intrinsic anti-epileptic effects of CBD in a mechanistic clinical study described in **Chapter 4**.

Retrospective studies suggest that CBD may also be effective in other syndromes, including CDKL5, Aicardi, Dup15q, Doose, and SYNGAP1 syndromes

and myoclonic absence epilepsy, but randomized controlled trials in these syndromes are not yet available.[33,34]

Other indications

Nabilone, a synthetic THC analogue, reduced levodopa-induced dyskinesias in a study with 7 patients with Parkinson's disease.[35] A later trial in 19 participants showed no difference between an oral cannabis extract (2:1 THC:CBD) and placebo.[36] CBD has demonstrated neuroprotective effects in *in vitro* and animal models of Parkinson's disease. In a clinical study, CBD did not improve motor function, but did improve psychotic symptoms and quality of life.[37] The Dutch Neurology Association guideline from 2020 advises against the use of medicinal cannabis in Parkinson's disease.[38]

There have been three trials with cannabinoids in patients with Huntington's disease. One study with CBD found no positive effect on the severity of chorea.[39] In a crossover study in 44 patients, nabilone improved the chorea score, but not the total motor score.[40] In a recent crossover study, Sativex® had no effect on relevant clinical features of Huntington's disease.[41]

THC reduced disturbed behavior in a study of twelve patients with Alzheimer's disease,[42] but did not show a positive effect on neuropsychiatric symptoms in patients with dementia in two other recent studies.[43]

THC did not provide beneficial effects in small studies in patients with cervical dystonia,[44] Gilles de la Tourette syndrome[45] and amyotrophic lateral sclerosis.[46]

LIMITATIONS OF SCIENTIFIC RESEARCH ON CANNABIS

Although medicinal cannabis has been investigated for a wide range of indications, this research has important methodological limitations. All studies have reported large placebo effects. Difficulties in blinding are inherent to research with psychoactive compounds like THC. For many indications, studies had (extremely) small populations and thus limited statistical power. These factors negatively influence the reliability individual trial results. Furthermore, the heterogeneity of interventions (i.e., cannabinoids involved, dose, route of administration, synthetic vs botanical origin), outcome measures, and study populations makes it difficult to compare studies, reduces the reliability of meta-analyses and fuels over-interpretation. These methodological shortcomings and research gaps are cited by the Dutch healthcare authorities as the reason for the lack of reimbursement for the costs of medicinal cannabis,[47] and therefore severely limit patient access to medicinal cannabis in practice.

TYPE OF CANNABIS PER INDICATION

The OMC and the pharmacies provide recommendations for specific strains of cannabis for specific indications, based on the experiences of patients, clinicians, and pharmacists. Although these recommendations may serve as a practical starting point for treatment, there is no solid evidence (nor a convincing pharmacological rationale) for the superiority of specific THC-containing strains over others for specific indications. In principle, any type of cannabis containing THC should produce the effects associated with THC. For the treatment of epileptic syndromes, only products that contain CBD (without THC) should be prescribed.

SIDE EFFECTS AND INTERACTIONS

The safety and tolerability of cannabis and cannabinoids depend on the population, frequency and duration of use, and the cannabinoid (especially THC) content of the product. Frequently observed side effects of THC-containing products are dizziness, fatigue, dry mouth, dry eyes, reduced balance, tachycardia, anxiety, paranoid delusions and hallucinations, nausea, and vomiting.[48] Products with a high CBD:THC ratio have been claimed to cause fewer side effects (less anxiety, delusions, hallucinations, and cognitive disturbances) compared with THC-dominant products.[49] This claim fits in the broader 'entourage effect' hypothesis, which entails that multiple constituents of the cannabis plant work together to increase the therapeutic and reduce the adverse effects, when compared to isolated compounds.[50] Research results in this area are inconsistent[49] and we thoroughly investigate the potential interaction between CBD and THC in **Chapter 2**.

The most common side effects of CBD are diarrhea, drowsiness, and increased transaminases. A sidenote to this is that the knowledge regarding the safety of CBD is largely based on studies in epilepsy patients, in which relatively high doses were used and where concomitant medication use and potential drug interactions may have played a role.[48] CBD carries a significant risk of drug interactions via the CYP450 system, specifically CYP2C19, 3A4 and 2C9.[51] In **Chapter 3**, we evaluate potential drug interaction between CBD and two commonly used analgesics.

The potency of recreational cannabis has been increasing over time. In the Netherlands, the average THC content of recreational cannabis has approximately doubled since the turn of the century.[52] Novel, concentrated cannabis products have emerged on the recreational drug market, including cannabis resins[53] and extracts,[54] as well as potent synthetic alternatives to

botanical products.[55] Various edible products containing THC are available, such as chocolates, brownies, and gummies. Such products are easily mistaken for regular foods and can be especially appealing to children.[56,57] As a result of these factors, the incidence of emergency department visits related to cannabinoid-induced toxicity has continued to increase.[57–60] In **Chapters 6 and 7**, we investigate a novel pharmaceutical treatment for acute cannabinoid intoxication.

In the long term, the use of THC-containing products may negatively affect cognitive and motor skills. Cannabis use can lead to dependence, with the risk being greatest in young adults. Adolescent cannabis use is likely a component cause that, in interaction with other risk factors, contributes to the development of schizophrenia.[61] Smoking cannabis also exposes the patient to mutagenic and carcinogenic combustion products. Recent literature highlights the cardiovascular risks of cannabis.[62] Use of cannabis during pregnancy increases the risk of congenital anomalies (in particular gastroschisis) and may have long-term negative consequences on the cognitive functioning and psychological health of the child.[48] THC (but not CBD) affects driving ability,[63] and legal limits for drug use in traffic apply, including for medicinal/prescription use.[64] Exceeding these limits constitutes an offense. A travel declaration is required when visiting other countries.

CONCLUSION AND THESIS OUTLINE

Therapeutic use of cannabinoids has progressed markedly over the past decades. Randomized controlled trials have shown that cannabinoid formulations can decrease chronic neuropathic pain and spasticity, and a purified CBD formulation has been approved for three rare epileptic syndromes. For most other neurological indications, however, evidence from preliminary studies has been negative or insufficient.

Enthusiasm for cannabinoid therapeutics has nonetheless outpaced the evidence. Existing studies are heterogeneous in design and quality, effect sizes are generally modest and key knowledge gaps persist. Although THC appears to be the principal cannabinoid responsible for analgesic and spasmolytic effects, there is no consensus on the optimal cannabinoid composition. Claims of therapeutic superiority and improved tolerability for CBD-rich formulations remain inadequately substantiated. Consequently, health authorities are unconvinced and reluctant to reimburse cannabinoid-based analgesic or antispasmodic therapy. Even in the epileptic syndromes for which CBD is approved, efficacy is at least partly dependent on a pharmacokinetic interaction with clobazam, and the extent of any intrinsic anti-seizure effects remains uncertain.

Parallel developments in recreational markets further accentuate the need for rigorous pharmacological evaluation. Wide-ranging health benefits are claimed for OTC CBD supplements without substantiating evidence or consideration of adverse effects and interactions. Escalating potency of cannabis products, including concentrated extracts, has resulted in rising emergency department visits for cannabinoid toxicity, yet systematic attention to its management lags behind.

For cannabinoid products to develop into effective, safe and widely adopted and reimbursed medicines, well-designed clinical trials addressing the remaining knowledge gaps in cannabinoid pharmacology are required. In this thesis, we attempt to contribute to this goal by advancing the understanding of the pharmacodynamics and side effects of individual cannabinoids, their interactions, and by evaluating a targeted countermeasure for toxicity. With **Chapters 2 and 3** we aim to determine the optimal CBD content in formulations used for the treatment of neuropathic pain. In **Chapter 2**, we investigate the presumed beneficial effects of CBD on the side effects of THC and evaluate the contribution of CBD to cannabinoid analgesia. In **Chapter 3**, conversely, we evaluate the potential of CBD to cause harm by interfering with other drugs that are likely to be used concomitantly by patients with neuropathic pain. We test whether CBD has a pharmacokinetic interaction with amitriptyline and tramadol, two commonly used analgesic drugs. In **Chapter 4**, we assess the effects of CBD on cortical excitability, a proxy measure of anti-seizure effectiveness. This is an indirect way of assessing whether the anti-seizure effects of CBD are intrinsic, as opposed to attributable to the interaction with clobazam. In **Chapter 5**, we comment on the design of a clinical study of CBD performed elsewhere and argue for closer adherence to basic principles of clinical pharmacology in cannabinoid trials. **Chapters 6 and 7** evaluate selonabant, a CB_1 receptor antagonist, as an emergency treatment for cannabinoid intoxication.

Collectively, these studies aim to replace some of the mythology surrounding cannabinoids with understanding of their pharmacology, and to further the development of cannabinoids into safe, effective and evidence-based therapeutic modalities.

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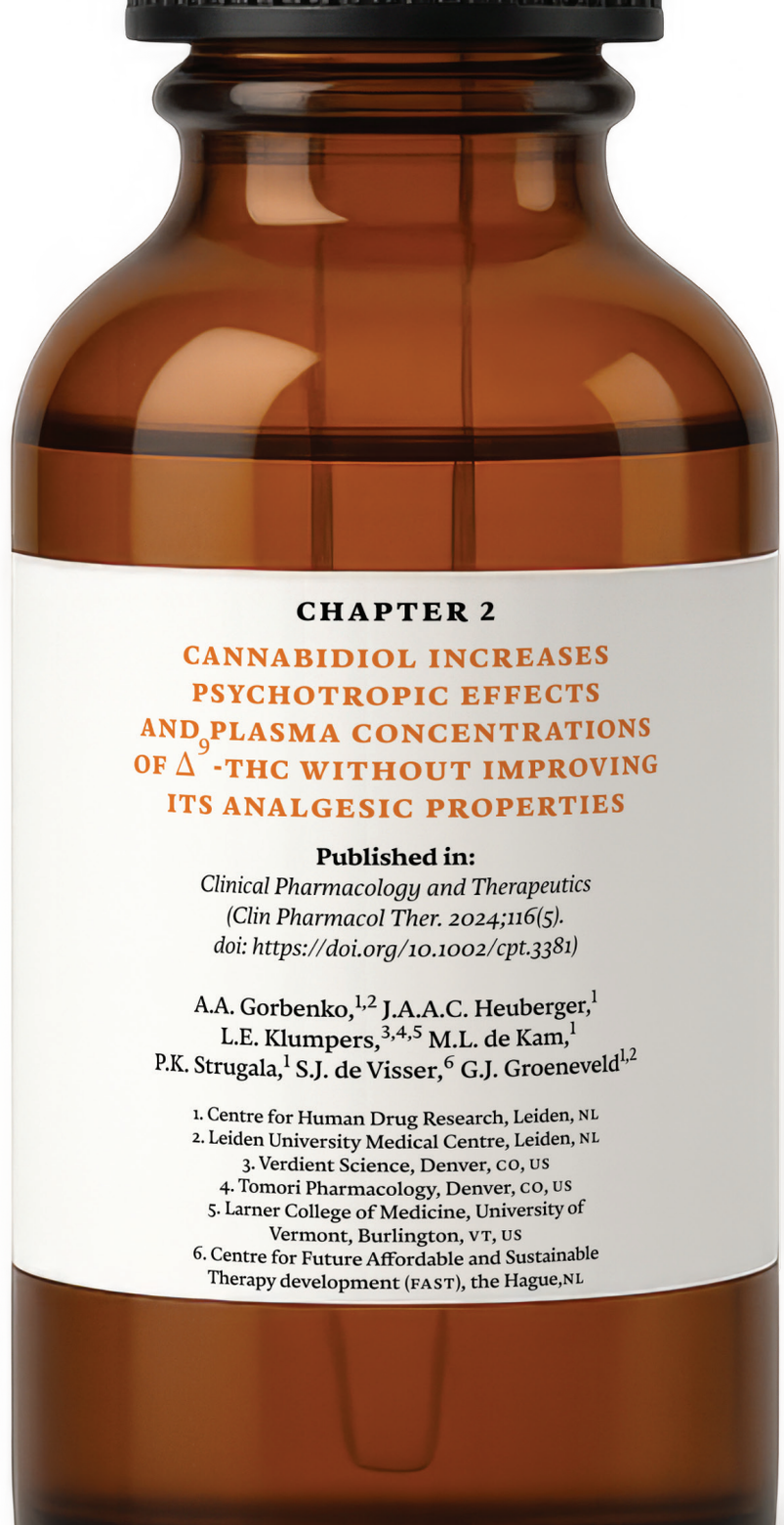

CHAPTER 2

CANNABIDIOL INCREASES PSYCHOTROPIC EFFECTS AND PLASMA CONCENTRATIONS OF Δ^9-THC WITHOUT IMPROVING ITS ANALGESIC PROPERTIES

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Abstract

Cannabidiol (CBD), the main non-intoxicating compound in cannabis, has been hypothesized to reduce adverse effects of Δ^9-tetrahydrocannabinol (THC), the main psychoactive and analgesic component of cannabis. This clinical trial investigated the hypothesis that CBD counteracts adverse effects of THC and thereby potentially improves tolerability of cannabis as an analgesic. A randomized, double blind, placebo-controlled, 5-way cross-over trial was performed in 37 healthy volunteers. On each visit, double placebo, THC 9 mg with placebo CBD, or THC 9 mg with 10, 30 or 450 mg CBD were administered orally. Psychoactive and analgesic effects were quantified using standardized test batteries. Pharmacokinetic sampling was performed. Data were analyzed using a mixed effects model. Co-administration of 450 mg CBD did not reduce, but instead significantly increased subjective, psychomotor, cognitive and autonomous effects of THC (e.g. VAS 'Feeling High' by 60.5% (95% CI: 12.7%, 128.5%, p<.01)), whereas THC effects with 10 and 30 mg CBD were not significantly different from THC alone. CBD did not significantly enhance THC analgesia at any dose level. Administration of 450 mg CBD significantly increased AUC_{last} of THC (AUC_{last} ratio: 2.18, 95% CI: 1.54, 3.08, p<.0001) and 11-OH-THC (AUC_{last} ratio: 6.24 (95% CI: 4.27, 9.12, p<.0001) compared to THC alone, and 30 mg CBD significantly increased AUC_{last} of 11-OH-THC (AUC_{last} ratio: 1.89, 95% CI: 1.30, 2.77, p=0.0013), and of THC (AUC_{last} ratio: 1.44, 95% CI: 1.01, 2.04, p=0.0446). Present findings do not support the use of CBD to reduce adverse effects of oral THC or enhance THC analgesia.

Study highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? Cannabis and cannabis products are used as an analgesic for chronic neuropathic pain. Cannabidiol (CBD), a non-intoxicating constituent of cannabis, is hypothesized to attenuate the effects of Δ^9-tetrahydrocannabinol (THC), the main psychoactive constituent. However, this effect is not found consistently and its mechanism and the required CBD dose remain unknown.

WHAT QUESTION DID THIS STUDY ADDRESS? The modulation of acute subjective, cognitive, psychomotor, autonomous, and analgesic effects of THC by three dose levels of CBD was investigated in healthy volunteers and compared with placebo.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE? This study found no evidence of CBD reducing adverse THC effects. On the contrary, THC effects were significantly increased by 450 mg of CBD, which was most likely explained by CBD inhibiting cytochrome P450-mediated metabolism of THC. Evidence of a pharmacokinetic interaction between CBD and THC was found at both 30 and 450 mg CBD dose levels. CBD did not enhance THC analgesia at any dose level.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE? These results provide evidence against the hypothesis that CBD attenuates THC effects, highlight the potential for drug interactions even at low doses of CBD, and add to the understanding of THC analgesia.

Introduction

Δ^9-Tetrahydrocannabinol (THC) is the main psychoactive component of cannabis plants. Its effects are mediated by partial agonism of the cannabinoid receptor 1 (CB_1) and include feeling high, altered perception and an elevated heart rate. THC shows promise as an analgesic in patients with chronic neuropathic pain,[1,2] although its effectiveness is limited to an undefined subgroup of patients and its therapeutic potential is further limited by adverse effects that are typically of a psychotropic nature, including intoxication, cognitive and psychomotor impairment, anxiety, delusions, and hallucinations.[3,4]

Cannabidiol (CBD), the main non-intoxicating compound in cannabis, has been hypothesized to reduce the adverse effects of THC.[5–7] The purported superior tolerability of CBD-rich cannabis has been the subject of numerous scientific publications[5,8,9] and is routinely referenced to in popular and commercial publications.[10] The complex pharmacology of CBD includes multiple pathways, which could plausibly contribute to such an effect. For instance, CBD is a negative allosteric modulator (NAM) of the CB_1 receptor,[11] potentially diminishing any CB_1-mediated THC effects. Additionally, CBD acts as an agonist at the serotonin $5\text{-}HT_{1A}$ receptor with potential for anxiolytic properties[12] and as a partial agonist at the dopamine D_2^{high} receptors with a suggested potential for antipsychotic action.[13] Furthermore, CBD potentially possesses analgesic effects via its activity at the TRPV1 and $5\text{-}HT_{1A}$ receptors.[14]

The results of clinical research to date are conflicting. Studies have shown CBD to inhibit THC-elicited paranoid and psychotic symptoms and memory impairment,[15] reduce anxiety,[16] and produce a lower degree of subjective intoxication[17,18] compared to THC alone. However, in other clinical trials, CBD failed to attenuate THC-induced anxiety,[4] subjective intoxication and cognitive task performance[19] as well as acute psychotic and memory-impairing effects of THC.[20]

Drug doses, THC:CBD ratios and routes of administration varied throughout the studies, further complicating the interpretation of the results. Consequently, there is no consensus on what effects of THC are attenuated by CBD, if any, and at which doses or dose ratios, and if different routes of administration alter this.

The goal of this study was to assess whether co-administration of CBD could reduce the adverse effects of THC while not compromising, or potentially even enhancing its analgesic properties. Effects of THC alone were therefore compared to the combination of THC with three doses of CBD, controlled with a placebo and a THC + placebo treatment arm. Subjective, cognitive, and psychomotor effects were measured using a validated CNS test battery[21] and the analgesic effects using a validated pain test battery.[22]

Methods

PARTICIPANTS AND STUDY DESIGN

The study was a double-blind, randomized, double-dummy placebo-controlled, 5-way cross-over study in which the effects of THC + placebo were compared to the effects of THC in combination with 3 doses of CBD and double 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 Participants (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 prospectively with the Netherlands National Trial Register (NTR) under registration number: NL9543.

Each participant provided written informed consent before any screening procedures were performed. All participants were healthy male and female volunteers aged 18 to 45 years with a body mass index of 18 to 30 kg/m^2. The participants underwent a full medical screening, including medical history anamnesis, a physical examination, blood chemistry and hematology, urinalysis and an electrocardiogram (ECG) to assess eligibility. Participants with a clinically significant known medical condition, particularly any psychotic disorder or existing condition affecting cold or pain sensitivity, were excluded. All included participants were cannabis users for at least 1 year prior to screening, with cannabis use not exceeding once per month on average in the 6 months prior to study participation. The participants refrained from cannabis use from at least 3 weeks prior to the first dosing day until the end of the study. Any participant who was a regular user of any illicit drugs other than the casual use of cannabis, or had a history of drug abuse or a positive drug screen at screening, was excluded. Any nutrients known to modulate CYP enzyme activity (e.g., grapefruit or Seville orange containing products or quinine containing drinks (tonic water or bitter lemon) were not permitted from 3 days before each study visit until discharge from the research unit.

Smoking and the use of xanthine-containing products were not allowed during dosing days.

The full list of inclusion and exclusion criteria is provided in the **Supplementary Materials**.

All females of childbearing potential and all males were required to practice effective contraception during the study and to continue contraception for at least 90 days after the last dosing (detailed contraception requirements provided in the **Supplementary Materials**). Urine pregnancy testing was conducted in female participants on each study day prior to dosing.

STUDY DRUGS

On each visit, participants received single doses of one of the five oral treatments: double placebo, THC 9 mg with placebo, or THC 9 mg with either CBD 10 mg, CBD 30 mg or CBD 450 mg. The doses were chosen based on concentration-effect relationship and receptor occupancy data for three potential mechanistic pathways through which we hypothesized that CBD could attenuate THC effects: 1) negative allosteric modulation of the CB_1 receptor, 2) partial agonism of the 5-HT_{1A} receptor and 3) partial agonism of the D_2^{high} receptor (full dose rationale is provided in the **Supplementary Materials**). CBD (or placebo) was always administered 30 minutes prior to THC (or placebo) in order to align the expected t_{max} of the two compounds. The washout period between study visits was at least 14 days.

THC was administered in oral tablets containing 1.5 mg THC (Namisol®) and CBD in oral tablets containing 20 mg or 150 mg CBD (Arvisol®); 20 mg CBD tablets were halved for the 10 mg CBD treatment. Both formulations, as well as matching placebo, were manufactured by Echo Pharmaceuticals. Namisol® had been previously administered in multiple studies in healthy volunteers and patient populations[23,24] and Arvisol® in healthy volunteers only (unpublished). Active and placebo tablets contained an identical amount of excipients. The drug substance in both Namisol® and Arvisol® was botanically derived. Release specifications for impurities are provided in the **Supplementary Materials**.

Fasting was required for at least 4 hours prior to every scheduled visit. Shortly after arrival, participants received a semi-standardized light breakfast (contents described in **Supplementary Materials**). Participants remained fasted for at least 2 hours before, and 1.5 hours after study drug administration (water was allowed as required).

PHARMACODYNAMIC ASSESSMENTS

Assessments were performed in 'test-blocks', where for each nominal timepoint (e.g., 1h post-dose), vital signs were measured 2 minutes prior to the timepoint, blood sampling for hormones and pharmacokinetics was performed exactly on the timepoint, and subjective, psychomotor, and cognitive tests were performed thereafter, with the analgesic tests performed last. Such a 'test-block' ended approximately 45 minutes after the nominal timepoint. Measurements were performed at approximately the same clock time during each visit to account for circadian effects. Within 3 weeks prior to the first study visit, participants had a training session to get acquainted with the pharmacodynamic tests and to minimize learning effects. Brief descriptions of each pharmacodynamic assessment are provided below; detailed descriptions can be found in the **Supplementary Materials**.

Subjective effects

Visual Analogue Scales (VAS) according to Bond and Lader were used for assessment of study participant's subjective state.[25] Three main factors, namely 'alertness', 'mood' and 'calmness', were calculated from 16 bipolar horizontal scales ranging from 0 to 100, where values of 0 and 100 represented opposing subjective states and a value of 50 represented the neutral state.[26] Subjective psychedelic effects including VAS 'Feeling High' were evaluated using the 13-item VAS 'Bowdle' with unipolar scales ranging from 0 to 100.[27] The VAS scales were performed twice pre-dose and at 1, 2, 3, 4, and 6h post-dose. The State-Trait Anxiety Inventory (STAI) was used to quantify present feelings of anxiety or tension and was performed twice pre-dose and 1, 2, 4, and 6h post-dose.[28] Once pre-dose and 6h post-dose, participants completed the Brief Symptom Inventory (BSI), which is a self-assessment instrument to measure psychopathology in adults across 9 different dimensions: general somatic symptoms, cognitive symptoms, interpersonal sensitivity, depressed mood, anxiety, hostility, phobic anxiety, paranoid thoughts, and psychoticism.

Psychomotor and cognitive effects

A selection of tests from the validated NeuroCart® CNS test battery was performed pre-dose and at 1, 2, 3, 4, and 6h post-dose for assessment of THC effects on psychomotor function and cognition. The body sway task measures postural stability.[29] The adaptive tracking test is used to evaluate visuomotor coordination and vigilance.[21] The Stroop task is used to assess attention,

perception, and inhibition. Two parameters were derived from the Stroop task: Stroop 1 relates to reaction time and Stroop 2 relates to the number of correct responses. The Simple Reaction Time (SRT) task is designed to measure the attention and speed of information processing of the participant.

Autonomous effects

Measurements of autonomous effects were performed pre-dose (twice for heart rate and once for cortisol and prolactin) and 0.5, 1, 2, 3, 4, 6, and 8h post-dose. Heart rate measurements were performed using Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400 automated devices after 5 minutes in supine position. Serum prolactin levels were determined as a potential marker of anti-psychotic effects of CBD, as antipsychotic drugs consistently increase prolactin levels due to their antidopaminergic properties[30] and CBD has been hypothesized to have potential antipsychotic properties due to its partial agonism of the D_2^{high} receptor.[13,31] Serum cortisol levels have been shown to increase after administration of THC compared to placebo in previous research.[32]

Analgesic effects

During each treatment period, a validated battery of pain tests, the PainCart®, was performed twice pre-dose and at 1, 2, 3, and 6h after dosing, consisting of a heat pain test, a pressure pain test, an electrical pain test, and the cold pressor pain test.[33,34] For all tests (except heat pain) participants were given an electronic visual analog scale (eVAS) slider to hold, with which they could indicate their current perceived pain intensity. The eVAS had a range of 0 – 100, with 0 defined as 'no pain,' sliding >0 defined the pain detection threshold (PDT), and 100 defined the pain tolerance threshold (PTT; 'worst pain tolerable'). When PTT was reached, the test automatically stopped and immediately relieved participants from their pain. Following the test, the participant was asked to rate the pain experienced during the test using the short form of the McGill pain questionnaire (SF-MPQ), a questionnaire that evaluates the affective and sensory components of pain with 4-point Likert-type scales. The SF-MPQ also evaluated the peak pain intensity of the test just performed using a 5-point Likert-type scale (SF-MPQ PPI), as well as using a visual analogue scale (SF-MPQ VAS).

The capsaicin 1% solution model was included as a model for thermal and mechanical allodynia by selectively sensitizing the TRPV1 channel.[33,34] A 3×3 cm surface on the dominant volar forearm was used for the application of the 1% capsaicin solution. The nondominant volar forearm served as a control (not treated with capsaicin). The size of the area of secondary mechanical allodynia around the 3x3 cm area where capsaicin was applied was assessed using Von Frey filaments. The heat pain test was performed on capsaicin-treated skin as well as the untreated skin.

PHARMACOKINETIC ASSESSMENTS

Venous blood samples were taken pre-dose and between 0.5, 1, 2, 3, 4, 6 and 8h following THC dosing. Approximately 2 mL of blood per sample was collected via a venous catheter in an antecubital vein. Plasma THC and its metabolites 11-OH-THC, 11-COOH-THC, and CBD and its metabolites 6α-OH-CBD, 6β-OH-CBD, 7-OH-CBD, 7-CBD-COOH and 2'-CBD-Glucuronide concentrations were measured using a validated LC-MS/MS method.[35] The lower limits of quantification (LLOQs), as well as reference material sources and analytical run acceptance criteria are provided in the **Supplementary Materials**.

R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019) was used to calculate pharmacokinetic parameters. When an actual sampling time differed from the protocol time by more than 10% and at least 5 minutes, the concentration was excluded from the descriptive statistics, but not from the non-compartmental analysis. For the calculation of PK parameters, concentration values below the LLOQ (BLQ) were replaced by 0, except when such values could be interpolated from two neighboring concentration values. Metabolite to parent ratios (MPRs) were calculated for 11-OH-THC with respect to THC and for 11-COOH-THC with respect to 11-OH-THC using AUC_{last} estimates.

SAMPLE SIZE, RANDOMIZATION AND BLINDING

VAS 'Feeling High' was used for the sample size calculation as this assessment has been shown sensitive to the effects of THC in previous studies, data from which were used to determine variability.[22,23] A sample size of 24 was calculated to have 81.5% power to detect a reduction of 25% of the VAS 'Feeling High', assuming a CV% of 50% (conservative estimate) and using a one sample t-test with a 0.05-sided significance level, presuming a log-normal distribution. To properly balance the cross-over study with 5 treatment arms, a sample size of n=30 (15 males and 15 females) was chosen. Additional details regarding the sample size are provided in the **Supplementary Materials**.

Study staff and subjects remained blinded until database lock. The balanced Williams design randomization code was generated using SAS version 9.4 by a study-independent statistician. 10 sequences were randomized in 3 blocks of 10, with 10 females in one block, 10 males in the second block and 5 males and 5 females in the third block. Blinded study staff assigned the randomization numbers to the participants sequentially after medical screening.

STATISTICAL ANALYSIS

To establish whether significant treatment effects could be detected, the repeatedly measured pharmacodynamic (PD) parameters were analyzed with a mixed effects model with fixed factors treatment, period, time and treatment by time, random factors participant, participant by treatment and participant by time and the average baseline value as covariate. Single measured PD data were compared with a mixed effects model with as fixed factors treatment, period, as random factors participant, and the baseline value as covariate. PK parameters were compared with a mixed effects model with treatment and period as fixed factors and subject as random factor on log-transformed data. Post-dose measurements that were performed outside a 10% time window around the scheduled protocol time were excluded from analysis. 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 square means estimates over time by treatment were presented with 95% confidence intervals as error bars. All calculations were performed using SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA). No adjustments for multiple comparisons were employed in accordance with the exploratory nature of this study.[36]

Results

PARTICIPANTS AND DEMOGRAPHICS

The clinical phase of the trial ran from July 2021 to May 2022. 108 participants were screened and 37 participants were enrolled in the study and dosed at least once. **Table S1** contains a summary of the baseline demographics. Of the 37 dosed participants, 8 withdrew from the study and 3 were excluded prior to completion; 26 participants (15 males and 11 females) completed the trial per protocol. Further details are contained in the study flow diagram (**Figure S2**).

PHARMACODYNAMIC OUTCOMES

Pharmacodynamic measurements of all participants (completers and dropouts) were analyzed. No measurements fell outside the 10% time window around the planned timepoints and had to be excluded from analysis.

Subjective effects

Statistics of the subjective effects are summarized in **Table 1**. VAS 'Alertness' was significantly reduced by THC with 450 mg CBD compared to THC alone (**Figure 1**). VAS 'Mood' was not significantly affected by any treatment. VAS 'Calmness' did not differ significantly between THC alone and any combination of THC and CBD. VAS 'Feeling High', VAS 'Internal perception' and VAS 'External perception' were significantly increased by THC with 450 mg CBD compared to THC alone. State anxiety did not differ significantly between THC alone and any combination of THC and CBD. (**Figure 1**). THC with 450 mg CBD significantly increased the BSI total score compared to THC alone. The statistics of the BSI subscales are provided in the **Table S3**.

Psychomotor and cognitive effects

Statistics of the psychomotor and cognitive effects are summarized in **Table 1**. Postural stability was significantly impaired by THC with 450 mg CBD compared to THC alone (**Figure 1**). Adaptive tracking performance did not differ significantly between THC alone and any combination of THC and CBD (**Figure 1**). Scores on the Stroop task (both Stroop 1 and Stroop 2 parameters) were not significantly affected by any treatment. Reaction time was significantly increased by THC with 450 mg CBD compared to THC alone (**Figure 1**).

Autonomous effects

Statistics of the autonomous effects are summarized in **Table 1**. Heart rate was significantly increased by THC with 450 mg CBD compared to THC alone (**Figure 2**). Serum cortisol and prolactin concentrations did not differ significantly between THC alone and any combination of THC and CBD (**Figure 2**).

Figure 1 Least Square Means of (A) VAS Alertness (displayed as mm change from baseline, (B) VAS Feeling High (absolute values in mm+2), (C) State-Trait Anxiety Inventory state scores, (D) postural stability, (E) Adaptive Tracking performance and (F) reaction time in the Simple Reaction Time Task displayed as % change from baseline. Means are displayed with 95% confidence intervals (for the treatments with the highest and lowest means only; confidence intervals for other treatments omitted for visual clarity).

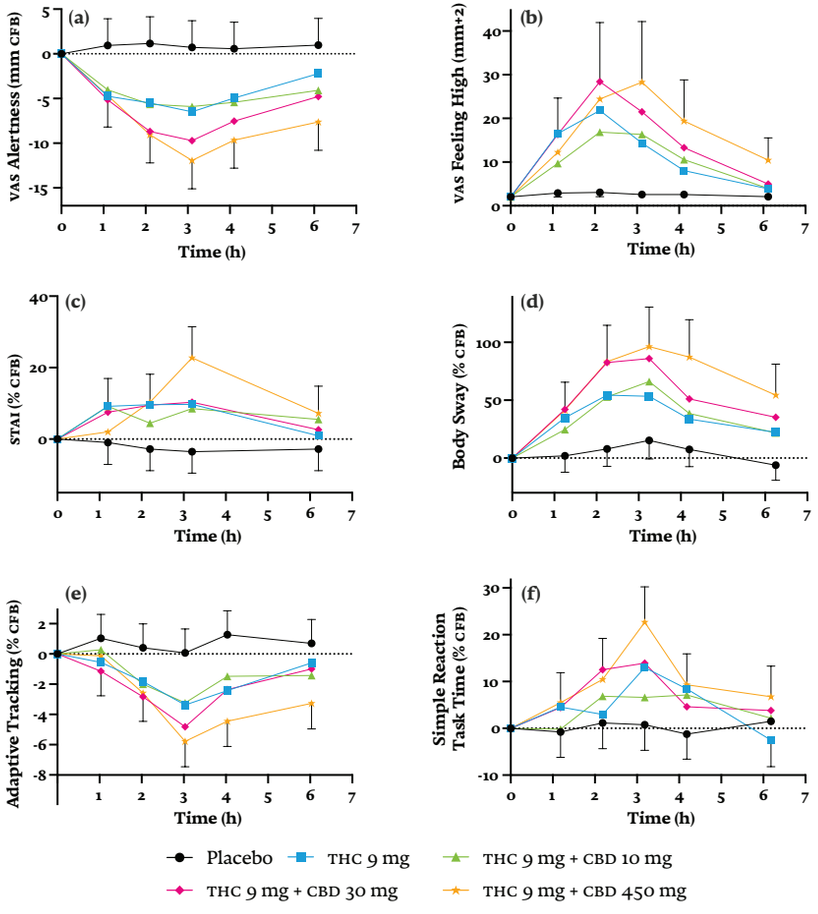


Figure 2 Least Square Means of the autonomous outcome measures. (A) Heart Rate, displayed as beats per minute change from baseline with 95% confidence intervals, (B) Plasma Cortisol concentrations and (C) Plasma Prolactin concentrations, displayed as % change from baseline with 95% confidence intervals (for the treatments with the highest and lowest means only; confidence intervals for other treatments omitted for visual clarity).

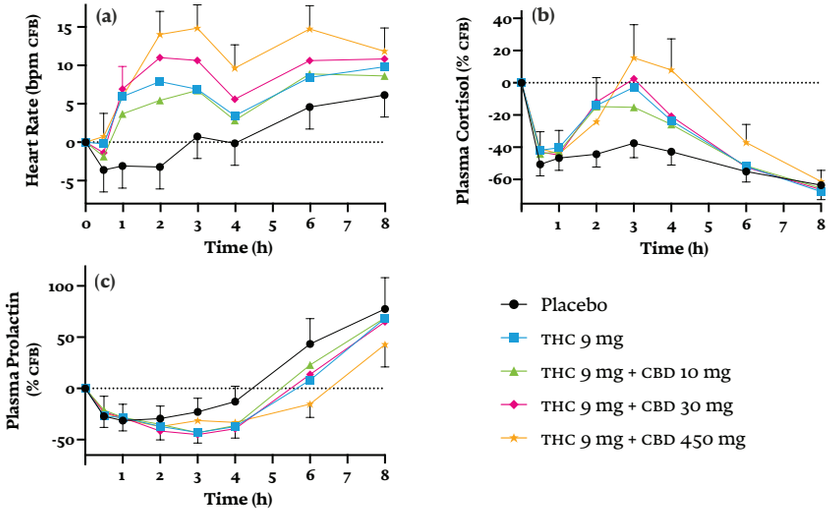


Table 1 Overall treatment effects on subjective, psychomotor, cognitive and autonomous outcome measures (estimated means, estimated mean difference, 95% CI, p-value).

Measurement	Treatment	N	LSM	Estimated difference vs placebo (95% CI), p-value	Estimated difference vs THC 9 mg (95% CI), p-value
SUBJECTIVE EFFECTS					
VAS Alertness (mm+2)	Placebo	32	51.0	-	-
	THC 9 mg	27	45.4	**-5.7 (-8.2, -3.1), p<.0001**	-
	THC + CBD 10 mg	32	45.1	**-5.9 (-8.4, -3.4), p<.0001**	-0.2 (-2.8, 2.3), p=.86
	THC + CBD 30 mg	30	43.0	**-8.0 (-10.5, -5.6), p<.0001**	-2.4 (-5.0, 0.2), p=.067
	THC + CBD 450 mg	30	41.6	**-9.5 (-11.9, -7.0), p<.0001**	**-3.8 (-6.4, -1.2), p<.01**
VAS Mood (mm+2)	Placebo	32	53.7	-	-
	THC 9 mg	27	53.6	-0.2 (-2.5, 2.2), p=.88	-
	THC + CBD 10 mg	32	52.9	-0.9 (-3.2, 1.4), p=.45	-0.7 (-3.1, 1.7), p=.56
	THC + CBD 30 mg	30	53.5	-0.2 (-2.5, 2.1), p=.87	-0.0 (-2.4, 2.4), p=.98
	THC + CBD 450 mg	30	52.5	-1.2 (-3.6, 1.1), p=.29	-1.1 (-3.5, 1.3), p=.38
VAS Calmness (mm+2)	Placebo	32	53.8	-	-
	THC 9 mg	27	56.7	**2.8 (0.1, 5.6), p=.045**	-
	THC + CBD 10 mg	32	57.1	**3.2 (0.5, 5.9), p=.02**	0.4 (-2.4, 3.2), p=.79
	THC + CBD 30 mg	30	59.1	**5.2 (2.5, 8.0), p<.001**	2.4 (-0.4, 5.2), p=.098
	THC + CBD 450 mg	30	57.2	**3.4 (0.6, 6.1), p=.02**	0.5 (-2.3, 3.4), p=.71
VAS Feeling High (mm+2)	Placebo	32	2.6	-	-
	THC 9 mg	27	11.0	**321.7% (199.2%, 494.3%), p<.0001**	-
	THC + CBD 10 mg	32	10.2	**291.7% (180.6%, 446.8%), p<.0001**	-7.1% (-34.4%, 31.6%), p=.68
	THC + CBD 30 mg	30	14.6	**459.3% (298.5%, 684.9%), p<.0001**	32.6% (-6.7%, 88.5%), p=.11
	THC + CBD 450 mg	30	17.7	**576.8% (382.0%, 850.3%), p<.0001**	**60.5% (12.7%, 128.5%), p<.01**
VAS Internal Perception (Log(mm+2))	Placebo	32	0.34	-	-
	THC 9 mg	27	0.42	0.08 (0.0004, 0.16), p=.049	-
	THC + CBD 10 mg	32	0.39	0.05 (-0.03, 0.13), p=.20	-0.03 (-0.11, 0.05), p=.47
	THC + CBD 30 mg	30	0.42	0.08 (-0.003, 0.15), p=.058	-0.003 (-0.08, 0.08), p=.94
	THC + CBD 450 mg	30	0.52	**0.18 (0.11, 0.26), p<.0001**	**0.11 (0.02, 0.19), p=.01**
VAS External perception (Log(mm+2))	Placebo	32	0.33	-	-
	THC 9 mg	27	0.58	**0.25 (0.15, 0.35), p<.0001**	-
	THC + CBD 10 mg	32	0.55	**0.22 (0.12, 0.31), p<.0001**	-0.03 (-0.13, 0.07), p=.54
	THC + CBD 30 mg	30	0.64	**0.31 (0.21, 0.41), p<.0001**	0.06 (-0.04, 0.16), p=.26
	THC + CBD 450 mg	30	0.74	**0.40 (0.31, 0.50), p<0.0001**	**0.15 (0.05, 0.26), p<.01**
STAI	Placebo	32	27.1	-	-
	THC 9 mg	27	29.8	**10.1% (4.1%, 16.3%), p<.001**	-
	THC + CBD 10 mg	32	29.7	**9.7% (3.9%, 15.7%), p=.001**	-0.4% (-5.8%, 5.4%), p=.90
	THC + CBD 30 mg	30	29.8	**10.2% (4.3%, 16.4%), p<.001**	0.1% (-5.4%, 6.0%), p=.96
	THC + CBD 450 mg	30	30.6	**13.2% (7.1%, 19.5%), p<.0001**	2.8% (-2.9%, 8.8%), p=.34
BSI Total	Placebo	32	1.8	-	-
	THC 9 mg	27	8.5	**6.8 (2.0, 11.5), p<.01**	-
	THC + CBD 10 mg	32	8.1	**6.3 (1.7, 10.9), p<.01**	-0.5 (-5.2, 4.3), p=.84
	THC + CBD 30 mg	30	12.1	**10.3 (5.6, 15.1), p<.0001**	3.6 (-1.3, 8.4), p=.15
	THC + CBD 450 mg	30	16.1	**14.3 (9.6, 19.1), p<.0001**	**7.6 (2.7, 12.5), p<.01**
PSYCHOMOTOR AND COGNITIVE EFFECTS					
Body Sway (mm)	Placebo	32	243.2	-	-
	THC 9 mg	27	322.1	**32.5% (16.1%, 51.1%) p<.0001**	-
	THC + CBD 10 mg	32	323.5	**33.0% (17.1%, 51.1%), p<.0001**	0.4% (-12.1%, 14.7%), p=.95
	THC + CBD 30 mg	30	365.8	**50.4% (32.2%, 71.1%), p<.0001**	13.6% (-0.7%, 29.9%), p=.06
	THC + CBD 450 mg	30	396.3	**62.9% (43.1%, 85.5%), p<.0001**	**23.0% (7.4%, 40.9%), p<.01**

(Continuation Table 1)

Measurement	Treatment	N	LSM	Estimated difference vs placebo (95% CI), p-value	Estimated difference vs THC 9 mg (95% CI), p-value
Adaptive tracking (%)	Placebo	32	31.7	-	-
	THC 9 mg	27	29.3	**-2.5 (-3.9, -1.0), p<.01**	-
	THC + CBD 10 mg	32	29.4	**-2.3 (-3.7, -0.8), p<.01**	0.2 (-1.3, 1.7), p=.81
	THC + CBD 30 mg	30	28.6	**-3.1 (-4.6, -1.7), p<.0001**	-0.7 (-2.2, 0.8), p=.38
	THC + CBD 450 mg	30	27.8	**-3.9 (-5.4, -2.5), p<.0001**	-1.5 (-3.0, 0.04), p=.06
Stroop 1 (ms)	Placebo	32	84.9	-	-
	THC 9 mg	27	92.7	7.8 (-13.7, 29.2), p=.47	-
	THC + CBD 10 mg	32	93.5	8.6 (-12.2, 29.4), p=.42	0.8 (-20.8, 22.5), p=.94
	THC + CBD 30 mg	30	81.8	-3.2 (-24.1, 17.7), p=.76	-10.9 (-32.6, 10.7), p=.32
	THC + CBD 450 mg	30	91.3	6.4 (-14.6, 27.3), p=.55	-1.4 (-23.2, 20.4), p=.90
Stroop 2	Placebo	32	0.3	-	-
	THC 9 mg	27	0.4	0.1 (-0.2, 0.4), p=0.40	-
	THC + CBD 10 mg	32	0.2	-0.1 (-0.4, 0.2), p=0.44	-0.3 (-0.6, 0.1), p=0.12
	THC + CBD 30 mg	30	0.2	-0.1 (-0.4, 0.2), p=0.56	-0.2 (-0.5, 0.1), p=0.17
	THC + CBD 450 mg	30	0.2	-0.1 (-0.4, 0.3), p=0.67	-0.2 (-0.5, 0.1), p=0.23
Simple Reaction Time Test (msec)	Placebo	32	237.4	-	-
	THC 9 mg	27	248.9	4.9% (0.2%, 9.7%), p=.04	-
	THC + CBD 10 mg	32	247.3	4.2% (-0.3%, 8.8%), p=.07	-0.6% (-5.1% 4.0%), p=.78
	THC + CBD 30 mg	30	255.1	**7.5% (2.8%, 12.3%), p<.01**	2.5% (-2.1% 7.3%), p=.29
	THC + CBD 450 mg	30	262.2	**10.5% (5.6%, 15.5%), p<.0001**	**5.3% (0.6% 10.3%), p=.03**
AUTONOMOUS EFFECTS					
Heart Rate (bpm)	Placebo	32	60.2	-	-
	THC 9 mg	27	66.0	**5.8 (3.4, 8.3), p<.0001**	-
	THC + CBD 10 mg	32	64.9	**4.7 (2.3, 7.1), p<.001**	-1.1 (-3.6, 1.4), p=.37
	THC + CBD 30 mg	30	67.7	**7.5 (5.1, 10.0), p<.0001**	1.7 (-0.8, 4.2), p=.18
	THC + CBD 450 mg	30	70.8	**10.0 (7.6, 12.5), p<.0001**	**4.2 (1.7, 6.8), p=.001**
Cortisol (nmol/L)	Placebo	32	168.3	-	-
	THC 9 mg	27	205.4	**22.0% (7.8%, 38.2%), p=.002**	-
	THC + CBD 10 mg	32	200.0	**18.8% (5.2%, 34.1%), p=.006**	-2.7% (-14.1%, 10.3%), p=.67
	THC + CBD 30 mg	30	206.5	**22.7% (8.6%, 38.6%), p=.001**	0.5% (-11.4%, 14.0%), p=.93
	THC + CBD 450 mg	30	229.9	**36.6% (20.8%, 54.3%), p<.0001**	**11.9% (-1.5%, 27.1%), p=.08**
Prolactin (µg/L)	Placebo	32	10.4	-	-
	THC 9 mg	27	9.0	**-13.7% (-22.9%, -3.6%), p=.01**	-
	THC + CBD 10 mg	32	9.3	**-10.6% (-19.8%, -0.2%), p<.05**	3.7% (-7.5%, 16.2%), p=.53
	THC + CBD 30 mg	30	8.9	**-14.5% (-23.4%, -4.5%), p<.01**	-0.9% (-11.6%, 11.1%), p=.88
	THC + CBD 450 mg	30	8.8	**-15.4% (-24.3%, -5.6%), p<.01**	-2.0% (-12.6%, 9.9%), p=.73

The figures in bold indicate statistical significance. Abbreviations: CBD, cannabidiol; CI, confidence interval; bpm, beat per minute; BSI, brief symptom inventory; LSM, least square mean; N, number; STAI, state-trait anxiety inventory; THC, Δ^9-tetrahydrocannabinol; VAS, visual analogue scale.

Analgesic effects

Statistics of the SF-MPQ VAS scores, pain tolerance thresholds and the area of secondary allodynia are summarized in **Table 2**, statistics of the SF-MPQ affective, sensory and PPI scores in **Table S4**, and statistics of the pain detection thresholds in **Table S5**.

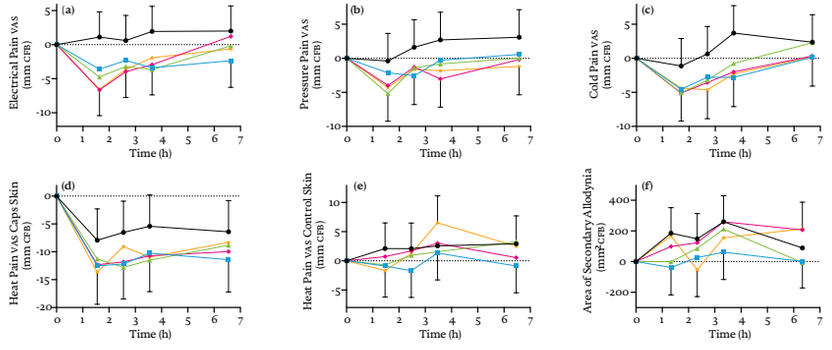
Area of secondary allodynia was significantly reduced by THC alone compared to placebo. Area of secondary allodynia was not significantly reduced by any CBD-containing treatment compared to placebo, and was significantly increased by THC with 30 mg CBD, compared to THC alone (**Figure 3**). The SF-MPQ VAS was significantly reduced by all THC-containing treatments compared to placebo following the electrical pain, pressure pain, cold pain and heat pain (on capsaicin-treated skin) tests, except THC alone on pressure pain and THC with 10 mg CBD on cold pain (**Figure 3**). SF-MPQ VAS following the heat pain test on control skin was significantly reduced by THC alone

compared to placebo (**Figure 3**). SF-MPQ affective and sensory scores were not significantly reduced compared to placebo by any treatment, except for the THC with 30 mg CBD following the electrical pain test (**Table S4**).

SF-MPQ PPI scores were significantly reduced compared to placebo by some, but not all THC-containing treatments following the electrical pain, cold pain and heat pain (on capsaicin-treated skin) tests (**Table S4**).

PDTs for electrical pain, pressure pain, cold pain or heat pain (capsaicin-treated skin and control skin) were not increased significantly by any of the study treatments (**Table S5**). PTTs for electrical pain, pressure pain or cold pain were not increased significantly by any of the study treatments. Conversely, the electrical PTT was significantly reduced by all combinations of THC and CBD compared to placebo. The pressure PTT was reduced significantly by THC alone, as well as THC with 30 and 450 mg CBD compared to placebo, and further reduced significantly by THC **with 450 mg** CBD compared to THC alone (**Figure 3**). The cold PTT was significantly reduced by THC with 10 mg CBD and THC with 450 mg CBD both compared to placebo and compared to THC alone (**Figure 3**).

Figure 3 Least Square Means of selected measurements of the nociceptive test battery. Short-Form McGill Pain Questionnaire (A) VAS Electrical Pain, (B) VAS Pressure Pain, (C) VAS Cold Pain, (D) VAS Heat Pain on capsaicin-treated skin and (E) VAS Heat Pain on control skin, displayed as mm change from baseline with 95% confidence intervals. (F) Area of Secondary Allodynia, displayed as mm² change from baseline with 95% confidence intervals. (G) Electrical Pain Tolerance Threshold, (H) Pressure Pain Tolerance Threshold, (I) Cold Pain Tolerance Threshold, displayed as % change from baseline with 95% confidence intervals (for the treatments with the highest and lowest means only; confidence intervals for other treatments omitted for visual clarity).



(Continuation Figure 3)

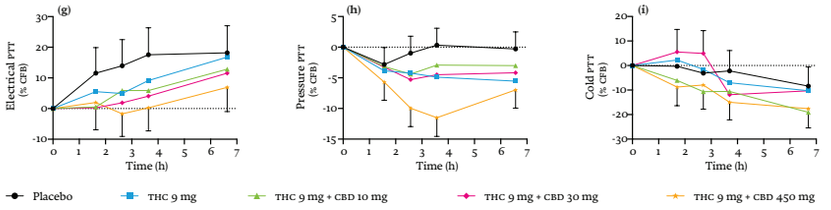


Table 2 Overall treatment effects on analgesic outcome measures (estimated means, estimated mean difference, 95% CI, p-value).

Measurement	Treatment	N	LSM	Estimated difference vs placebo (95% CI), p-value	Estimated difference vs THC 9 mg (95% CI), p-value
AREA OF SECONDARY ALLODYNIA					
Area of secondary Allodynia (mm²)	Placebo	32	745.6	-	-
	THC 9 mg	27	587.6	**-158.0 (-313.0, -3.1), p=.046**	-
	THC + CBD 10 mg	32	647.6	-98.1 (-245.1, 49.0), p=.19	60.0 (-94.6, 214.5), p=.44
	THC + CBD 30 mg	30	747.1	1.4 (-148.7, 151.6), p=.98	**159.5 (3.2, 315.7) p=.046**
	THC + CBD 450 mg	30	695.6	-50.1 (-201.2, 101.1), p=.51	108.0 (-49.9, 265.9), p=.18
SF-MPQ PEAK PAIN INTENSITY VAS SCORES					
SF-MPQ VAS Electrical Pain (mm)	Placebo	32	47.2	-	-
	THC 9 mg	27	42.9	**-4.34 (-7.30, -1.37), p<.01**	-
	THC + CBD 10 mg	32	42.8	**-4.37 (-7.27, -1.47), p<.01**	-0.03 (-3.03, 2.97), p=.99
	THC + CBD 30 mg	30	42.7	**-4.50 (-7.43, -1.57), p<.01**	-0.16 (-3.18, 2.86), p=.92
	THC + CBD 450 mg	30	42.6	**-4.61 (-7.56, -1.65), p<.01**	-0.27 (-3.32, 2.78), p=.86
SF-MPQ VAS Pressure Pain (mm)	Placebo	32	40.1	-	-
	THC 9 mg	27	37.3	-2.9 (-6.1, 0.4), p=.09	-
	THC + CBD 10 mg	32	36.5	**-3.6 (-6.8, -0.3), p=.03**	-0.7 (-4.0, 2.6), p=.66
	THC + CBD 30 mg	30	36.2	**-3.9 (-7.1, -0.7), p=.02**	-1.0 (-4.3, 2.3), p=.54
	THC + CBD 450 mg	30	36.1	**-4.0 (-7.2, -0.7), p=.02**	-1.1 (-4.5, 2.2), p=.50

(Continuation Table 1)

Measurement	Treatment	N	LSM	Estimated difference vs placebo (95% CI), p-value	Estimated difference vs THC 9 mg (95% CI), p-value
SF-MPQ VAS Cold Pain (mm)	Placebo	32	49.6	-	-
	THC 9 mg	27	45.7	**-3.9 (-7.1, -0.7), p=.02**	-
	THC + CBD 10 mg	32	46.5	-3.1 (-6.2, 0.03), p=.052	0.8 (-2.4, 4.0), p=.62
	THC + CBD 30 mg	30	45.6	**-4.0 (-7.1, -0.8), p=.01**	-0.1 (-3.3, 3.1), p=.96
	THC + CBD 450 mg	30	45.4	**-4.2 (-7.3, -1.0), p=.01**	-0.3 (-3.6, 3.0), p=.86
SF-MPQ VAS Heat Pain (capsaicin skin) (mm)	Placebo	32	35.6	-	-
	THC 9 mg	27	30.6	**-5.0 (-8.5 -1.6), p<.01**	-
	THC + CBD 10 mg	32	31.1	**-4.5 (-7.9, -1.2), p<.01**	0.5 (-3.0, 4.0), p=.78
	THC + CBD 30 mg	30	31.0	**-4.6 (-8.0, -1.2), p<.01**	0.4 (-3.1, 3.9), p=.83
	THC + CBD 450 mg	30	31.7	**-3.9 (-7.4, -0.5), p=.03**	1.1 (-2.4, 4.6), p=.54
SF-MPQ VAS Heat Pain |(control skin) (mm)	Placebo	32	24.5	-	-
	THC 9 mg	27	21.5	**-2.9 (-5.8, -0.1), p=.04**	-
	THC + CBD 10 mg	32	23.3	-1.2 (-3.9, 1.6), p=.41	1.8 (-1.0, 4.6), p=.21
	THC + CBD 30 mg	30	23.6	-0.9 (-3.7, 1.8), p=.51	2.0 (-0.8, 4.9), p=.16
	THC + CBD 450 mg	30	24.2	-0.3 (-3.1, 2.5), p=.85	2.7 (-0.2, 5.6), p=.07
PAIN TOLERANCE THRESHOLDS					
Electrical Stair Pain Tolerance Threshold (mA)	Placebo	32	17.2	-	-
	THC 9 mg	27	16.2	-5.4% (-12.8%, 2.5%), p=.17	-
	THC + CBD 10 mg	32	15.8	**-7.9% (-14.8%, -0.4%), p=.04**	-2.6% (-10.2%, 5.7%), p=.53
	THC + CBD 30 mg	30	15.5	**-9.5% (-16.3%, -2.1%), p=.01**	-4.3% (-11.8%, 3.9%), p=.29
	THC + CBD 450 mg	30	15.2	**-11.7% (-18.5%, -4.3%), p<.01**	-6.6% (-14.0%, 1.5%), p=.11
Pressure Pain Tolerance Threshold (kPa)	Placebo	32	42.3	-	-
	THC 9 mg	27	38.6	**-3.7 (-6.6, -0.8), p=.01**	-
	THC + CBD 10 mg	32	39.8	-2.4 (-5.2, 0.3), p=.08	1.3 (-1.6, 4.2), p=.39
	THC + CBD 30 mg	30	38.9	**-3.4 (-6.2, -0.6), p=.02**	0.3 (-2.6, 3.2), p=.83
	THC + CBD 450 mg	30	34.7	**-7.6 (-10.4, -4.8), p<.0001**	**-3.9 (-6.9, -0.9), p=.01**
Cold Pain Tolerance Threshold (s)	Placebo	32	16.5	-	-
	THC 9 mg	27	16.3	-0.8% (-8.4%, 7.5%), p=.85	-
	THC + CBD 10 mg	32	15.1	**-8.5% (-15.3%, -1.1%), p=.03**	**-7.8% (-14.9%, -0.0%), p<.05**
	THC + CBD 30 mg	30	16.5	0.3% (-7.3%, 8.4%), p=.95	1.0% (-6.8%, 9.6%), p=.80
	THC + CBD 450 mg	30	14.9	**-9.2% (-16.1%, -1.8%), p=.02**	**-8.5% (-15.7%, -0.7%), p=.03**

The figures in bold indicate statistical significance. Abbreviations: CBD, cannabidiol; CI, confidence interval; LSM, least square mean; N, number; SF-MPQ, Short-Form McGill Pain Questionnaire; THC, Δ^9-tetrahydrocannabinol; VAS, visual analogue scale.

PHARMACOKINETICS

Pharmacokinetic parameters of THC, 11-OH-THC, 11-COOH-THC, and CBD are summarized in **Tables S6-9**, concentration-time profiles are displayed in **Figure 4** (linear y-axis), as well as **Figures S10-13** (logarithmic y-axis) and statistical comparisons of PK parameters between treatments are provided in **Table 3**. Administration of 450 mg CBD significantly increased the AUC_{last} of THC, 11-OH-THC and 11-COOH-THC, as well as the C_{max} of 11-OH-THC and 11-COOH-THC, and significantly increased the metabolite to parent ratio for both THC metabolites, when compared to THC alone. Administration of CBD 30 mg significantly increased the AUC_{last} of THC, 11-OH-THC and 11-COOH-THC, the C_{max} of 11-OH-THC and 11-COOH-THC, and significantly changed the metabolite to parent ratio for 11-COOH-THC compared to administration of THC alone. The 10 mg CBD dose did not significantly change any pharmacokinetic parameters compared to THC alone. The pharmacokinetic parameters and concentration-time profiles of CBD metabolites are displayed in **Tables/Figures S14-S26**. Number and percentage of BLQ samples per analyte are provided in **Table S27**.

Figure 4 Concentration-time profiles of (A) THC, (B) 11-OH-THC, (C) 11-COOH-THC and (D) CBD following oral administration, displayed as means with standard deviation.

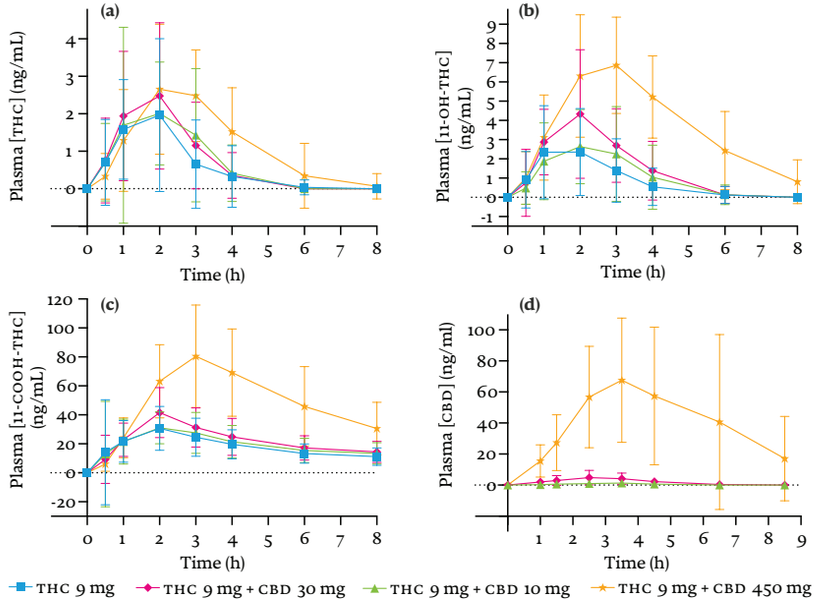


Table 3 Overall treatment effects on pharmacokinetic parameters (estimated means, estimated mean ratios, 95% CI, p-value).

Parameter	Treatment	N	LSM	Estimated LSM ratio vs THC 9 mg (95% CI), p-value
THC				
AUC_{last}	THC 9 mg	25	3.26	-
	THC + CBD 10 mg	30	3.03	0.93 (0.66, 1.32), p=0.68
	THC + CBD 30 mg	27	4.68	**1.44 (1.01, 2.04), p=0.04**
	THC + CBD 450 mg	28	7.09	**2.18 (1.54, 3.08), p<.0001**
C_{max}	THC 9 mg	25	2.63	-
	THC + CBD 10 mg	30	2.50	0.95 (0.76, 1.19), p=0.65
	THC + CBD 30 mg	27	2.64	1.00 (0.80, 1.26), p=0.99
	THC + CBD 450 mg	28	3.25	1.23 (0.98, 1.55), p=0.07
11-OH-THC				
AUC_{last}	THC 9 mg	25	4.00	-
	THC + CBD 10 mg	29	4.27	1.07 (0.73, 1.56), p=0.73
	THC + CBD 30 mg	28	7.58	**1.89 (1.30, 2.77), p=0.0013**
	THC + CBD 450 mg	28	24.95	**6.24 (4.27, 9.12), p<.0001**
C_{max}	THC 9 mg	25	3.18	-
	THC + CBD 10 mg	29	3.23	1.02 (0.87, 1.18), p=0.85
	THC + CBD 30 mg	28	4.64	**1.46 (1.25, 1.70), p<.0001**
	THC + CBD 450 mg	28	7.52	**2.36 (2.02, 2.75), p<.0001**
MPR	THC 9 mg	25	1.12	-
	THC + CBD 10 mg	29	1.21	1.08 (0.72, 1.63), p=0.71
	THC + CBD 30 mg	28	1.67	1.49 (0.99, 2.24), p=0.06
	THC + CBD 450 mg	28	3.24	**2.88 (1.93, 4.31), p<.0001**
11-COOH-THC				
AUC_{last}	THC 9 mg	27	127.86	-
	THC + CBD 10 mg	31	142.22	1.11 (1.00, 1.24), p=0.06
	THC + CBD 30 mg	29	162.69	**1.27 (1.14, 1.42), p<.0001**
	THC + CBD 450 mg	28	355.41	**2.78 (2.49, 3.10), p<.0001**
C_{max}	THC 9 mg	27	31.46	-
	THC + CBD 10 mg	31	35.48	1.13 (0.95, 1.33), p=0.15
	THC + CBD 30 mg	29	38.60	**1.23 (1.04, 1.45), p=0.02**
	THC + CBD 450 mg	28	80.60	**2.56 (2.17, 3.03), p<.0001**
MPR	THC 9 mg		32.51	-
	THC + CBD 10 mg		33.48	1.03 (0.73, 1.45), p=0.87
	THC + CBD 30 mg		19.31	**0.59 (0.42, 0.84), p=0.004**
	THC + CBD 450 mg		13.38	**0.43 (0.30, 0.60), p<.0001**

The figures in bold indicate statistical significance. Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; CBD, cannabidiol; CI, confidence interval; C_{max}, maximum concentration; LSM, least square mean; MPR, metabolite to parent ratio; N, number; THC, Δ^9-tetrahydrocannabinol.

Discussion

In this study, in contrast to what is commonly hypothesized in (popular) literature, CBD did not reduce the adverse effects of THC, and CBD did not enhance the analgesic properties of THC. While the lower doses of 10 and 30 mg of CBD did not significantly influence the subjective (including anxiety), psychomotor, cognitive, or autonomous effects of 9 mg THC, the high dose of 450 mg CBD significantly increased THC effects on most measures. The enhanced THC effects were accompanied, and most plausibly explained by significantly elevated plasma concentrations of THC and its psychoactive metabolite 11-OH-THC.

A cytochrome P450-mediated drug-drug interaction with CBD as perpetrator drug and THC as victim drug appears the most likely explanation for the pharmacokinetic findings of this study. CBD has been shown to inhibit CYP3A4-, CYP2C9-, CYP2CB6-, and CYP2C19-mediated metabolism in vitro and[37–40] in humans,[41] and CYP2C9 is the major enzyme responsible for metabolism of THC.[42] The significant changes in THC metabolite to parent ratios observed in this study support the presence of a cytochrome P450-mediated drug-drug interaction. Furthermore, pharmacokinetic interactions have been reported in two recent clinical trials, where CBD was co-administered with THC[43] or with a cytochrome P450 drug cocktail.[41]

Previous studies reporting pharmacokinetic interactions with CBD either administered 640 mg CBD[41,43] or took place in the context of treatment of rare epilepsy syndromes, where daily doses up to 50 mg/kg were administered.[44–46] Most patient populations or recreational cannabis users are unlikely to use CBD in such high doses. Our results, however, show that pharmacokinetic drug interactions could be caused by CBD doses as low as 30 mg, which are easily available to consumers in the United States as CBD-containing gummies, oils and tinctures, and other oral formulations referred to as edibles. In fact, some online retailers of such products recommend a starting dose of 20-30 mg CBD,[47] potentially putting consumers at risk for drug interactions. Theoretically, recreational cannabis use could similarly result in sufficient CBD intake to influence CYP450 metabolism, although the risk would depend on the CBD content of the cannabis variety, and the amount of cannabis consumed, both of which are highly variable.

Although the simplest and most obvious explanation for our study results is a PK interaction between oral CBD and THC in absence of a PD interaction, the study design cannot conclusively rule out the presence of a PD

interaction that is distinct from the PK interaction. Such hypothetical PD interaction could either be negative, meaning CBD *reduced* THC effects (as was hypothesized prior to the study) or positive, meaning CBD *increased* THC effects. Both cases contradict the use of CBD to attenuate THC effects. If the negative interaction was present, then its magnitude must have been relatively small, as it was clearly overshadowed by the increased psychotropic effects of increased THC and metabolite exposures. If the positive interaction was present, then the THC effects would be in effect enhanced by *both* PK and PD interactions. What is certain then, is that CBD is not useful for attenuation of adverse THC effects when administered orally.

It is possible that the findings of this study are specific to the oral administration route, and findings in studies with other administration routes differ.[9,48] To our knowledge, studies with inhaled cannabinoids have not reported PK interactions between CBD and THC, nor increases in THC effects when co-administered with CBD. Possibly, the difference in findings is due to the substantial formation of the active metabolite 11-OH-THC following oral administration of THC,[49] and further elevation of 11-OH-THC levels via CBD-induced CYP inhibition. In contrast, comparatively little 11-OH-THC is formed following THC inhalation.[50] Nevertheless, similar to oral dosing studies, inhalation studies have not yet produced convincing evidence of CBD attenuating THC effects.[9,48,51] Regardless of the administration route, the hypothesis that CBD attenuates THC effects remains contentious, and our results add to a growing body of evidence against it. Besides, alternative explanations have emerged for the purported superior long-term safety of CBD-rich cannabis. For example, CBD-rich cannabis varieties could cause fewer long-term side effects simply by virtue of containing smaller absolute amounts of THC, rather than due to a pharmacological interaction.[48]

A key strength of this study was the wide, and pharmacologically relevant, dose range of CBD administered. The pharmaceutical drug formulations in this study contained low levels of impurities, minimizing the risk of other cannabis constituents biasing the study results. The extensive set of both subjective and objective validated CNS tests, the use of a validated pain test battery and a dense PK sampling schedule around the t_{max} resulted in detailed assessment of oral THC/CBD interaction effects over time, both at the level of PK and PD, and the cross-over design allowed for within-participant comparison of effects.

Our study is not without limitations. A larger sample size may have confirmed the presence of increased THC effects at the 30 mg CBD dose level – a possibility which appears plausible due to the confirmed presence of the PK interaction and the consistent, although not statistically significant increases across multiple measures of THC effects at the 30 mg CBD dose level. The administration of CBD 30 minutes prior to THC, while done to align the t_{max} of the study drugs, may have enhanced the PK interaction compared to simultaneous administration, as CYP450 inhibition is a time-dependent process. However, in all likelihood the staggered administration was not a decisive factor in the study outcomes, as simultaneous administration studies have led to similar conclusions.[43] Also, because CBD is a time-dependent inhibitor of many CYPs,[38,39,41] the interaction may be more profound in chronic administration compared to single doses administered in this study. Another limitation is that no CBD-only cross-over arms were included. This could obscure the distinction between 'pure' CBD effects and THC/CBD interaction effects. However, it is highly likely that the observed increase in THC effects is explained by a THC/CBD interaction, rather than PD effects of CBD alone, since CBD is not known to cause psychotropic effects on its own.[52] Furthermore, a relatively high proportion of the study participants dropped out of the study due to adverse effects or the study being too burdensome, which may have introduced a selection bias towards participants who are less sensitive to adverse effects of THC. The drop-outs were disproportionately female; although sex differences in sensitivity to THC effects have been described previously,[53] more research is needed on the differences in THC effects between sexes.

A substantial proportion of plasma concentration values for THC and 11-OH-THC fell below the limit of quantification in this study. As BLQ values were replaced by '0' when calculating pharmacokinetic parameters, resulting AUC_{last}, estimates are likely to be lower, than if bioanalytical method with lower quantification limits had been used. The exact magnitude of this effect is unknown, but we can deduce that it must have differed between the treatments in this study. The underestimation of the AUC_{last} will be greater when THC was administered alone, as this treatment had the lowest THC and metabolite exposures and the highest proportion of BLQ values – and for the opposite reasons, will be smaller when 450 mg CBD was co-administered. Therefore, some degree of overestimation of the point estimate of the

AUC_{last} ratios between treatments will have occurred – although it can be assumed to have been limited. Per definition, plasma concentrations reported as BLQ are relatively low, and therefore would contribute relatively little to AUC estimate. Most importantly, the presence of a pharmacokinetic drug interaction in this study is not under question. First, a decreasing proportion of BLQ values at an increasing CBD dose is in itself an indication of an increase in concentration and therefore a pharmacokinetic interaction. And second, the drug interaction is also evident from the significant treatment effects on the mean 11-OH-THC C_{max}, a pharmacokinetic parameter which is not meaningfully impacted by varying proportions of BLQ values between treatments.

This is the first study to evaluate analgesic effects of a wide dose range of CBD when co-administered with THC. We found relatively small, but significant analgesic effects on the peak pain intensity VAS scores of the McGill Pain Questionnaire following the pressure, cold, and electrical pain tests, and after the heat test on capsaicin-sensitized skin, which occurred to a similar extent following all THC-containing treatments, regardless of CBD dose. This points to THC, and not CBD, being the cannabinoid responsible for the analgesia, and to the analgesia not being linearly dependent on plasma THC concentrations, since the magnitude of the analgesia across treatments was similar despite varying THC and 11-OH-THC concentrations. On the other hand, we did not find any analgesic effects on nociceptive thresholds for any treatment and regardless of presence and dose of CBD. In fact, we occasionally found THC-containing treatments had small, but significant *hyperalgesic* effects on cold pressor, pressure and electrical pain tolerance thresholds. These findings are consistent with previous observations by our group and by others[22,54] that THC can paradoxically decrease nociceptive thresholds.

The absence of THC analgesia on nociceptive thresholds in our study should not be interpreted as contradictory to earlier evidence of efficacy in patient populations.[2] Pain is a complex subjective phenomenon which, in addition to nociception, also involves cognitive and affective components, and in case of neuropathic pain, additional neurological pathology like central sensitisation.[55] Therefore, results obtained with evoked pain tests in healthy volunteers do not lend themselves to a straightforward translation to patients. Rather, our findings provide insights into the mechanisms of cannabinoid-induced analgesia. The absence of THC analgesia on nociceptive thresholds, a measure obtained *during* the administration of the painful stimulus, combined with clear analgetic effects when pain scores were measured shortly *after* the stimulus, suggests that THC exerts its analgesic effects at the level of pain experience or pain memory, rather than at the level of nociception. This aligns with previous research suggesting that THC may target preferentially the affective qualities of pain, e.g. via dissociative effects resulting from reduces sensory-limbic functional connectivity.[56] Furthermore, we found THC to reduce mechanical allodynia, which is a prominent symptom of many neuropathic pain syndromes.[55] This finding may partially explain how THC exerts its analgesic effects in patients with neuropathic pain.[2]

In conclusion, in this study CBD did not reduce the (adverse) effects of THC, but rather increased them at higher doses, likely by way of a pronounced pharmacokinetic interaction, while not enhancing the analgesic effects of THC. In a future study we aim to learn more about the potential phenotypical differences between neuropathic pain patients who respond to cannabinoid-induced analgesia versus patients for whom cannabinoid-based treatments do not work well.

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CHAPTER 2: SUPPLEMENTARY MATERIALS

Full supplementary materials available at the publisher's website:
https://tinyurl.com/4jmssfhd https://tinyurl.com/265upkx3

Selected supplementary text, figures and tables are provided below.

DOSE RATIONALE

The dose of THC was chosen based on data and experience from previous trials conducted at our unit, CHDR. An oral dose of 9 mg THC is expected to be significantly psychoactive in most study participants (see power calculation), while being sufficiently low as to have a minimal risk in causing unpleasant adverse effects like nausea and vomiting. Symptoms like paranoia and psychotic effects are not expected at this dose level, in combination with the in/exclusion criteria applied for this study.

We hypothesized that a modulating effect of CBD on the psychotropic effects of THC is possible via three distinct pathways:

Negative allosteric modulation (NAM) of the CB_1 receptor by CBD. In the in vitro study published by Laprairie et al.,[1] approximately 50% of the maximal NAM effect was achieved at a THC:CBD ratio of 2:1, and a near maximal NAM effect was observed at THC:CBD ratios of 1:1.5 to 1:2. Thus, we aimed to achieve similar ratios at target site in humans. The bioavailability of CBD and THC are known to be similar,[2] and for simplicity, we assumed absorption speed and distribution to target tissues to be identical for both compounds. However, a correction was made to account for the metabolism of THC to the active metabolite 11-OH-THC. It is known that 11-OH-THC is formed in a 0.6-0.8:1 THC:11-OH-THC ratio after oral administration (for simplicity, rounded off to 1:1 THC:11-OH-THC ratio). The exact potency of 11-OH-THC is not known,[3] and we assumed equipotency with THC. The presence of the equipotent 11-OH-THC in a ~1:1 ratio to THC would effectively double the CB_1-agonistic activity, and to correct for this, the THC amount in the human dose ratios was adjusted to 50% of that in the desired target tissue ratios:

NAM effect	THC:CBD target tissue ratio	THC:CBD corrected for 11-OH-THC (human dose ratio)	THC:CBD human dose
~50%	2:1	1:1	9 mg : 10 mg
Near maximal	1:1.5	1:3	9 mg : 30 mg

These dose ratios are reflected in the selected doses of 9 mg THC with 10 mg CBD for the low dose and 30 mg CBD for the middle dose. These ratios would cover the potential NAM effect of the CB_1 receptor by THC. However, in practice, most clinical studies investigating the effect of CBD on THC-induced effects have not reported any changes on the most CB_1-specific effect: feeling high. According to a review by Freeman et al.,[4] only two studies reported changes on high or stoned, whereas more studies reported changes on feelings of anxiety (see next bullet) or cognitive changes. Therefore other potential mechanisms through which CBD could mitigate THC side effects were also evaluated.

The agonism of the 5-HT_{1A} receptor by CBD. The 5-HT_{1A} receptor has been implicated in anxiolytic properties of CBD in previous research.[5] Based on the research of Russo et al., where 75% receptor occupancy and a clear agonistic effect were seen at target site [CBD] of 16 µM (32-fold higher than the IC_{50} for the CB_1 NAM effect), we hypothesize that an oral CBD dose of at least 320 mg should be sufficient for a clear agonistic effect on the 5-HT_{1A} receptor.[6] Findings from animal studies are congruent with this hypothesis.[7,8] The dose was rounded up to 450 mg (due to available tablet strength of 150 mg CBD) with the intention of observing a near-maximal agonistic effect and to have a dose that is expected to achieve an estimated maximum effect when comparing the data from previous single dose studies of CBD in anxiety as shown in the following table.

Study	Dose(s) tested
Zuardi, 1993	CBD 300 mg[9]
Crippa, 2004	CBD 400 mg[10]
Bergamaschi, 2011	CBD 600 mg[11]
Crippa, 2011	CBD 400 mg[12]
Zuardi, 2017	CBD 300 mg, 600 mg, 900 mg[13]
Linares, 2019	CBD 150 mg, 300 mg, 600 mg[14]
de Faria, 2020	CBD 300 mg[15]

The partial agonism of dopamine D_2^{high} receptors leading to antipsychotic effect at a dose that is comparable to the calculated dose for the agonism of the 5-HT_{1A} receptor.[16]

An additional consideration is that the clinically prescribed THC+CBD combination formulations typically use a 1:1 ratio of the two compounds due to the literature on nabiximols (Sativex®). Based on the above, we chose CBD doses of 10 mg, 30 mg and 450 mg for this study.

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Figure S1 Study flow diagram.

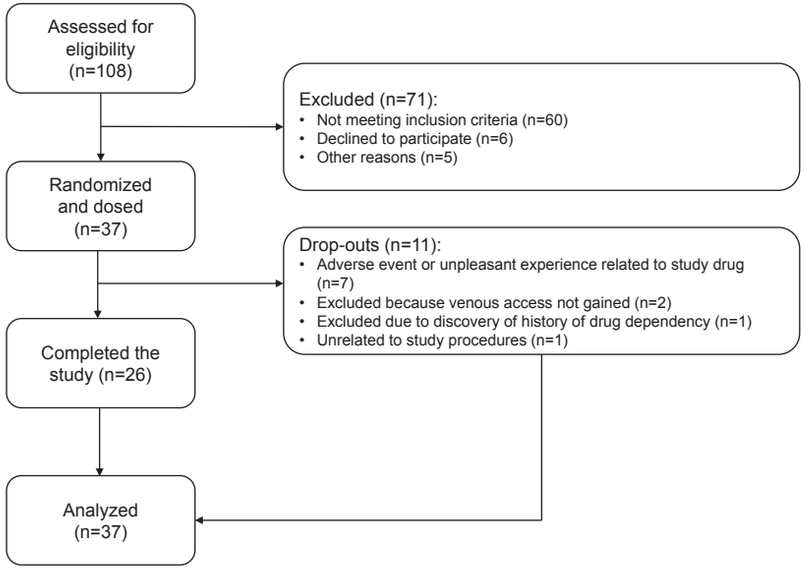
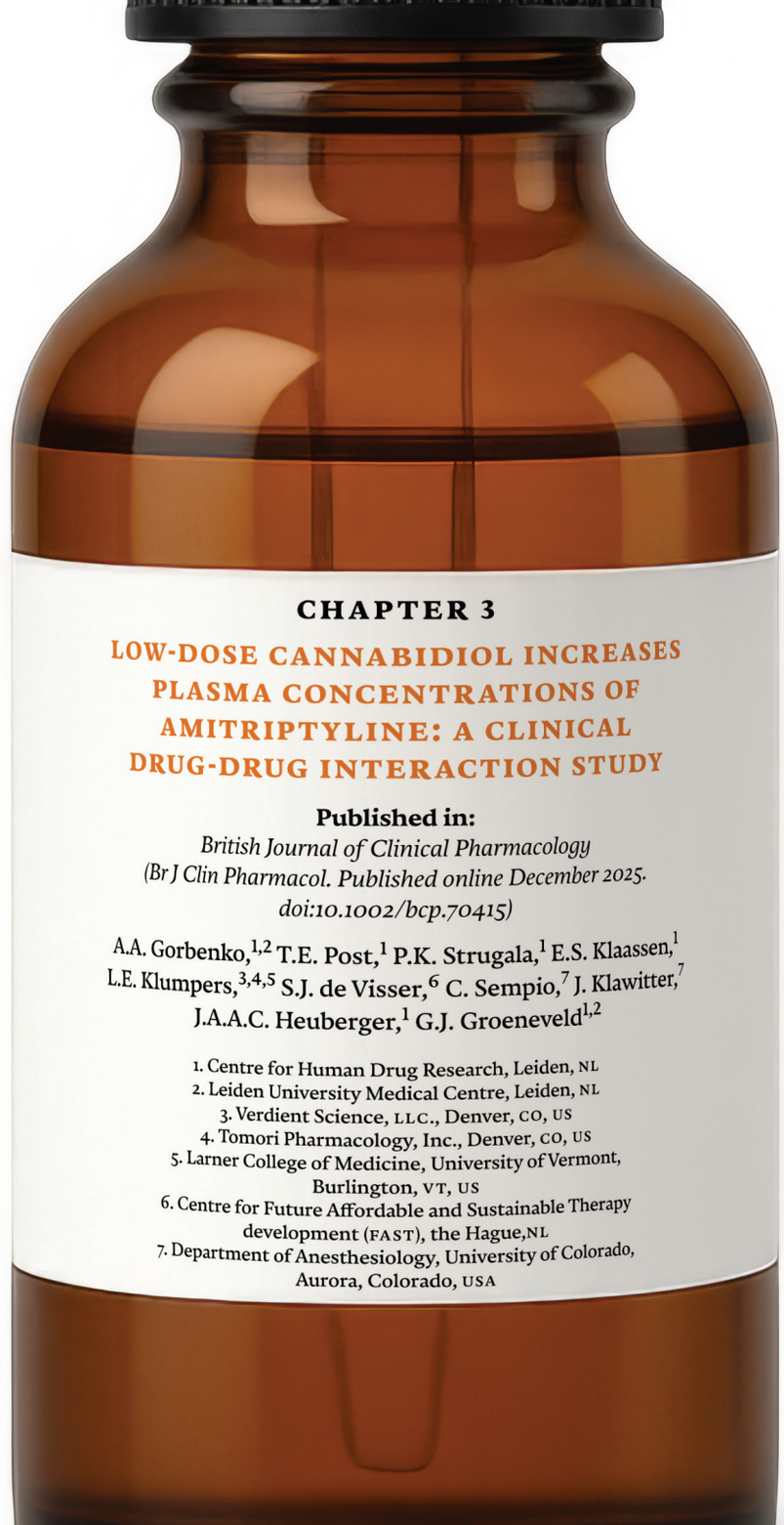


Table S1 Baseline demographics.

All participants (n = 37)	
AGE (YEARS)	
Mean (SD)	26.0 (6.0)
Median	26
Min, Max	18, 41
HEIGHT (CM)	
Mean (SD)	175.1 (11.4)
Median	175.6
Min, Max	149.4, 192.2
WEIGHT (KG)	
Mean (SD)	72.1 (11.0)
Median	71.7
Min, Max	50.1, 99.9
BMI (KG/M^2)	
Mean (SD)	23.5 (2.4)
Median	23.1
Min, Max	19.8, 29.5
SEX	
Female	19 (51.4%)
Male	18 (48.6%)
RACE	
Asian	3 (8.1%)
Black or African American	2 (5.4%)
Mixed	2 (5.4%)
White	30 (81.1%)

Abbreviations: BMI, body mass index; max, maximum; min, minimum; N, number; SD, standard deviation.



CHAPTER 3

LOW-DOSE CANNABIDIOL INCREASES PLASMA CONCENTRATIONS OF AMITRIPTYLINE: A CLINICAL DRUG-DRUG INTERACTION STUDY

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Abstract

AIM Cannabidiol (CBD), the main non-intoxicating compound from the cannabis plant, is regularly used by patients with chronic pain who also take analgesics. CBD has previously been shown to inhibit CYP-mediated drug metabolism. This study aimed to characterize the potential pharmacokinetic interaction of CBD with amitriptyline and tramadol, two commonly used analgesics.

METHODS This was an open-label, fixed-sequence, 2-way crossover study in 13 healthy participants. On Day 1, 25 mg amitriptyline and 50 mg tramadol were co-administered orally in a fasted condition, followed by a 7-day washout period. On Day 8, 30 mg CBD was administered orally 1 hour prior to amitriptyline and tramadol. Pharmacokinetic sampling was performed for CBD, amitriptyline, tramadol and their respective active metabolites nortriptyline and O-desmethyltramadol for up to 24 hours post-dose. The areas-under-the-curve (AUCs) were compared between visits using a mixed effects model.

RESULTS 12 participants (4M/8F) completed the study; 1 participant (M) dropped out for personal reasons. CBD significantly increased the AUC_{0-24h} (least square means (LSM) ratio 1.13, 95% CI: 1.01, 1.26, p=0.033) and the C_{max} (LSM ratio 1.17, 95% CI: 1.01, 1.36, p=0.041) of amitriptyline. CBD did not significantly change the AUC_{0-24h} and C_{max} of nortriptyline and tramadol, and the C_{max} of O-desmethyltramadol.

CONCLUSIONS A single dose of 30 mg CBD significantly influenced the metabolism of amitriptyline in healthy volunteers. In patients, CBD-induced drug interactions may be more pronounced in chronic dosing and dependent upon prandial status.

Bullet point summary

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT:

- Cannabidiol (CBD) is a CYP enzyme inhibitor known to increase plasma concentrations of THC, anti-epileptics, and other drugs.
- Amitriptyline and tramadol are commonly used for treatment of chronic neuropathic pain.
- As CYP substrates, amitriptyline and tramadol are potentially liable to drug interactions with CBD.

WHAT THIS STUDY ADDS:

- Single doses of 30 mg CBD significantly increased plasma concentrations of amitriptyline in healthy volunteers.
- Concentrations of nortriptyline, tramadol and O-desmethyltramadol did not increase significantly.
- Even a relatively low dose of CBD can influence the metabolism of drugs used by patients with chronic pain, leading to higher plasma concentrations.

Introduction

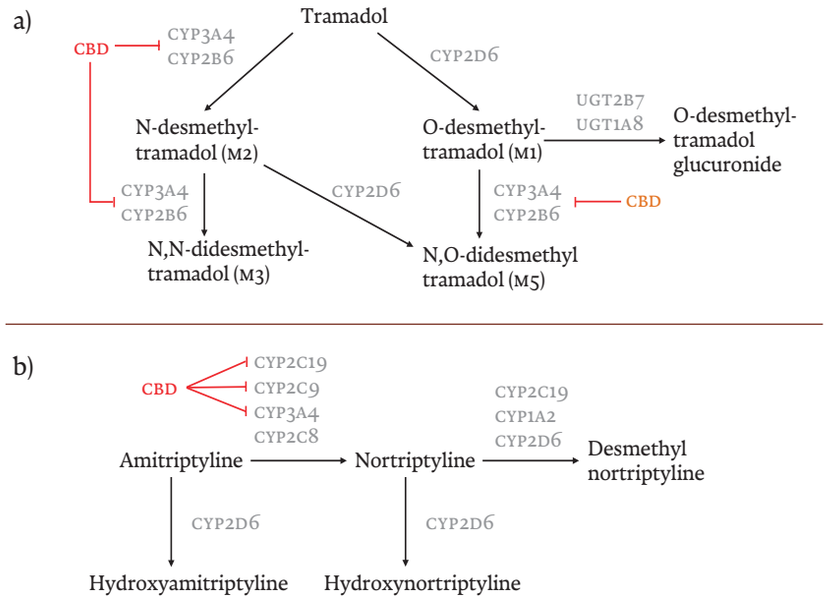
Cannabidiol (CBD) is the main non-intoxicating compound from the cannabis plant.[1] CBD is commonly used in combination with traditional analgesics by virtue of its frequent presence in medicinal cannabis, which is a moderately effective treatment for neuropathic pain.[2] Moreover, CBD-containing products such as oils, gummies and beverages are available as over-the-counter (OTC) health supplements[3] and are increasingly used for treatment of pain, as well as anxiety, and improvement of sleep and mood,[4] despite lacking clear evidence of therapeutic efficacy.[5]

Although CBD use is highly prevalent,[6] it may not be harmless. Besides the risk of liver injury after long-term use,[7] CBD is also a potent CYP-enzyme inhibitor, having been shown to inhibit mostly CYP2C19-, CYP2C9-, CYP3A4-, CYP1A2-, and CYP2B6-mediated metabolism in vitro,[8–11] as well as CYP2C19, CYP2C9, CYP3A4 and CYP1A2 in humans administered a validated CYP substrate cocktail.[12] Although some in-vitro studies suggest a degree of CYP2D6 inhibition,[8,9,13] the only available study in humans failed to show it.[12] Clinical publications report interactions between CBD and tacrolimus,[14] clobazam[15] and other anti-epileptics.[16] Typically, the interactions between CBD and substrate drugs result in increased substrate concentrations and therefore increased toxicity, although in the case of clobazam, in has even been argued that the interaction could explain the *efficacy* of CBD in the treatment of Dravet syndrome,[17] and the European Medicines Agency (EMA) approved CBD for this indication only in conjunction with clobazam.[18]

Patients with chronic neuropathic pain who use medicinal cannabis or OTC CBD supplements are, therefore, at risk of drug interactions between CBD and conventional analgesics that are metabolized by CYP-enzymes. Amitriptyline is one such commonly prescribed analgesic that is liable to a pharmacokinetic interaction with CBD, as it is metabolized by CYP2C19 (mainly), CYP2C9, and CYP3A4, into nortriptyline, an active metabolite. CBD is a known inhibitor of these three enzymes and can be expected to inhibit the conversion of amitriptyline to nortriptyline, resulting in elevated amitriptyline concentrations and an increased amitriptyline to nortriptyline ratio (**Figure 1**). This is associated with an increased potential for adverse effects, including sedation, orthostatic hypotension, and cardiotoxicity.[19]

Tramadol is another analgesic commonly used by patients with chronic neuropathic pain that has potential for drug-interactions with CBD. Tramadol is converted for approximately 80% into O-desmethyltramadol (O-DSMT) by CYP2D6, and for approximately 20% into N-desmethyltramadol (N-DSMT) by CYP3A4 and CYP2B6. O-DSMT has a significantly higher affinity for the μ-opioid receptor than the parent compound and is primarily responsible for the analgesic effects of tramadol, whereas N-DSMT is inactive.[20] O-DSMT subsequently undergoes phase II metabolism, either directly or after conversion to the mildly active N,O-desmethyltramadol (N,O-DSMT), by CYP3A4 and CYP2B6. CBD is an inhibitor of CYP3A4 and CYP2B6, and therefore we hypothesized that CBD would 1) further increase the proportion of parent compound converted to O-DSMT by CYP2D6 (which we assumed to be unaffected by CBD)[12] and 2) inhibit the N-demethylation of O-DSMT to N,O-DSMT, with both 1) and 2) resulting in increased concentrations of O-DSMT (**Figure 1**). Elevated O-DSMT levels can cause toxicity such as sleepiness, confusion, shallow breathing and even life-threatening respiratory depression.[21]

Figure 1 Expected action of CBD on tramadol and amitriptyline metabolism.



Although previous clinical research clearly demonstrated CBD-induced CYP-mediated drug interactions, the high doses of CBD administered limit the translatability to patients with neuropathic pain and OTC CBD users. Some

studies administered a single dose of 640 mg CBD,[12,22] while others involved the long-term treatment of rare epilepsy syndromes, with daily doses up to 50 mg/kg CBD.[14–16] In contrast, patients with chronic neuropathic pain who participated in a clinical trial of Sativex®, a formulation containing both THC and CBD in an (approximately) equal measure, self-administered an average of 27.3 mg CBD daily.[23] It is likely that this lower CBD intake is also more representative of OTC CBD use, with a typical strength per serving ranging between 5 and 50 mg CBD,[3] although products up to 100 mg CBD per serving are available.[24] Our own previous research showed that 30 mg CBD affects the metabolism of THC (CYP3A4 and CYP2C9 substrate), demonstrating that commonly used low CBD doses may already put individuals at risk of drug interactions.[25]

The extent of pharmacokinetic interactions between CBD in commonly used doses and analgesics is not yet known. This lack of knowledge puts patients with chronic neuropathic pain, as well as other patient populations commonly treated with amitriptyline and tramadol, at an increased risk of adverse effects. The aim of this study was to characterize the potential of CBD to induce CYP-mediated drug interactions with amitriptyline and tramadol. We used an open-label, fixed sequence two-way crossover design in healthy volunteers, utilizing a CBD dose that is representative of common use in patients with neuropathic pain and OTC CBD users.

Methods

PARTICIPANTS AND STUDY DESIGN

This study was an open-label, fixed sequence, 2-way crossover study in 13 healthy participants. On Day 1, 25 mg amitriptyline and 50 mg tramadol were orally administered concurrently, followed by a wash-out period of at least 7 days. On Day 8, 30 mg of oral CBD oil was administered 1 hour prior to amitriptyline and tramadol. On Days 1 and 8, participants presented to the clinical research unit in the morning and were discharged in the evening, briefly returning in the morning of Days 2 and 9 for pharmacokinetic sampling.

The study was conducted at the Centre for Human Drug Research in Leiden, the Netherlands. The study was authorised under the Clinical Trial Regulation (536/2014) 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 Participants (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. This study was submitted via the Clinical Trials Information System (CTIS) and therefore registered prospectively on the corresponding public portal, under EU trial number 2023-508428-36-00. This research has been funded by the Dutch Organisation for knowledge and innovation in health, healthcare and well-being (ZonMw), grant number 848120001.

Each participant provided written informed consent before any screening procedures were performed. Eligible participants included healthy male and female volunteers aged 18 to 45 years, with a body mass index of 18 to 32 kg/m^2 and a minimum weight of 50 kg. The participants underwent a full medical screening, including medical history, a physical examination, blood chemistry and haematology, urinalysis and an electrocardiogram (ECG) to assess eligibility. Participants with a history of clinically significant epileptic disorders or traumatic brain injury were excluded, as tramadol and amitriptyline are known to lower the seizure threshold.[26] Any participant who was a regular user of any illicit drugs (including cannabis), had a history of drug abuse or a positive urine drug test at screening, was excluded. Urine drug tests were repeated pre-dose on Days 1 and 8.

A CYP genotyping sample was taken at screening and analysed by the Department of Clinical Pharmacy and Toxicology, Leiden University Medical Centre (Leiden, The Netherlands). The list of tested CYP enzyme variants is provided in the **Supplementary Materials** and phenotyping into poor, intermediate, extensive and ultra-rapid metabolizers was performed according to the guidelines of the Dutch Pharmacogenetics Working Group.[27,28] Participants with a poor metabolizer phenotype for CYP2D6 were excluded, as their significantly reduced bioavailability of O-desmethyltramadol,[29] would hinder the assessment of the effect of cannabidiol on its metabolism. Furthermore, participants with the CYP2D6 ultrarapid metabolizer phenotype were also excluded, as this phenotype increases the metabolism of amitriptyline and its metabolite nortriptyline into their hydroxy metabolites, leading to low drug concentrations (and suboptimal therapeutic efficacy) in the clinical setting.[19] Therefore, the CYP2D6 ultrarapid metabolizer phenotype was expected to hinder the assessment of cannabidiol effects on CYP2C19, CYP2C9, and CYP3A4-mediated metabolism of amitriptyline to nortriptyline. Also, individuals with a poor metabolizer phenotype for CYP2C19 and CYP3A4 were excluded, as the low inherent enzyme activity in such individuals would hinder the assessment of inhibitory effects of cannabidiol.

No prescription and OTC medications were permitted within 14 days prior to dosing and during the course of the study, with the exception of paracetamol (up to 4 g/day) and ibuprofen (up to 1 g/day). No vitamin, mineral, herbal, and dietary supplements were permitted within 7 days prior to dosing. Participants abstained from nicotine, alcohol and caffeine starting at 24 hours prior to dosing and until the last PK sample of the visit. Any nutrients known to modulate CYP enzyme activity (e.g., grapefruit or Seville orange containing products or quinine containing drinks such as tonic water or bitter lemon) were not permitted from 3 days before the first visit until the end of study. Participants were required to fast for at least 8 hours overnight before dosing and for 3 hours post-dose.

All females of childbearing potential and all males were required to practice effective contraception during the study and to continue contraception for at least 90 days after the last dosing. Urine pregnancy testing was conducted in female participants on each study day prior to dosing.

STUDY DRUGS

The cannabidiol formulation used in this study was Clinican®, an almond oil-based formulation containing 10% cannabidiol and manufactured by Clinical Cannabis Care (Breukelen, the Netherlands) and administered orally with 240 mL of water following an overnight fast. Single doses of 30 mg CBD were administered. Although CYP-enzyme inhibition was expected to increase with multiple administrations of CBD, owing to accumulation and the time-dependency of the inhibition, single administrations of CBD were deemed sufficient for this study, based on observed PK interactions induced by single doses of CBD in our previous research.[25] Furthermore, we estimated that the 30 mg CBD dose is at the upper end of the range of CBD intake of patients who use CBD-rich varieties of medicinal cannabis (or formulations containing THC and CBD, like Sativex®) for treatment of neuropathic pain symptoms,[23] and as well as an approximately median dose for OTC CBD users. Cannabidiol was administered one hour prior to amitriptyline and tramadol (during visit 2 only) to offset the slower absorption expected from an oil-based cannabinoid formulation, compared to the victim drugs.

Generic oral formulations of tramadol (50 mg tramadol hydrochloride capsule) and amitriptyline (25 mg hydrochloride tablet) were used in this study. These doses were at the low end of the therapeutic range, allowing for a safety margin for possible increases in plasma concentrations when co-administered with CBD. Tramadol and amitriptyline were not known to induce or inhibit CYP-enzymes in general, and no specific pharmacokinetic interactions between the two victim drugs were known to the investigators. Co-administration of tramadol and amitriptyline was hypothesized to increase the risk of serotonergic syndrome and seizures, as both drugs have a monoaminergic effect and reduce the seizure threshold. However, this risk was deemed clinically irrelevant when low, single doses were administered to healthy volunteers. Based on these consideration regarding pharmacokinetics and safety, the two victim drugs were administered together in a cocktail design.

A wash-out period of at least 7 days was considered sufficient, as it spanned over 5 terminal half-lives of tramadol (~5-6 hours),[30] O-DSMT (~7 hours),[30] amitriptyline (~25 hours),[31] and nortriptyline (~26 hours).[32]

PHARMACOKINETIC ASSESSMENTS

Sampling for pharmacokinetics was performed pre-dose and at 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours following the administration of amitriptyline and tramadol. Approximately 4 mL blood was collected via an i.v. catheter placed in an antecubital vein in the arm in polypropylene K2EDTA tubes. Following collection, samples were immediately cooled on ice. Within 30 minutes of collection, plasma was separated by centrifugation at approximately 2000 g for 10 minutes and promptly transferred into amber glass vials and stored at -70 to 80 °C. The samples were analysed by the iC42 Laboratory (Department of Anesthesiology, University of Colorado, Aurora, Colorado, USA). CBD and CBD metabolites were analysed using a validated assay that was previously published (CBD metabolite concentrations not reported in this manuscript).[33] Concentrations of amitriptyline, nortriptyline, tramadol and O-desmethyltramadol were determined using a validated assay, that was based on a previously published high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method.[34] Details regarding the bioanalytical assays used to measure CBD, amitriptyline, nortriptyline, tramadol and O-desmethyltramadol are located in the **Supplementary Materials** section.

R 3.6.1 for Windows (R Foundation for Statistical Computing/R development Core Team, Vienna, Austria, 2019), PKNCA package version 0.9.5, was used to calculate pharmacokinetic parameters. PK parameter calculations were based on actual sampling time. When an actual sampling time differed

from the protocol time by more than 10% and at least 5 minutes, the concentration was excluded from the descriptive statistics, but not from the non-compartmental analysis. AUC was calculated using the linear-log trapezoidal rule. For the calculation of PK parameters, concentrations below the limit of quantification (BLQ) that occurred prior to the first quantifiable concentrations were considered as zero. BLQ concentrations that occurred between the first and last non-BLQ values or occurred after the last non-BLQ value, were dropped (treated as 'missing'). Metabolite/parent ratios were calculated based on the AUC_{last} and corrected for the molecular weight ratio.

SAFETY ASSESSMENTS

Safety and tolerability were assessed by monitoring of adverse events (AE) and vital signs.

SAMPLE SIZE

A sample size calculation was performed based on the AUCs of O-desmethyltramadol and amitriptyline, as these parameters were expected to be most impacted by CBD. We expected the co-administration of CBD to result in an approximately 50% increase in AUC of O-desmethyltramadol and amitriptyline, based on our previous report.[25] The sample calculation for a crossover study design required intra-individual variability of the AUC of O-desmethyltramadol and amitriptyline to be known; however, such data was not publicly available. Therefore, the intra-individual variability was assumed to be (at most) equal to the known inter-individual variability (which was a conservative estimate, as inter-individual variability typically exceeds intra-individual variability), and the calculated power is likely to be an underestimation.

For the sample size calculation for O-desmethyltramadol, the AUC and variability reported in a previous trial with 25 participants dosed with 100 mg tramadol were used, in which a mean (SD) AUC of 669 (181) ng*h/mL was reported for O-desmethyltramadol.[35] Although the tramadol dose in this trial differed from the intended dose in our study, the estimate of the variability derived from this trial was deemed appropriate for the purpose of this sample size calculation. We calculated a sample size of 12 to have a power of 0.998 to detect a difference in means of 350 ng*h/mL (corresponding to a difference of 52%), assuming a standard deviation of differences of 225 ng*h/mL, using a paired t-test with a 0.05 2-sided significance level, in a crossover study design.

For the sample size calculation for amitriptyline, the AUC and variability reported in a previous trial with 28 participants dosed with 10 mg of amitriptyline were used, in which a mean (SD) AUC of 156 (87.8) ng*h/mL was reported for amitriptyline.[36] Although the amitriptyline dose in this trial differed from the intended dose in our study, the estimate of the variability derived from this trial was deemed appropriate for the purpose of this sample size calculation. We calculated a sample size of 12 to have a power of 0.764 to detect a difference in means of 85 ng*h/mL (corresponding to a difference of 54%), assuming a standard deviation of differences of 100 ng*h/mL, using a paired t-test with a 0.05 2-sided significance level, in a crossover study design.

In addition to the statistical considerations provided above, the sample size of 12 participants was expected to be sufficient for a 2-way crossover drug-drug interaction study of CBD as perpetrator drug, based on previously published literature on design of drug-drug interaction studies.[16,37]

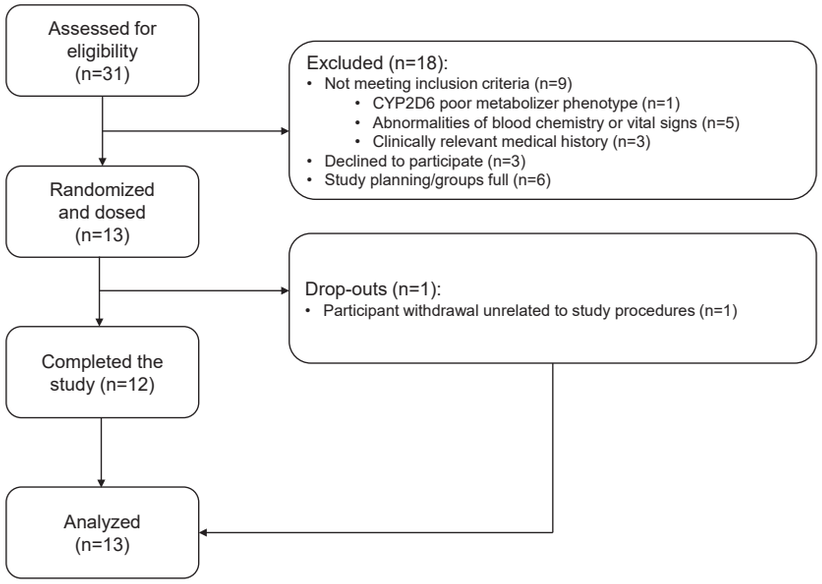
STATISTICAL ANALYSIS

To establish whether significant treatment effects could be detected, the PK parameters were compared with a mixed effects model analysis of variance (ANOVA) with treatment as fixed factor and participants as random factor on log-transformed data. 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. All calculations were performed using SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

The clinical phase of the trial ran from December 2023 to March 2024. A total of 31 participants were screened, of which 13 (5 males/8 females) were enrolled in the study and dosed. **Table 1** contains a summary of the baseline demographics. Of the 13 dosed participants, 1 (male) withdrew from the study following the first visit due to personal reasons and was replaced; 12 participants successfully completed the trial per protocol. Further details on participant enrolment are contained in the study flow diagram (**Figure 2**). All participants included in the study had either the extensive metabolizer or the intermediate metabolizer phenotype for CYP2C19, CYP2D6 and CYP3A4 (**Table 1**).

Figure 2 CONSORT study flowchart.



Baseline concentrations of all analytes were below the limit of quantification (BLQ) for all participants, except for one participant with a baseline amitriptyline concentration of 0.85 ng/ml (lower limit of quantification: 0.75 ng/ml) during visit 2, which was treated as BLQ in the analysis. Number and percentage of BLQ samples per analyte are provided in the **Supplementary Materials**.

Mean pharmacokinetic parameters of CBD, amitriptyline, nortriptyline, tramadol and O-DSMT are provided in **Table 2**, and their concentration-time profiles are displayed in **Figure 3**. CBD significantly increased the AUC_{0-24h} of amitriptyline (LSM ratio 1.13, 95% CI: 1.01, 1.26, p=0.033), as well as its C_{max} (LSM ratio 1.17, 95% CI: 1.01, 1.36, p=0.041). Furthermore, amitriptyline C_{last} (LSM ratio 1.14, 95% CI: 0.99, 1.32, p=0.058) and tramadol C_{max} (LSM ratio 1.10, 95% CI: 0.99, 1.22, p=0.064) increased after treatment with CBD but did not reach statistical significance. (**Table 3**). CBD had no statistically significant effects on other pharmacokinetic parameters of amitriptyline, tramadol and their metabolites, including the metabolite to parent ratios (**Table 3**).

No serious adverse events occurred during the study. All AEs were transient and mild. The most common AEs were fatigue, reported by 10 (76.9%) participants after treatment with amitriptyline and tramadol and 10 (83.3%) participants after treatment with CBD, amitriptyline and tramadol, and headache, reported by 3 (21.3%) participants and 3 (25.0%) participants, respectively. Dizziness was reported more frequently after dosing with amitriptyline and tramadol (4 (30.8%) participants), than after dosing with CBD, amitriptyline and tramadol (1 (8.3%) participant). An overview of AEs is provided in the **Supplementary Materials**.

Table 1 Baseline participant demographics and CYP-enzyme phenotypes.

All participants (N = 13)	
AGE (YEARS)	
Mean (SD)	24.4 (4.4)
Median (Min,Max)	22 (21, 35)
HEIGHT (CM)	
Mean (SD)	177.4 (9.7)
Median (Min,Max)	174.8 (164.5, 194.4)
WEIGHT (KG)	
Mean (SD)	69.02 (8.17)
Median (Min,Max)	68.05 (58.95, 87.85)
BMI (KG/M^2)	
Mean (SD)	22.0 (2.3)
Median (Min,Max)	21.3 (18.6, 25.8)
GENDER	
Female	8 (61.5%)
Male	5 (38.5%)
RACE	
Asian	1 (7.7%)
White	12 (92.3%)
CYP2C19 PHENOTYPE	
EM	10 (76.9%)
IM	3 (23.1%)
CYP2D6 PHENOTYPE	
EM	10 (76.9%)
IM	3 (23.1%)
CYP3A4 PHENOTYPE	
EM	12 (92.3%)
IM	1 (7.7%)

Abbreviations: BMI, body mass index; EM, extensive metabolizer; IM, intermediate metabolizer; max, maximum; min, minimum; N, number; SD, standard deviation.

Table 2 Pharmacokinetic parameters of cannabidiol, amitriptyline, nortriptyline, tramadol and O-desmethyltramadol.

	Parameter	Unit	N	Mean	Geometric mean	SD	CV (%)	Geometric CV (%)	Median	Min	Max
CANNABIDIOL											
Visit 2	AUC_{last}	h*ng/mL	12	6.9	5.3	5.4	78.0	87.4	6.2	1.7	20.3
	C_{max}	ng/mL	12	3.5	2.9	2.4	68.1	68.4	3.0	1.1	9.8
	C_{last}	ng/mL	12	1.1	1.0	0.3	25.3	25.0	1.1	0.8	1.7
	t_{lag}	h	12						0.0	0.0	4.0
	t_{max}	h	12						2.0	2.0	5.0
AMITRIPTYLINE											
Visit 1	AUC_{last}	h*ng/mL	13	143.9	138.6	39.4	27.3	30.2	142.7	79.3	199.1
	C_{max}	ng/mL	13	12.9	12.3	3.9	30.3	33.6	12.8	6.1	20.7
	C_{last}	ng/mL	13	3.6	3.4	1.1	30.6	31.3	3.3	2.0	5.5
	t_{lag}	h	13						0.0	0.0	1.0
	t_{max}	h	13						4.0	3.0	6.0
Visit 2	AUC_{last}	h*ng/mL	12	160.3	153.0	50.1	31.3	33.0	152.9	88.2	224.4
	C_{max}	ng/mL	12	14.8	14.0	4.8	32.4	37.1	13.6	6.5	21.4
	C_{last}	ng/mL	12	4.0	3.8	1.3	31.8	32.3	3.6	2.3	6.2
	t_{lag}	h	12						0.0	0.0	1.0
	t_{max}	h	12						4.0	2.0	5.0
NORTRIPTYLINE											
Visit 1	AUC_{last}	h*ng/mL	13	77.9	75.3	20.9	26.8	27.9	73.0	40.7	126.0
	C_{max}	ng/mL	13	4.5	4.4	1.2	25.7	26.4	4.5	2.5	7.1
	C_{last}	ng/mL	13	3.4	3.3	0.9	27.6	30.8	3.3	1.5	5.5
	t_{lag}	h	13						1.0	0.0	3.0
	t_{max}	h	13						6.0	4.0	12.0
	MPR	-	13	0.62	0.57	0.24	38.9	44.8	0.60	0.24	1.06
Visit 2	AUC_{last}	h*ng/mL	12	84.5	80.6	27.6	32.7	32.8	77.3	48.0	133.7
	C_{max}	ng/mL	12	4.7	4.5	1.5	32.3	31.7	4.7	2.7	8.3
	C_{last}	ng/mL	12	3.5	3.3	1.4	40.4	39.7	3.2	1.9	6.2
	t_{lag}	h	12						1.0	0.0	2.0
	t_{max}	h	12						6.0	4.0	12.0
	MPR	-	12	0.60	0.56	0.24	39.8	44.4	0.53	0.23	0.99
TRAMADOL											
Visit 1	AUC_{last}	h*ng/mL	13	1168.4	1130.0	305.4	26.1	27.8	1239.2	753.8	1553.9
	C_{max}	ng/mL	13	140.6	136.9	33.9	24.1	24.8	134.8	89.4	205.9
	C_{last}	ng/mL	13	8.9	7.4	5.0	56.5	74.0	8.4	2.1	16.7
	t_{lag}	h	13						0.0	0.0	0.0
	t_{max}	h	13						2.0	1.0	4.0

(Continuation Table 2)

	Parameter	Unit	N	Mean	Geometric mean	SD	CV (%)	Geometric CV (%)	Median	Min	Max
Visit 2	AUC_{last}	h*ng/mL	12	1211.0	1152.7	392.7	32.4	34.0	1159.2	727.7	1773.7
	C_{max}	ng/mL	12	153.3	150.1	31.4	20.5	22.6	154.9	89.6	195.4
	C_{last}	ng/mL	12	8.7	6.8	5.7	65.4	9.6	8.0	1.9	17.4
	t_{lag}	h	12						0.0	0.0	0.0
	t_{max}	h	12						2.0	1.0	3.0
O-DESMETHYLTRAMADOL											
Visit 1	AUC_{last}	h*ng/mL	13	412.0	400.4	105.2	25.5	25.0	388.6	283.5	605.4
	C_{max}	ng/mL	13	36.7	35.0	12.6	34.3	32.0	31.1	24.1	63.2
	C_{last}	ng/mL	13	4.8	4.4	2.0	41.8	46.3	4.1	2.0	9.1
	t_{lag}	h	13						0.0	0.0	0.0
	t_{max}	h	13						3.0	1.0	5.0
	MPR	-	13	0.40	0.37	0.15	37.7	34.9	0.33	0.26	0.74
Visit 2	AUC_{last}	h*ng/mL	12	411.1	399.5	102.9	25.0	25.4	396.1	282.2	603.9
	C_{max}	ng/mL	12	39.5	37.6	13.8	34.9	31.8	33.6	25.8	70.3
	C_{last}	ng/mL	12	4.4	4.0	2.0	46.0	48.5	3.9	2.0	7.9
	t_{lag}	h	12						0.0	0.0	0.0
	t_{max}	h	12						2.5	1.0	4.0
	MPR	-	12	0.39	0.37	0.14	36.0	34.4	0.32	0.27	0.64

Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; CBD, cannabidiol; C_{max}, maximum concentration; C_{last}, last observed concentration; CV, coefficient of variation; Geo, geometric; max, maximum; min, minimum; MPR, metabolite to parent ratio; N, number; SD, standard deviation; T_{lag}, absorption lag time; T_{max}, time to attain C_{max}.

Figure 3 Concentration-time profiles of A) cannabidiol, B) amitriptyline, C) nortriptyline, D) tramadol and E) O-desmethyltramadol following oral administration, displayed as means with standard deviations.

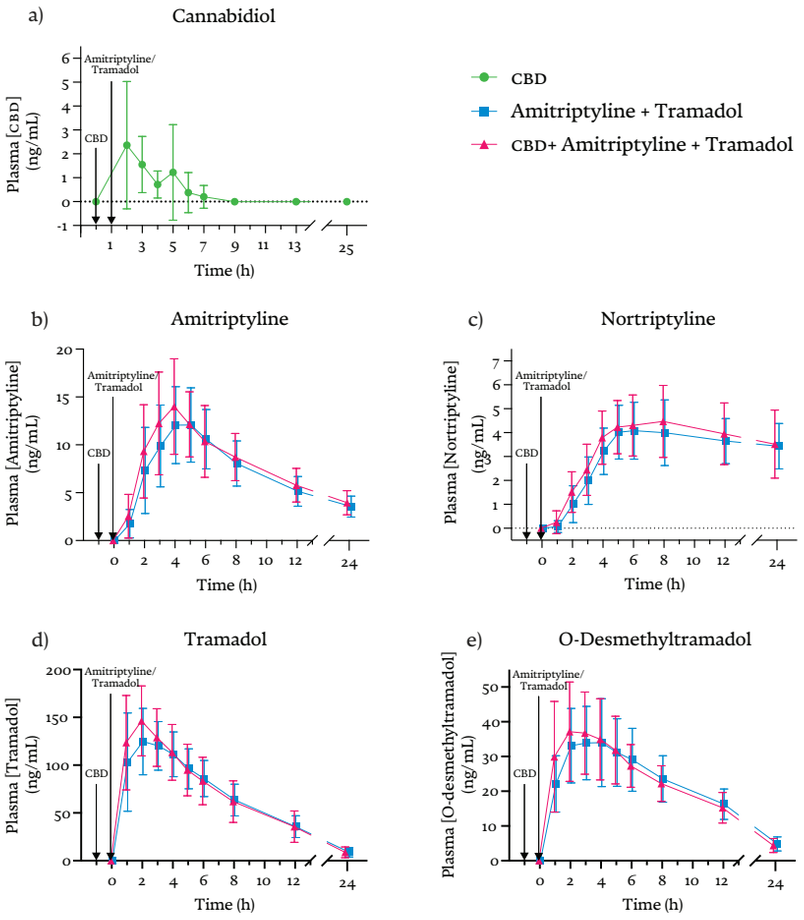


Table 3 Overall treatment effects on pharmacokinetic parameters.

			Back Transformed				
						95% CI	
Analyte	**Parameter**	**P-value**	**LSM Visit 2**	**LSM Visit 1**	**Ratio of LSMs**	**Lower**	**Upper**
Amitriptyline	AUC_{last}	**0.033**	156.24	138.56	1.13	1.01	1.26
	C_{max}	**0.041**	14.46	12.33	1.17	1.01	1.36
	C_{last}	0.058	3.89	3.40	1.14	0.99	1.32
Nortriptyline	AUC_{last}	0.309	79.54	75.31	1.06	0.94	1.18
	C_{max}	0.580	4.51	4.36	1.04	0.91	1.18
	C_{last}	0.926	3.28	3.30	0.99	0.86	1.15
	MPR	0.294	0.54	0.57	0.93	0.82	1.07
Tramadol	AUC_{last}	0.259	1175.77	1130.01	1.04	0.97	1.12
	C_{max}	0.064	150.95	136.87	1.10	0.99	1.22
	C_{last}	0.690	7.16	7.41	0.97	0.80	1.16
O-desmethyl-tramadol	AUC_{last}	0.830	396.86	400.41	0.99	0.91	1.08
	C_{max}	0.261	36.84	34.99	1.05	0.96	1.16
	C_{last}	0.206	4.04	4.43	0.91	0.79	1.06
	MPR	0.351	0.36	0.37	0.95	0.86	1.06

The figures in bold indicate statistical significance. Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; C_{max}, maximum concentration; CI, confidence interval; C_{last}, last observed concentration; LSM, least square mean; MPR, metabolite to parent ratio.

Discussion

CBD is known to cause potentially toxic pharmacokinetic drug interactions with multiple CYP-substrate drugs due to its CYP-enzyme inhibiting properties. However, the potential of CBD to cause pharmacokinetic interactions with analgesics that are commonly used for treatment of chronic neuropathic had not been previously evaluated. The aim of this study was to characterize the potential pharmacokinetic interaction between CBD and common analgesics amitriptyline and tramadol at a CBD dose that is expected to be representative of common use in patients with neuropathic pain and OTC CBD users. In line with our hypothesis, 30 mg CBD significantly increased the plasma concentrations of amitriptyline, indicative of CBD-induced inhibition of CYP-mediated metabolism. Furthermore, the C_{max} of tramadol increased also, although not statistically significantly. The magnitude of the observed increases was limited, and it is unclear from the current study whether it is clinically meaningful. The observed slight increases in amitriptyline and

tramadol concentrations may not be noticed by a patient and would certainly not cause toxicity. Thus, at exposures achieved in this study, CBD is safe in patients who use amitriptyline or tramadol.

However, there are reasons to expect, that some patients may reach CBD exposures far exceeding those achieved in this study. Firstly, repeated or chronic CBD dosing is likely to be prevalent in patient populations, whereas single doses were administered in this study. CBD has a long terminal half-life of ~60 hours, and moderate accumulation is known to occur after repeated dosing.[38] Additionally, CBD is a time-dependent CYP-inhibitor,[8,9] meaning that a prolonged exposure from repeated dosing will produce more inhibition than single doses of CBD. Secondly, as CBD is a highly lipophilic compound,[39] its absorption is strongly influenced by the prandial state of the individual. Therefore, patients who take CBD in a fed state will reach higher CBD concentrations for any given dose and thereby increase the risk, and magnitude, of interactions.

The size of the interaction observed in this study was smaller compared to our previous report, where 30 mg CBD increased the mean AUC of 11-OH-THC, main active metabolite of THC, another CYP2C19 substrate, by 71% when administered 30 minutes before THC.[25] Metabolism of THC is not identical to the metabolism of amitriptyline and tramadol, and the discrepant findings in the two studies may reflect differences in the way these drugs interact with CBD. Importantly, substantially lower plasma CBD concentrations in the current study (mean±SD C_{max} 3.5±2.4 ng/ml) compared to the previous study (mean±SD C_{max} of 5.6±4.6 ng/ml) likely contributed to a smaller effect size. The higher CBD concentrations in the previous trial could be attributed to the semi-standardized light breakfast was administered prior to dosing, whereas participants in the current study were dosed in a fasted condition. Additionally, it is possible that the difference in drug formulation played a role in absorption: an oil formulation was administered in this study versus a tablet formulation in the previous study. The relative contribution of prandial status and formulation remains uncertain in absence of pharmacokinetic data of both formulations administered under similar prandial conditions.

The strengths of this study included the use of a CBD dose that is relevant to patients with chronic neuropathic pain (as well as the general population), and the choice of two widely prescribed analgesics as victim drugs, which makes the results of this research relevant to a broad patient population. The open-label, fixed-sequence, two-way crossover design was well suited to address the research question and the sample size was adequate for the hypothesized effect size. Exclusion of individuals with poor metabolizer phenotypes for CYP2D6, CYP2C19 and CYP3A4, as well as CYP2D6 ultra-rapid metabolizers, removed the variability that such phenotypes would have introduced to the outcomes, since CBD was a priori expected to have little to no effect on victim drug metabolism in participants with such phenotypes.

Limitations include a pharmacokinetic sampling schedule that lacked sampling for CBD concentrations at earlier timepoints than 2 hours post-dose, which limited the characterization of the absorption phase of CBD and is likely to have affected the estimates of its pharmacokinetic parameters. However, characterization of CBD PK was not a primary goal of this study. Moreover, sampling up to 24 hours post-dose proved too short for full characterization of the pharmacokinetic profile of amitriptyline and especially nortriptyline. For amitriptyline, the terminal elimination phase was not fully captured by the sampling schedule, and for nortriptyline, most of the elimination phase. The estimates of the C_{max} are unaffected, but the estimates of the AUC are limited by the duration of the sampling period and are certain to be underestimation of the AUC_{inf}. This limited sampling schedule is unlikely to have influenced the conclusions of this study, however. Inhibition of CYP-mediated metabolism should be apparent from altered concentrations of amitriptyline and nortriptyline within 24 hours post-dose, if at all present, as evidenced by previous research comparing the metabolism of amitriptyline in patients with different CYP2D6 and CYP2C19 genotypes.[40] Therefore, it is highly unlikely that an extended sampling period would have revealed an interaction that was missed in the current study. A larger sample size may have allowed for the detection of significant changes in the amitriptyline C_{last} and tramadol C_{max}.

The proven potential of CBD to cause CYP-mediated drug interactions, as well as the high (and growing) prevalence of its use,[4] warrant future clinical studies of CBD-induced drug interactions, despite the modest interaction observed in this study. Future investigations should especially focus on interaction between CBD and commonly used analgesics, anxiolytics, hypnotics and antidepressants. Because OTC CBD is often used for treatment of pain and anxiety and to improve sleep and mood,[4] it is precisely the typical prescription drugs for these indications, which are the most exposed to interactions with CBD. Finally, the large potential of CBD to cause drug-drug interaction induces us to speculate that the putative efficacy of CBD as an analgesic,

anxiolytic or hypnotic could be in part explained by CBD-induced, CYP-mediated drug-drug interactions with conventional drugs used concomitantly, as has also been hypothesized for CBD's anti-epileptic properties. Speculative, but not implausible, are positive reinforcement loops, where CBD-induced interactions noticeably increase the effects of concomitant drugs, which leads patients to escalate CBD intake. Uncovering such interactions has potential to improve individual pharmacotherapy, as adjustments of the victim drug dose appear to be a more sound pharmacotherapeutic strategy than reliance on unpredictable CYP-mediated interactions induced by an expensive drug with adverse effects of its own.

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CHAPTER 3: SUPPLEMENTARY MATERIALS

Full supplementary materials available at the publisher's website:

https://tinyurl.com/2a55urfd

Selected supplementary tables are provided below.

Table S1 Number and proportion of post-dose values below the limit of quantification (BLQ) per analyte.

	Total		Day 1		Day 8	
	Samples (n)	n (%) BLQ	Samples (n)	n (%) BLQ	Samples (n)	n (%) BLQ
Amitriptyline	225	7 (3.1%)	117	4 (3.4%)	108	3 (2.8%)
Nortriptyline	225	27 (12%)	117	17 (14.5%)	108	10 (9.3%)
Tramadol	225	-	117	-	108	-
O-DSMT	225	-	117	-	108	-
CBD	108	71 (65.7%)	-	-	108	71 (65.7%)

Abbreviations: BLQ, below the limit of quantification; CBD, cannabidiol; n, number; O-DSMT, O-desmethyltramadol.

Table S2 Overview of adverse events.

	Amitriptyline + tramadol (n=13)		CBD + amitriptyline + tramadol (n=12)	
System Organ Class/ Preferred Term	Events N	Participants N (%)	Events N	Participants N (%)
ANY EVENTS	23	12 (92.3)	16	10 (83.3)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	10	10 (76.9)	10	10 (83.3)
Fatigue	10	10 (76.9)	10	10 (83.3)
INFECTIONS AND INFESTATIONS	1	1 (7.7)	-	-
Tonsillitis	1	1 (7.7)	-	-
METABOLISM AND NUTRITION DISORDERS	-	-	1	1 (8.3)
Increased appetite	-	-	1	1 (8.3)
NERVOUS SYSTEM DISORDERS	12	7 (53.8)	4	4 (33.3)
Dizziness	5	4 (30.8)	1	1 (8.3)
Headache	4	3 (23.1)	3	3 (25.0)
Presyncope	1	1 (7.7)	-	-
Sedation	1	1 (7.7)	-	-
Somnolence	1	1 (7.7)	-	-
VASCULAR DISORDERS	-	-	1	1 (8.3)
Hypotension	-	-	1	1 (8.3)

Abbreviations: CBD, cannabidiol; n, number.

CHAPTER 4

CANNABIDIOL LACKS DIRECT EFFECT ON CORTICAL EXCITABILITY: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED, 3-WAY CROSSOVER TRIAL

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Abstract

Cannabidiol (CBD) is approved as an adjunctive treatment of seizures associated with Dravet syndrome, Lennox-Gastaut Syndrome and tuberous sclerosis. Its therapeutic and adverse effects are thought to arise, at least partly, from a pharmacokinetic interaction with clobazam, another anti-seizure medication (ASM). The goal of this study was to evaluate the intrinsic anti-epileptic and sedative properties of CBD. A randomized, double blind, placebo controlled, 3-way crossover trial was conducted in 25 healthy males. On each visit, single doses of 30 mg CBD, 700 mg CBD, or placebo were administered orally. The effects of CBD on cortical excitability were measured using transcranial magnetic stimulation (TMS) combined with electromyography (EMG) and electroencephalography (EEG). Sedative properties were assessed using a validated CNS test battery. Pharmacokinetic sampling was performed. Data were analyzed using a mixed effects model. CBD did not have significant effects on single pulse and paired pulse TMS-EMG parameters, compared to placebo. Some significant clusters were seen on paired pulse TMS-EEG at 3h post-dose for 30 mg CBD, and at 3 and 5h post-dose for 700 mg CBD. CBD did not have significant effects on any tests assessing its sedative properties. These results suggest that CBD may lack intrinsic anti-epileptic and sedative properties and that its effects could be primarily a product of interactions with other drugs, notably clobazam.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

CBD reduces seizure frequency in Dravet syndrome, Lennox–Gastaut syndrome, and tuberous sclerosis. However, its intrinsic anti-epileptic action remains debated, as a pharmacokinetic interaction with clobazam is suspected to (at least in part) explain its efficacy and adverse effects.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study investigated whether CBD modulated human cortical excitability or produced measurable CNS effects in healthy volunteers at over-the-counter (30 mg) or therapeutic (700 mg) doses.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

CBD did not alter cortical excitability, as measured with TMS-EMG and single-pulse TMS-EEG. Although CBD-induced effects on paired-pulse TMS-EEG were detected, these lack a clear physiological interpretation. Compared with the pronounced signatures typically observed after single doses of established antiseizure medicines, these findings suggest that CBD alone does not meaningfully decrease cortical excitability. Furthermore, CBD did not affect vigilance, eye movements, balance, memory, or subjective state.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study provides further support to the idea that the anti-epileptic and adverse effects of CBD may be caused in large part by its interaction with clobazam, rather than by intrinsic pharmacological actions of CBD itself.

Introduction

Cannabidiol (CBD) is a non-intoxicating constituent of the cannabis plant.[1] CBD is available as an over-the-counter (OTC) health supplement and is increasingly used to reduce anxiety and to improve sleep and mood,[2] despite a lack of evidence for such use.[3] CBD is approved by the Federal Drug Administration (FDA) in the US and the European Medicines Agency (EMA) as an adjunctive treatment of seizures associated with Dravet syndrome (DS), Lennox-Gastaut Syndrome (LGS) and tuberous sclerosis (TSC).[4–6]

Although CBD has been demonstrated to reduce seizure frequency in the three rare epileptic syndromes,[1] there is no consensus on the underlying mechanism through which CBD exerts its anti-epileptic effects.[7] Several hypotheses have been proposed, including CBD-induced modulation of intracellular Ca^{2+} (either via antagonism of the G protein-coupled receptor 55 (GPR55),[8,9] or via desensitization of the vanilloid receptor 1 (TRPV1)),[8] inhibition of adenosine reuptake,[8] modulation of voltage-gated sodium channels (Na_v)[10–12] and modulation of the $GABA_A$ chloride current.[13]

At least in part, however, the therapeutic effects of CBD are thought to arise from an interaction with clobazam, another antiseizure medication (ASM), rather than from any intrinsic anti-epileptic action of CBD itself.[7,14] In the registration trials in DS and LGS patients, 56% of the patients assigned to CBD treatment used clobazam concomitantly,[7] and substantially greater reductions in seizure frequency were observed in these patients.[15] A pharmacokinetic (PK) interaction between the two drugs seems the most obvious explanation: clinical studies show that CBD, a CYP3A4 and CYP2C19 inhibitor, increases concentrations of N-desmethylclobazam, the active metabolite of the clobazam, in the order of 2.6 to 6-fold.[16–19] The *adverse* effects of CBD appear to be similarly driven by its interaction with clobazam. A meta-analysis of the data from the DS, LGS and TSC trials found the odds of adverse events to be almost four times higher in patients receiving CBD concomitantly with clobazam, with primarily the incidence of somnolence and sedation being increased,[1] both of which are typical adverse effects associated with clobazam.[20]

The magnitude of intrinsic anti-epileptic effects of CBD remains unclear due to the widespread concomitant clobazam use in the clinical trials in DS, LGS and TSC patients.[7,21] Illustrative of such uncertainty is the decision by the EMA to only approve CBD for treatment of DS and LGS (although not TSC), when combined with clobazam.[6] Our own research group has even argued that, based on clinical trial simulations, the PK interaction with clobazam sufficiently explains the full antiseizure effect of CBD in LGS, and that CBD might lack intrinsic anti-epileptic effects altogether.[14,22]

The main goal of this study was to evaluate the intrinsic anti-epileptic activity of CBD by measuring its effects on cortical excitability in healthy volunteers. Transcranial magnetic stimulation (TMS) is a technique that allows for non-invasive stimulation of the motor cortex.[23] Responses to such stimulation can be measured with electromyography (EMG) over contralateral muscles, allowing for assessment of neuronal excitability along the cortico-spinal tract, or using electroencephalography (EEG), which measures cortical excitability more directly.[24] Cortical excitability is a relevant measure of anti-seizure drug effects, as it is increased across different epilepsy types[25] and has been shown to normalize in patients who became seizure free after successful treatment with ASMs.[26] Demonstrating drug-induced changes in cortical excitability in healthy volunteers likely has high translational value to patient populations, as various approved, widely prescribed, efficacious ASMs have been found to reduce cortical excitability in healthy volunteers.[23,27] Accordingly, TMS-EMG and TMS-EEG are increasingly utilized in early-phase clinical trials of novel ASMs to identify potential anti-seizure effects in healthy volunteers.[24,28]

A secondary aim of this study was to assess the sedative properties of CBD using a validated test battery for assessment of central nervous system (CNS) drug effects, at CBD doses representative of both OTC and anti-seizure use.

Methods

PARTICIPANTS AND STUDY DESIGN

This study had a double-blind, randomized, placebo-controlled, 3-way crossover design. Each participant had three visits to the clinical research unit, during which the effects of two doses of CBD were compared to placebo. There was a 2-week washout period between each visit. The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands, between December 2023 and March 2024. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, the Netherlands) and conducted in compliance with the International Conference on Harmonization Good Clinical Practice guidelines. The study was registered under the European Union Clinical Trials Information System number 2023-508311-23-00.

Each participant provided written informed consent before study participation. Healthy males, aged 18-55 years, were selected following a medical screening according to protocol-specific inclusion and exclusion criteria (**Supplementary Materials**). Females were excluded due to potential menstrual cycle-related confounding effects on cortical excitability.[29] Participants with an increased risk of TMS-related complications based on the TMS safety questionnaire[30] were excluded.

STUDY DRUGS

The active formulation used in this study was Clinican®, a 10% CBD almond oil solution, with an orange flavoring liquid added to disguise the taste. The placebo formulation consisted of almond oil with an orange flavoring liquid. On each visit, participants received single oral doses of either 30 mg CBD (as 0.3 mL 10% CBD oil + 7 mL placebo), 700 mg CBD (as 7 mL 10% CBD oil + 0.3 mL placebo) or placebo (as 7.3 mL placebo).

The low dose of 30 mg CBD was deemed representative of OTC use.[31] The high dose of 700 mg CBD corresponded to the high end of the approved range for treatment of DS and LGS (twice daily dosing up to 10 mg/kg),[6] assuming a 70 kg individual.

Fasting was required for at least 4 hours prior to every scheduled visit. Shortly after arrival, participants received a semi-standardized light breakfast as described previously.[32] Participants remained fasted for at least 2 hours before and 1.5 hours after study drug administration, except for a small biscuit (~50 kCal) eaten directly after dosing to remove the taste of the study drug.

PHARMACODYNAMIC ASSESSMENTS

TMS

TMS measurements were done pre-dose for baseline and at 3 and 5 hours following dosing. TMS was performed in accordance with current guidelines,[33] using a MagPro R30 with MagOption stimulator and an MCF-B65 butterfly coil (2 × 75 mm) (MagVenture GmbH, Hueckelhoven, Germany). The motor hotspot of the dominant abductor digiti minimi (ADM) muscle was stimulated, as assessed by the Edinburgh Handedness Questionnaire.[34] The TMS coil was positioned tangentially to the skull and laterally at a 45° angle with the midline. After determining the resting motor threshold (RMT)[35] at the start of each measurement, a single pulse protocol was applied immediately followed by a paired pulse protocol. The single pulse protocol consisted of 75 single pulses at an intensity of 120% the RMT. The paired pulse protocol consisted of 75 pairs of pulses in randomized order with inter-stimulus intervals (ISI) of 2, 15 and 100 msec. Conditioning and test pulses were delivered at an intensity of 120% RMT, except for ISIs 2 and 15 msec, where 80% of RMT was used for the conditioning pulses. A random interval of approximately 4 sec (range 3.5 to 4.5 sec) was kept between single pulses and pairs of paired pulses. Adapted noise was applied to minimize auditory evoked potential generation.[36] EEG was continuously recorded during TMS using a 40-channel recording system (Refa-40, TMSi B.V., the Netherlands). Electrodes were placed according to the international 10-20 system (32-lead cap, ANT Waveguard), but replacing electrodes placed at the earlobes (i.e., A1 and A2) with electrodes placed at the mastoids (i.e., M1 and M2). The electrode impedance was kept below 5 kΩ and the ground electrode was placed between electrodes Fz and Fpz. EEG signals were recorded from 32 electrodes with a sample frequency of 2048 Hz. ADM muscle activity was continuously recorded using Ag/AgCl electrodes placed in a belly-tendon montage.

The following TMS-EMG parameters were extracted: mean single pulse peak-to-peak MEP amplitude (μV); paired pulse long intra-cortical inhibition at ISI 100 msec ($LICI_{100}$), defined as the percentage ratio of the mean MEP amplitude after the test pulses and the mean MEP amplitude after the conditioning pulses; and paired pulse short intra-cortical inhibition at ISI 2 msec ($SICI_2$) and intracortical facilitation at ISI 15 msec (ICF_{15}), defined as the percentage ratio of the mean MEP amplitude after the test pulses and the mean amplitude of the unconditioned single pulse MEPs. Details of the TMS-EEG data synthesis are provided in the **Supplementary Materials**.

NeuroCart ® CNS test battery

The CNS test battery was done twice pre-dose for baseline and repeated 2, 4 and 6 hours following dosing. Saccadic eye movements were employed as a sensitive measure of sedation.[37] Smooth pursuit eye movements and the adaptive tracking test assessed visuomotor coordination and vigilance.[38,39] The body sway task measured postural stability.[40] Visual Analogue Scales (VAS) according to Bond and Lader assessed study participants' subjective state using a series of horizontal bipolar scales ranging from 0 to 100, where values of 0 and 100 represented opposing subjective states and a value of 50 represented the neutral state.[41] Subjective psychedelic effects were evaluated using the VAS according to Bowdle on a scale of 0 to 100 mm.[42,43] Working

memory was evaluated using a computerised version of the N-Back test[44] and the Visual Verbal Learning Test (VVLT) evaluated episodic memory and learning behaviour. Further test procedure descriptions are provided in the **Supplementary Materials**.

PHARMACOKINETIC ASSESSMENTS

Venous blood samples were collected pre-dose and at 2, 3, 4, 5 and 6 hours post-dose. Plasma CBD concentrations were determined using a validated assay with a lower limit of quantification of 0.75 ng/mL by the iC42 Laboratory (Department of Anesthesiology, University of Colorado, Aurora, Colorado, USA).[45] Further details regarding the bioanalytical method are provided in the **Supplementary Materials**.

PK parameters were calculated using PKNCA package (version 0.9.5) in R v4.0.3 (R Foundation for Statistical Computing/R development Core Team, Vienna, Austria, 2019). All PK parameter calculations were based on actual sampling time. For the calculation of the PK parameters, concentrations below the limit of quantification were dropped (treated as missing). AUC was calculated using the log-linear trapezoidal rule.

SAMPLE SIZE, RANDOMIZATION AND BLINDING

The sample size calculation was based on TMS data obtained previously, and specifically the MEP amplitude.[27] A sample size of 24 had a power of 0.80 to detect a difference in means of -300 µV, assuming a SD of differences of 500 µV, using a paired t-test with a 0.05 two-sided significance level. This effect size of 300 µV was deemed a relevant magnitude, as we have shown previously that known effective ASMs (valproic acid, levetiracetam and lorazepam) produce effect sizes in this range.[27] The sample size calculation was performed with SAS v9.4 (SAS Institute Inc., Cary NC, USA).

Study staff and subjects remained blinded until database lock. The balanced Williams design randomization code was generated using SAS v9.4 by a study-independent statistician. Blinded study staff assigned the randomization numbers to the participants sequentially after medical screening.

STATISTICAL ANALYSIS

All pharmacodynamic parameters except for TMS-EEG, were analyzed with a mixed effects model with treatment, period, time and treatment by time as fixed factors, and participant, participant by treatment and participant by time as random factors, and the average baseline value as covariate. For the VAS Bowdle parameters (VAS 'Internal Perception', VAS 'External Perception', VAS 'Feeling High'), a constant value of 2 mm was added to each measurement to allow log-transformation and satisfy the model's normality assumption for residuals. The analysis results of VAS 'Feeling High' were subsequently back-transformed, reporting the estimated difference as a percentage change. Similarly, the MEP amplitude, all paired pulse TMS-EMG parameters and body sway were also log-transformed for analysis, with the estimated difference presented as percentage change after back-transformation. The general treatment effect and specific contrasts were reported with the estimated difference and 95% confidence intervals, the least square mean estimates, and the corresponding p-values. All calculations were performed using SAS for Windows v9.4 (SAS Institute, Inc., Cary, NC, USA). No adjustments for multiple comparisons were employed in accordance with the exploratory nature of this study.[46]

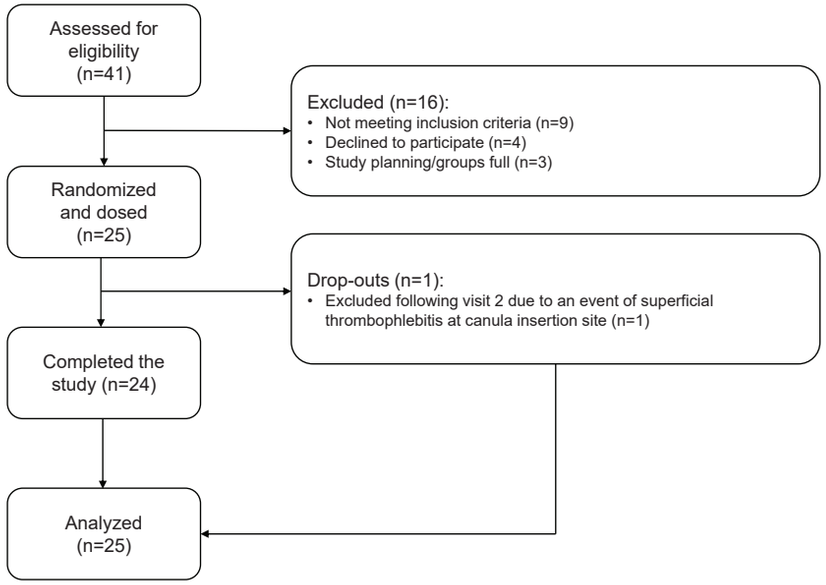
The effects of CBD and placebo on single and paired pulse TEPs were compared using cluster-based permutation analysis, a nonparametric method suited for multi-dimensional TMS-EEG data.[47] Dependent samples *t*-tests were used for electrode and time comparisons, clustering *t*-values with $P < 0.05$. Significant clusters were determined using permutation testing (1,500 permutations), and results were reported if less than 5% of the summed *t*-values obtained by permutation were larger than the cluster value observed in the original data. *P*-values of significant clusters are reported. In addition to analyzing the entire period between 0-300 ms, the same analysis was applied to specific time periods of interest (TOIs) around the characteristic TEP components (N15: 0-20 ms; P30: 20-40 ms; N45: 40-55 ms; P55: 55-80 ms; N100: 80-130 ms; P180: 130-230 ms).

Results

PARTICIPANTS AND DEMOGRAPHICS

A total of 41 male participants were screened, of which 25 were enrolled in the study and dosed at least once. A summary of the baseline demographics is provided in **Table S1**. Of the 25 dosed participants, one was excluded prior to completion due to an event of superficial thrombophlebitis (considered unrelated to the study drug by the investigator) and was replaced; 24 participants completed the trial per protocol (**Figure 1**).

Figure 1 Study flow diagram.



PHARMACODYNAMIC OUTCOMES

Single doses of 30 or 700 mg CBD had no significant effects, when compared to placebo, on the single pulse TMS-EMG parameters (peak-to-peak MEP amplitude (**Figure 2**) and rMT) and paired pulse TMS-EMG parameters ($LICI_{100}$, $SICI_2$ and ICF_{15}) (**Table 1**).

For single pulse TMS-EEG, single doses of 30 mg CBD significantly decreased the N15 TEP component (i.e. less negative) (**p=0.02**) compared to placebo in an ipsilateral centroparietal cluster at the 3 h post-dose timepoint (**Figure 3**).

For paired pulse TMS-EEG (ISI 100 ms), single doses of 700 mg CBD significantly decreased the N45 (i.e. less negative) (**p=0.01**) and increased the P60 TEP component (i.e. more positive) (**p=0.03**) compared to placebo in a contralateral centroparietal cluster at the 3 h post-dose timepoint. Similarly, at the 5 h post-dose timepoint, 700 mg CBD significantly increased the P30 (i.e. more positive) (**p=0.04**) and decreased the N45 (i.e.. less negative) (**p=0.04**) compared to placebo in a contralateral fronto-centroparietal cluster at ISI 100 ms (**Figure 4**).

Single doses of 30 or 700 mg CBD had no significant effects, when compared to placebo, on the CNS test battery parameters (saccadic and smooth pursuit eye movements, adaptive tracking test performance, postural stability, VAS 'Alertness', VAS 'Mood', VAS 'Calmness', VAS 'Internal Perception', VAS 'External Perception', 'Feeling High' and n-Back and VVLT test performance) (**Table 1**).

Figure 2 Change from baseline of the least square means (LSM) of the motor-evoked potential (MEP) amplitude (μV), using single pulse transcranial magnetic stimulation, for cannabidiol (CBD) and placebo.

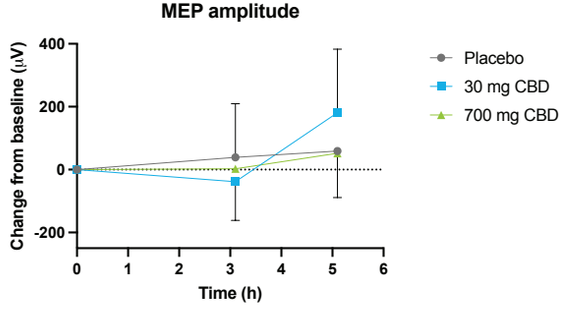
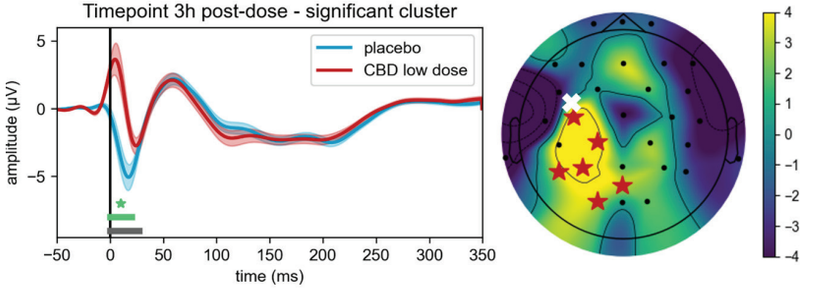
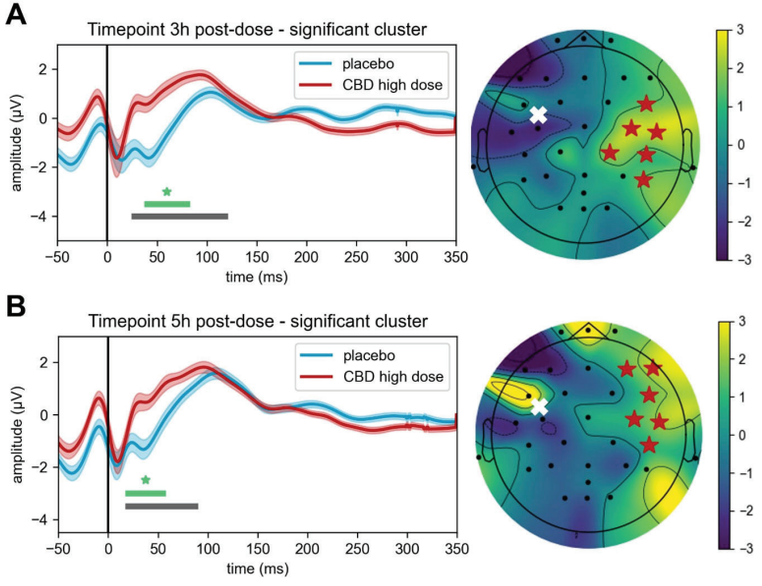


Figure 3 Grand average and topographical distribution of the significant single pulse TMS-EEG cluster. Overview of the significant cluster found at timepoint 3 hours post-dose when comparing TEPs of the placebo (in blue) and cannabidiol low-dose (in red) conditions for single pulse TMS. On the left side, the grand average (mean±SEM) over all significant electrodes is presented. On the right side, the difference in topographical distribution (cannabidiol – placebo) at the time of the cluster is presented. The thick green bar represents the time window of significant differences, the thick grey bar the time window of the entire cluster (including non-significant parts), the white cross the stimulation site, and the black dots the electrode positions with the significant electrodes as red stars.



Abbreviations: CBD, cannabidiol; ISI, interstimulus interval; TMS-EEG, transcranial magnetic stimulation – encephalography; SEM, standard error of the mean.

Figure 4 Grand average and topographical distribution of significant paired pulse TMS-EEG clusters. Overview of the significant clusters found when comparing TEPs of the placebo (in blue) and cannabidiol high-dose (in red) conditions for paired pulse TMS (ISI 100 ms) at A) timepoint 3 hours post-dose, and B) timepoint 5 hours post-dose. On the left side, the grand average (mean±SEM) over all significant electrodes is presented. On the right side, the difference in topographical distribution (cannabidiol – placebo) at the time of the cluster is presented. The thick green bar represents the time window of significant differences, the thick grey bar the time window of the entire cluster (including non-significant parts), the white cross the stimulation site, and the black dots the electrode positions with the significant electrodes as red stars.



Abbreviations: CBD, cannabidiol; ISI, interstimulus interval; TMS-EEG, transcranial magnetic stimulation – encephalography; SEM, standard error of the mean.

Table 1 Overall treatment effects on the pharmacodynamic outcome measures.

	Estimated difference (95% CI) p-value		LS Means		
Parameter	**CBD 30 mg vs Placebo**	**CBD 700 mg vs Placebo**	**Placebo**	**CBD 30 mg**	**CBD 700 mg**
SINGLE PULSE TMS-EMG					
Peak-to-peak amplitude (μV)	1.9% (-17.6%, 26.1%) p=0.86	-2.9% (-21.9%, 20.7%) p=0.78	740.27	754.56	718.54
Resting motor threshold (%MSO)	0.7 (-0.6, 2.1) p=0.28	-1.2 (-2.6, 0.1) p=0.08	58.0	58.8	56.8
PAIRED PULSE TMS-EMG[1]					
Short intracortical inhibition 2 ms (%)	-0.1% (-20.9%, 26.2%) p=0.99	6.5% (-15.7%, 34.5%) p=0.59	35.25	35.21	37.52
Intracortical facilitation 15 ms (%)	-7.2% (-27.2%, 18.1%) p=0.53	-14.9% (-33.2%,8.3%) p=0.18	131.34	121.84	111.72
Long intracortical inhibition 100 ms (%)	9.5% (-25.2%, 60.1%) p=0.63	-3.6% (-34.1%, 41.2%) p=0.85	3.40	3.73	3.28
Saccadic peak velocity (degrees/s)	-0.11 (-8.66, 8.43) p=0.98	-1.74 (-10.39, 6.91) p=0.69	489.74	489.63	488.00
Smooth pursuit eye movement (%)	-0.01 (-1.93, 1.90) p=0.99	-1.20 (-3.15, 0.74) p=0.22	51.03	51.02	49.83
Adaptive tracking (%)	0.01 (-1.16, 1.18) p=0.98	-0.59 (-1.77, 0.59) p=0.31	31.63	31.65	31.04
Body sway (mm)	-3.5% (-15.0%, 9.6%) p=0.58	-3.5% (-15.3%,9.9%) p=0.58	254.87	246.04	245.94
VAS BOND AND LADER					
VAS 'Alertness' (mm)	-0.6 (-1.9, 0.7) p=0.34	-0.9 (-2.2, 0.4) p=0.16	49.9	49.2	48.9
VAS 'Calmness' (mm)	1.5 (-0.5, 3.5) p=0.13	0.8 (-1.3, 2.8) p=0.45	52.1	53.6	52.9
VAS 'Mood' (mm)	-0.1 (-1.3, 1.1) p=0.89	-0.9 (-2.1, 0.4) p=0.18	51.9	51.8	51.0
VAS BOWDLE					
VAS 'External perception' (log(mm))	0.0083 (-0.0052, 0.0217) p=0.22	0.0045 (-0.0093, 0.0182) p=0.52	0.3336	0.3418	0.3380

(Continuation Table 1)

	Estimated difference (95% CI) p-value		LS Means		
Parameter	**CBD 30 mg vs Placebo**	**CBD 700 mg vs Placebo**	**Placebo**	**CBD 30 mg**	**CBD 700 mg**
VAS 'Internal perception' (log(mm))	-0.0016 (-0.0120, 0.0087) p=0.74	-0.0022 (-0.0129, 0.0084) p=0.67	0.3266	0.3250	0.3244
VAS 'Feeling High' (mm)	-4.7% (-11.2%, 2.3%) p=0.18	1.6% (-5.4%, 9.2%) p=0.64	2.16	2.06	2.20
N-BACK					
Reaction time 0-back (msec)	8.79 (-11.37, 28.94) p=0.38	10.45 (-9.36, 30.27) p=0.29	383.31	392.09	393.76
Reaction time 1-back (msec)	18.85 (-2.07, 39.76) p=0.08	15.95 (-5.32, 37.22) p=0.14	415.99	434.84	431.94
Reaction time 2-back (msec)	16.24 (-17.24, 49.71) p=0.33	-12.55 (-46.43, 21.34) p=0.46	513.96	530.20	501.42
Ratio correct 0-back	0.001 (-0.017, 0.020) p=0.88	-0.004 (-0.023, 0.015) p=0.67	0.964	0.965	0.960
Ratio correct 1-back	0.019 (-0.015, 0.054) p=0.26	0.022 (-0.013, 0.057) p=0.20	0.924	0.943	0.946
Ratio correct 2-back	0.014 (-0.030, 0.057) p=0.53	0.019 (-0.026, 0.063) p=0.40	0.895	0.908	0.913
VISUAL VERBAL LEARNING TEST					
Immediate recall trial 3 (N correct)	-0.6 (-2.0, 0.9) p=0.42	0.3 (-1.1, 1.8) p=0.65	17.6	17.0	18.0
Delayed recall (N correct)	-0.0 (-1.4, 1.4) p=0.99	-0.6 (-2.1, 0.8) p=0.37	13.2	13.2	12.6
Delayed recognition (reaction time correct) (msec)	25.6 (-21.9, 73.2) p=0.28	-0.5 (-48.7, 47.7) p=0.98	805.7	831.3	805.2
Delayed recognition (N correct)	0.2 (-1.1, 1.6) p=0.73	0.1 (-1.3, 1.5) p=0.85	24.2	24.5	24.3

Abbreviations: CBD, cannabidiol; CI, confidence interval; LS, least square; MSO, maximum stimulator output; N, number; TMS-EMG, transcranial magnetic stimulation-electromyography; VAS, visual analogue scale.

Footnotes: 1. All paired pulse TMS-EMG parameters were calculated as percentages, log-transformed for analysis and estimated differences back-transformed as percentage change for interpretation. The estimated differences in the TMS-EMG parameters reported here are thus percentage of a percentage (unit).

PHARMACOKINETIC OUTCOMES

After administration of 30 mg CBD, the mean±SD AUC_{last} was 20.3±8.4 h*ng/mL and the mean±SD C_{max} was 8.8±4.2 ng/mL (**Table 2**). Following the administration of 700 mg CBD, the mean±SD AUC_{last} was 931±413 h*ng/mL and the mean±SD C_{max} was 395±203 ng/mL (**Table 2**). The median (min, max) t_{max} for both dose levels was 3 (2, 4) hours. PK parameters increased more than dose-proportionally (**Table 2**). The concentration-time profiles of cannabidiol are displayed in **Figure 5**.

Figure 5 Plasma concentration levels over time following oral administration of A) 30 mg cannabidiol and B) 700 mg cannabidiol, displayed on a logarithmic scale.

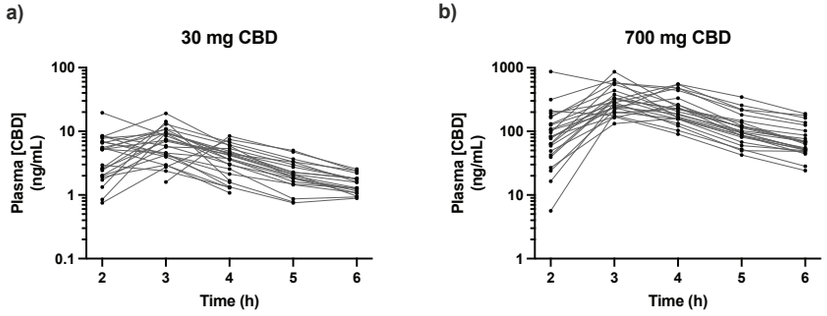


Table 2 Pharmacokinetic parameters of CBD following oral administration.

Treatment	Parameter	Unit	N	Mean	SD	CV%	Geometric Mean	Geometric CV%	Median	Min	Max
30 mg CBD	AUC_{last}	h*ng/mL	25	20.3	8.4	41.3	18.7	46.3	18.6	6.2	42.5
	C_{max}	ng/mL	25	8.8	4.2	47.4	7.9	49.9	8.43	2.9	19.6
	t_{max}	h	25						3	2	4
700 mg CBD	AUC_{last}	h*ng/mL	24	931	413.0	44.4	858	42.1	775	436	2213
	C_{max}	ng/mL	24	395	202.8	51.3	352	51.8	330	160	866
	t_{max}	h	24						3	2	4

Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; CBD, cannabidiol; C_{max}, maximum concentration; CV, coefficient of variation; max, maximum; min, minimum; N, number; SD, standard deviation; T_{max}, time to attain C_{max}.

Discussion

This study aimed to evaluate the intrinsic anti-epileptic properties of CBD by measuring its effects on cortical excitability with TMS-EMG and TMS-EEG, as well as CBD effects on vigilance, visuomotor coordination and memory.

EFFECTS OF CBD ON TMS-EMG MEASURES

No significant treatment effects of CBD were found on any TMS-EMG measure of cortical excitability, whereas literature reports consistent effects of various ASMs on TMS-EMG parameters,[23] and our research group has previously demonstrated such effects following single doses of lorazepam, valproic acid and levetiracetam in a similar population and experimental setup to the current study.[27] Therefore, the lack of significant changes following CBD administration suggests that CBD lacks an intrinsic anti-epileptic effect. More specific inferences regarding the mechanism of action can be made, as it is known that voltage-gated sodium channel blockers (e.g. carbamazepine and lamotrigine) increase the rMT, positive allosteric modulators of the $GABA_A$ receptor (i.e., benzodiazepines) decrease the MEP amplitude and ICF and increase SICI, and the specific $GABA_B$ receptor agonist baclofen increases LICI.[23] Levetiracetam, an ASM with an unclarified mechanism of action, decreased the MEP amplitude.[27] The absence of such findings in this study suggests that the putative anti-epileptic effect of CBD is not mediated by voltage-gated sodium channels, $GABA_A$ receptors or $GABA_B$ receptors, and has a different mechanism from levetiracetam.

EFFECTS OF CBD ON TMS-EEG MEASURES

CBD had significant treatment effects on TMS-EEG at both dose levels. The significant clusters at the 700 mg dose level are found both 3 and 5 hours post-dose and have the characteristics of a drug-induced effect. The significant clusters are consistent across the two timepoints, as they are found exclusively after paired-pulsed stimulation with an ISI of 100 ms, in a largely (although not perfectly) similar topographical distribution, and in (partially) overlapping temporal regions of interest (in both cases including the N45 peak). The magnitude of the effect is greater at 3 hours post-dose, which is consistent with the observed pharmacokinetic t_{max} of CBD.

Conversely, the significant cluster after single pulse stimulation at the 30 mg CBD dose level is more likely to represent a chance finding, as it is not reproduced after administration of 700 mg CBD. It could also be a stimulation artifact, which are more common in the TEP components closer to the test stimulus. In any case, the finding at 30 mg CBD appears irrelevant to the evaluation of anti-epileptic effects of CBD, since much higher doses are required to reduce seizure frequency in patients.

Interpretation of the significant clusters at the 700 mg CBD dose level is challenging. There is a general lack of understanding how paired-pulse TMS-EEG findings correspond to the underlying physiological processes. It is unclear whether the observed significant changes on the paired pulse TEPs signify a reduction or an increase of cortical excitability. One earlier study utilizing TMS-EEG found that baclofen, a $GABA_B$ receptor agonist, enhanced the N45 peak in a contra-lateral topographic region after paired-pulse stimulation at ISI of 100 ms,[48] which is similar to the enhancement of the N45 peak we observed with 700 mg CBD. However, it would be premature to conclude that CBD therefore is a $GABA_B$ receptor agonist. The same study reported effects on the N100 and P180 peaks (paired pulse), as well as single pulse TMS-EEG – which are all absent in our study. Furthermore, baclofen affects the LICI and has well-documented sedative effects, which we would expect to measure using TMS-EMG and the CNS test battery respectively, if CBD indeed acted as a $GABA_B$ receptor agonist.

One speculative explanation is that TMS-EEG, a method that measures cortical electrical activity directly and exclusively, was the only method sensitive enough to detect the effects of CBD. In contrast, TMS-EMG measures a peripheral response to central stimulation, and the CNS test battery offers even less direct tests of CNS function. However, we deem it unlikely that CBD, a drug that markedly reduces seizure frequency in hard-to-treat epileptic syndromes, would have such extremely subtle effects in comparison to other proven efficacious ASMs.

EFFECTS OF CBD ON THE CNS TEST BATTERY

The CNS test battery results certainly support the common characterization of CBD as a 'non-intoxicating' cannabinoid.[49] CBD did not differ significantly from placebo on any measure of vigilance, sedation, visuo-motor coordination, postural stability, subjective drug effects and working and episodic memory. This makes it highly unlikely that somnolence and lethargy, two frequently observed adverse effects of CBD, are attributable to CBD itself, and supports the hypothesis that their incidence is primarily driven by the

interaction with clobazam or other ASMs. To our knowledge, this study is the first to investigate CBD effects on postural stability and eye movements; the absence of significant treatment effects is in line with the general non-impairing character of CBD.

STRENGTHS AND LIMITATIONS

The main strength of our study lies in the use of TMS-EMG and TMS-EEG, which are validated tools for assessment of drug effects on cortical excitability. Whereas the assessment of anti-epileptic effects of CBD as a monotherapy in epilepsy patients may not be feasible for ethical and practical reasons, the use of TMS-EMG and TMS-EEG made such an assessment possible in healthy individuals instead. Further strengths of the study include a cross-over design, which allowed for within-subject comparisons, adequate power, and a relevant dose selection, with the high dose in line with doses used for treatment of epileptic syndromes (on a weight-adjusted basis) and the low dose representative of OTC CBD use for self-care. The validated CNS test battery assessed pharmacodynamic effects that had never been assessed for CBD previously.

Our study, however, is not without limitations. Most importantly, changes in cortical excitability in healthy (male) volunteers are a surrogate marker for anti-epileptic drug effects, and not the actual outcome measure of interest – which is seizure frequency reduction in patients. Although increased cortical excitability due to reduced cortical inhibition appears typical for most types of epilepsy,[23] a paradoxically reduced cortical excitability has been found in a population of LGS patients.[50] Therefore, a translation of our study results to patient populations should happen with caution: although CBD did not clearly reduce cortical excitability in this study, it does not rule out that CBD could have an intrinsic anti-epileptic effect. However, the results of this study make this less likely, as most anti-epileptic drugs typically show clear and characteristic effects on cortical excitability in healthy participants, whereas CBD does not.[23]

It is possible that CBD does in fact reduce cortical excitability, but this study erroneously failed to show this. A potential reason could be the administration of single doses of CBD, whereas reductions of cortical excitability may require a chronic treatment regimen to manifest. Another possibility is that CBD reduces cortical excitability in patients, just not in healthy individuals. However, we consider these possibilities less likely, since single doses of typical ASMs (levetiracetam, valproic acid, lorazepam) clearly reduced cortical excitability in healthy individuals in a similar study set-up.[27] Clinical studies with a longer treatment duration could provide clarity on these questions.

Although the findings of this manuscript cast doubt on the intrinsic anti-epileptic effects of CBD, it is important to mention the evidence to the contrary. Meta-analyses that pooled patients not using clobazam from registration studies in LGS and DS found significant seizure reduction compared to placebo – although with a considerably smaller effect size compared to clobazam users.[1,15,21] However, such subgroup meta-analyses are acknowledged to have important methodological limitations, i.e. their post-hoc nature, lack of randomization for clobazam use and the pooling across different seizure syndromes.[21] The more recent registration study in patients with TSC found that CBD significantly reduced seizure frequency, while including a lower – although still sizeable – proportion of clobazam users (approx. 25%, vs 56% in DS/LGS trials).[51] Nevertheless, it remains unclear whether the overall treatment effect for CBD would differ significantly from placebo, if clobazam users were excluded from analysis. Uncontrolled retrospective studies found no association between concomitant clobazam use and seizure frequencies, but such study designs cannot be relied upon to provide definitive conclusions.[52,53] Ultimately, the conclusive proof that CBD is an anti-seizure medication in absence of concomitant clobazam can only come from randomized controlled trials in defined populations that are adequately powered to answer this specific question.

PHARMACOKINETICS

This study reached clinically meaningful CBD exposures, as measured plasma concentrations at the 700 mg dose level were in line with findings in adult epileptic patients, who responded to treatment with CBD.[54] The CBD plasma concentrations increased more than dose proportionally, and the C_{max} and the AUC at the 700 mg dose level were approximately double of what would be expected based on the concentrations measured at 30 mg CBD and assuming a linear dose-exposure relationship. Whereas previous publications have established the dose-proportionality of Epidyolex® in the therapeutic range (5-10 mg/kg twice daily),[55] our finding suggests a less than dose proportional exposure at the sub-therapeutic dose of 30 mg (0.43 mg/kg, assuming a 70 kg individual). Alternatively, the non-linearity in absorption could be specific to the formulation used in this study. A speculative, although hypothetically

plausible, explanation is the possibility of auto-inhibition of CBD metabolism, as CBD is both an inhibitor and substrate of the CYP2C19 and CYP3A4 enzymes.[56,57]

CONCLUSION

This study found no evidence that CBD *reduces* cortical excitability. There were no significant treatment effects on TMS-EMG and single pulse TMS-EEG measures of cortical excitability, whereas other ASMs are known to produce typical and mechanism-specific changes on these measures. CBD does seem to *affect* cortical excitability as measured with paired pulse TMS-EEG – although further interpretation is challenging, because of the limited current understanding of how paired pulse TMS-EEG measures relate to the underlying physiology. The absence of an obvious reduction in cortical excitability casts doubts on the extent, or possibly, the very existence of intrinsic anti-epileptic effects of CBD. Such doubts are further exacerbated by the unclarified mechanism of action, a lack of clinical trials demonstrating efficacy in absence of concomitant ASMs, and the prominent pharmacokinetic interaction with clobazam. The absence of sedative effects on a sensitive battery of CNS tests suggests that typical *adverse* effects associated with CBD may also be primarily a product of its interactions with other drugs.

In conclusion, although addition of CBD reduces seizure frequency in DS, LGS and TCS patients, it remains to be demonstrated that CBD has actual anti-seizure properties by itself, and adequately powered, randomized, controlled trials specifically designed to address this question are needed.

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CHAPTER 4: SUPPLEMENTARY MATERIALS

Full supplementary materials available at the publisher's website:

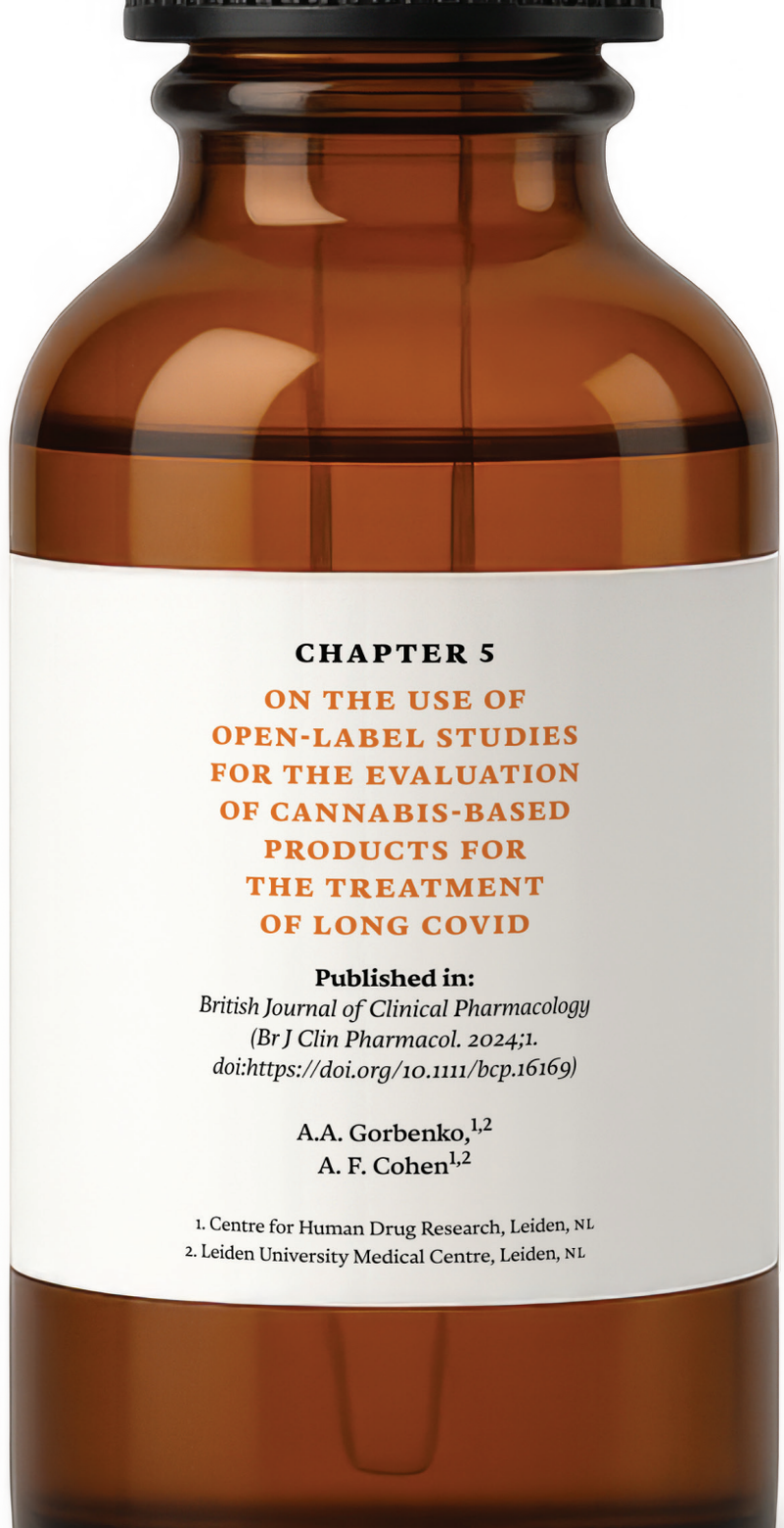
https://tinyurl.com/5hatecs8

Selected supplementary materials are provided below.

Table S1 Baseline demographics.

	All participants (N = 25)
AGE (YEARS)	
Mean (SD)	24.2 (4.6)
Median (Min, Max)	22 (19, 39)
HEIGHT (CM)	
Mean (SD)	183.0 (6.5)
Median (Min, Max)	183.1 (171.8, 199.2)
WEIGHT (KG)	
Mean (SD)	77.3 (10.3)
Median (Min, Max)	78.1 (58.4, 95.1)
BMI (KG/M^2)	
Mean (SD)	23.1 (2.8)
Median (Min, Max)	22.6 (19.4, 28.8)
SEX, N (%)	
Male	25 (100%)
RACE, N (%)	
Mixed	1 (4.0%)*
Other	1 (4.0%)**
White	23 (92.0%)

Abbreviations: BMI, body mass index; max, maximum; min, minimum; N, number; SD, standard deviation.
**Black or African-American/White*
***North African*



CHAPTER 5

ON THE USE OF OPEN-LABEL STUDIES FOR THE EVALUATION OF CANNABIS-BASED PRODUCTS FOR THE TREATMENT OF LONG COVID

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Dear Editor,

The publication in your journal by Thurgur et al. on the feasibility of treatment of patients with Long COVID symptoms with a cannabidiol (CBD)-dominant cannabis-based medicinal product[1] was concerning to us.

Our concerns primarily revolve around the manuscript's alignment with the pharmacological focus of the journal. Pharmacology traditionally examines drug substances and their effects on (patho-)physiological processes, including measurable outcomes in defined populations. However, in this open-label study, a diverse herbal composition was administered to a heterogeneous population without a well-defined pathophysiological basis. This design makes it challenging to draw any conclusions on drug effects and safety and limits the study's contribution to the field of pharmacology.

The study in question utilized a cannabis extract containing multiple potentially pharmacologically active cannabinoids other than CBD, like THC, cannabichromene, cannabigerol, and cannabidivarin. This raises questions about the specificity of the treatment towards the main hypothesized mechanisms of action: the purported anti-inflammatory effects of CBD and down-regulation of long COVID-related proteins by CBD and cannabivarin. The extract contains THC, which is known to be pharmacologically active and intoxicating in doses administered in this study, and little is known with regards to cannabigerol and cannabichromene.[2,3]

Furthermore, a dose justification is lacking. Authors have administered up to 150 mg of CBD, 'based on current clinical practice'. However, a referral to current clinical practice is only acceptable when clear evidence of clinical efficacy is present, and no such evidence exists (yet) for anti-inflammatory properties of CBD. Instead, the authors should have considered via which mechanism CBD is hypothesized to exert its anti-inflammatory effects, at which target tissue concentrations, and what human doses are needed to reach these target tissue concentrations.

The inclusion and exclusion criteria allowed for recruitment of a broad and diverse range of participants with varying symptoms. This introduced variability that could be expected to affect the study's applicability and reproducibility. The pathophysiological target intended for CBD intervention wasn't quantified and may not have been present at all. Moreover, the pathophysiological basis of long COVID still remains to be established,[4] which raises further questions about the rationale of generally targeting 'inflammation' for its treatment.

The relevance of the above considerations is in no way diminished by the primary goal of the study being the assessment of trial 'feasibility', which was defined by the authors as a combination of recruitment, safety and tolerability. Conclusion drawn from the current study may no longer apply in a future study, if a sound pharmacological rationale reveals the need for a different dose, formulation or study population.

In summary, we suggest that a closer adherence to basic principles of clinical pharmacology would greatly increase the relevance and scientific contribution of future studies on the topic.

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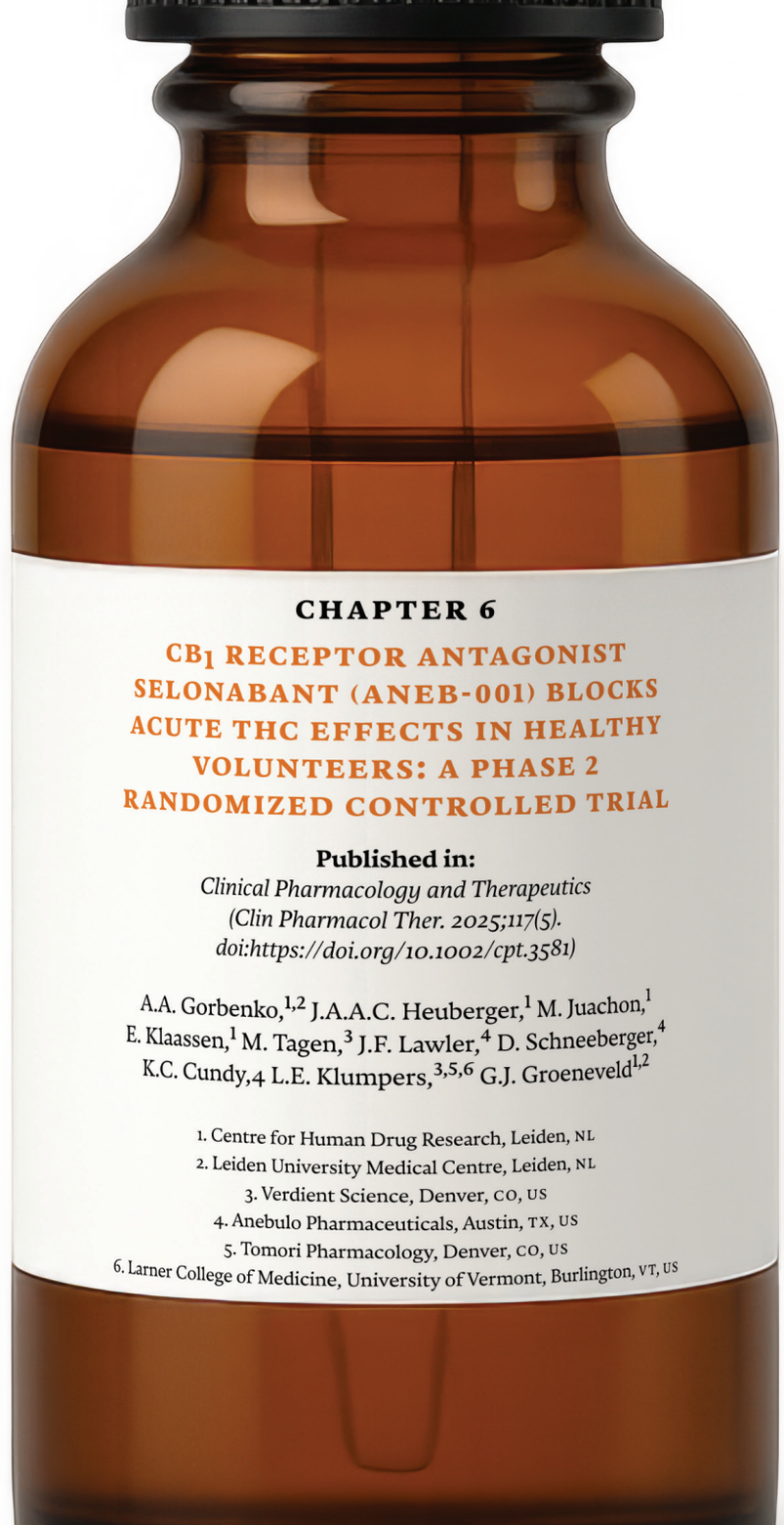

CHAPTER 6

CB_1 RECEPTOR ANTAGONIST SELONABANT (ANEB-001) BLOCKS ACUTE THC EFFECTS IN HEALTHY VOLUNTEERS: A PHASE 2 RANDOMIZED CONTROLLED TRIAL

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Abstract

Emergency department visits due to cannabinoid-induced toxicity, including acute cannabinoid intoxication (ACI) have increased worldwide as more states have liberalized cannabis policy. ACI symptoms include anxiety, panic attacks, tachycardia, and psychosis, primarily mediated through cannabinoid type 1 receptor (CB_1) agonism by Δ^9-tetrahydrocannabinol (THC). This Phase 2 randomized, double-blind, placebo-controlled study assessed the potential of CB_1 receptor antagonist selonabant (ANEB-001) to block THC-induced effects in healthy adults.

In Part A of the study, 10.5 mg THC was co-administered with 50 mg (N=20) or 100 mg (N=20) selonabant, or matching placebo (N=20). In Part B, 21 mg THC was co-administered with 30 mg (N=9) or 10 mg (N=7) selonabant, or matching placebo (N=9). THC-related effects were assessed using visual analogue scales (VAS) for feeling high and alertness, objective measures of postural stability and heart rate and analyzed using a mixed effects model. Selonabant significantly reduced VAS 'Feeling High' (up to -82.8% (95% CI: -91.0%, -67.2%, p<0.0001) at 30 mg selonabant) and increased VAS 'Alertness' (up to 10.8 mm (95% CI: 4.7, 16.8 mm, p=0.001) at 30 mg selonabant) vs placebo. Selonabant 10 and 30 mg significantly reduced body sway (up to -30.6% (95% CI: -44.1%, -13.9%, p=0.002) at 30 mg selonabant) vs placebo. Effects on heart rate were not significant. Selonabant was generally safe and no clinically meaningful changes in mood occurred. Nausea and vomiting occurred more frequently at high selonabant doses; 10 mg selonabant was both well-tolerated and efficacious. Present results support further development of selonabant for emergency treatment of ACI.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The incidence of cannabinoid-induced toxicity, including acute cannabinoid intoxication is increasing due to a liberalization of cannabis policy. Currently, there are no approved treatments that specifically target the cause of acute cannabinoid intoxication.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study assessed whether CB_1 receptor antagonist selonabant blocked the acute effects of Δ^9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis, in healthy volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Selonabant largely blocked the acute effects of THC intoxication at oral THC doses of up to 21 mg and was safe and well tolerated.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These result provide a basis for further development of selonabant as a treatment for cannabinoid-induced toxicity, such as acute cannabinoid intoxication.

Introduction

Cannabis is the most widely used recreational drug in the world.[1,2] Cannabis use has further increased worldwide following legalization and decriminalization in many states; as of November 2023, the number of cannabis users in the US alone exceeded 60 million.[3] In parallel, the potency of cannabis has increased over time, with the average content of Δ^9-Tetrahydrocannabinol (THC), its main psychoactive constituent, tripling between the years 1994 and 2014 in the US.[4] Consumers now also have access to cannabis extracts, which typically contain more than triple the amount of THC compared to cannabis flowers,[5] cannabis resins, which had an average THC concentration of 24.8% in the European Union in 2022,[6] and various edible cannabis products, such as chocolates, brownies, and gummies, which are visually appealing to children and easily mistaken for regular foods.[7,8] Additionally, synthetic cannabinoids have emerged as unregulated alternatives to botanical cannabis products.[9] Synthetic cannabinoids are more potent agonists of the CB_1 receptor compared to THC and are associated with more frequent and severe toxicities.[9] As a result of these factors, the incidence of emergency department visits related to cannabinoid-related toxicity, including acute cannabinoid intoxication (ACI) and cannabis hyperemesis syndrome has continued to grow.[8,10–12] As of 2018, there were approximately 1.7 million emergency department visits in the US associated with cannabis exposure.[13] Of these cannabis-related emergency department cases, approximately 25% are attributable to a direct effect of cannabis.[14]

Typical effects of cannabis in recreational users may include euphoria, sensory distortion and increased appetite.[15] Common adverse effects of ACI in adults are cognitive and motor impairment, anxiety, paranoia, tachycardia, and postural hypotension.[16] In adults presenting to emergency departments, adverse effects of high cannabis doses more commonly include neuropsychiatric symptoms, such as panic attacks, depersonalization, and acute psychosis.[16] In children, unintentional exposure to THC can result in severe complications, such as profound sedation, seizures, and respiratory depression.[16] Currently there are no approved therapies available for targeted treatment of cannabinoid-induced toxicity and management typically consists of supportive care.

THC acts primarily though partial agonism of the cannabinoid 1 receptor (CB_1)[17] and therefore antagonism of this receptor represents an opportunity for targeted treatment of cannabinoid-induced toxicity. In prior clinical studies, several CB_1 antagonists, including drinabant, surinabant, and rimonabant, were shown to reduce THC effects when administered prophylactically, several hours prior to cannabis inhalation.[18–20] However, the potential of a CB_1 antagonist as an acute treatment for established cannabinoid intoxication has not been explored to date. Although the CB_1 antagonist rimonabant was originally approved in Europe for treatment of obesity, chronic dosing was found to be associated with long term psychiatric adverse effects.[21] In contrast, emergency treatment of cannabinoid-induced toxicity represents an opportunity for acute use of a potent CB_1 antagonist while potentially avoiding the potential risk of long-term adverse effects.

Selonabant (ANEB-001; formerly V24343) is a CB_1 receptor antagonist that was originally under development as a potential treatment for obesity. In early phase trials (unpublished), selonabant was well-tolerated and significantly reduced caloric intake and body weight in healthy overweight or mildly obese subjects. To explore the potential of single oral doses of selonabant as an emergency treatment for ACI, a Phase 2 clinical study was conducted in healthy adult volunteers, in which selonabant was co-administered with oral THC. Here we report on the initial part of the larger study, designed to assess safety, PK, and the potential for coadministration of selonabant to block the effects of THC over a range of dose levels.

Methods

STUDY DESIGN

This study was a Phase 2 double-blind, randomized, placebo-controlled parallel-arm study, in which the effects of single oral doses of selonabant were compared to placebo co-administered with a single oral dose of THC. As a part of a larger study, the current report focuses on data from the co-administration of selonabant with THC. In Part A, 60 participants were randomized to three parallel treatment arms (20 participants/arm). All participants received a single oral dose of a 10.5 mg THC co-administered with one of three treatments: a single oral dose of selonabant (50 mg or 100 mg) or matching placebo. In Part B, two sequential cohorts of participants (target N=15 per cohort) each received a single oral dose of 21 mg THC, co-administered with 30 mg selonabant or placebo (2:1 active/placebo) in Cohort 1 and 10 mg selonabant or placebo in Cohort 2.

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 prospectively with the IRCTN registry under registration number: ISRCTN45282100 and registered to clinicaltrials.gov under NCT05282797.

STUDY PARTICIPANTS

Each subject provided written informed consent before any screening procedures were performed. All participants were healthy volunteers between 18 and 45 years of age with a body mass index of 18 to 30 kg/m^2. Initially, only males were included; during Part A the protocol was amended to allow inclusion of females. The participants underwent a full medical screening, including patient reported medical history, a physical examination, blood chemistry and hematology, urinalysis and an electrocardiogram (ECG) to assess eligibility. Participants with a clinically significant known medical condition, particularly any psychotic disorder, clinically significant mood disorder, suicidal ideation in the past 5 years, or any life-time suicide attempts, were excluded.

All included participants were occasional cannabis users for at least 1 year prior to screening, with a lifetime cannabis use of at least 10 times and recent cannabis use not exceeding once per week on average in the 6 months prior to study participation. The participants refrained from cannabis use from at least 3 weeks prior to dosing until the end of the study. Any participant who was a regular user of any illicit drugs (other than the casual use of cannabis) or had a history of drug abuse or a positive drug screen at screening, was excluded. The full list of inclusion and exclusion criteria is provided in the **Supplementary Materials**.

STUDY DRUGS

Selonabant was administered as oral capsules containing 10 or 50 mg selonabant. Matching placebo capsules were administered to participants randomized to placebo.[18,19] THC was administered as oral tablets containing 1.5 mg THC per tablet (Namisol®, Echo Pharmaceuticals). All treatments were administered in a fasted state.

Selonabant (ANEB-001; formerly V24343) was previously studied in Phase 1 clinical trials of safety, PK, tolerability, and efficacy as a treatment for obesity (unpublished data from Vernalis).[22] This is the first clinical study to examine selonabant when administered in combination with THC. The selonabant dose levels in Part A of the study were selected based on the selonabant doses demonstrating acceptable tolerability and efficacy in reducing body weight. The initial THC dose of 10.5 mg was chosen based on historical data showing induction of typical psychotropic effects while being safe and well-tolareted.[23] Based on results from Part A of the study, Part B subsequently evaluated lower dose levels of selonabant in combination with a higher dose level of THC.

PHARMACODYNAMIC ASSESSMENTS

Pharmacodynamic assessments of THC effects were performed on the dosing day twice pre-dose for baseline measurements and at 1, 2, 3, 4, 5, and 8 hours post-dose. In order to minimize potential learning effects, all participants were acquainted with the pharmacodynamic tests within 3 weeks prior to dosing.

Primary pharmacodynamic assessments

The Bowdle Visual Analogue Scale (VAS), an instrument for evaluating subjective psychedelic effects, was performed in this study to assess the subjective outcome of 'Feeling High', using a scale of 0 – 100 mm.[24,25] The Bond and Lader VAS was used to evaluate scores from a series of horizontal bipolar scales related to how a person feels, ranging from 0 to 100, where values of 0 and 100 represented opposing subjective states and a value of 50 represented the neutral state. From these measurements, the outcome for 'Alertness' was calculated as described in previous publications.[26]

Postural stability was measured objectively using a pot string meter based on the Wright ataxiameter.[27] With a string attached to the waist, subjects were asked to stand still with their eyes closed for a period of 2 minutes. All anteroposterior body movements over time were integrated and expressed as body sway in mm.

Heart rate measurements were performed pre-dose as baseline and at 20 min, 40 min, 1, 2, 3, 4, 5, 8 and 22-24 hours post-dose using Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400 automated devices after 5 minutes in supine position.

Secondary pharmacodynamic assessments

Secondary pharmacodynamic assessments included VAS 'Internal Perception' and VAS 'External Perception' according to Bowdle,[24,25] VAS 'Mood' and VAS 'Calmness' according to Bond and Lader,[26] saccadic and smooth pursuit eye movements,[28,29] pupillometry,[30] state-trait anxiety inventory (STAI),[31] adaptive tracking,[32,33] and N-back,[34] performed as described elsewhere.

PHARMACOKINETIC ASSESSMENTS

Venous blood samples were taken pre-dose and 0.5, 1, 2, 3, 4, 6, and 8 hours following dosing. Plasma selonabant, THC and 11-OH-THC concentrations were determined using a validated LC-MS/MS method (described further in the **Supplementary Materials**).

SAFETY ASSESSMENTS

Safety and tolerability were assessed by adverse event (AE) monitoring, clinical laboratory tests, vital signs, ECGs and physical and neurological examinations. The Beck Depression Inventory, the second edition, Dutch version (BDI-II-NL) was used to measure depressive symptoms and the Columbia Suicide Severity Rating Scale (C-SSRS), was performed throughout the study in order to monitor suicidal ideation and behavior.

SAMPLE SIZE AND RANDOMIZATION

VAS 'Feeling High' was used for the sample size calculation, as it was shown previously to be sensitive to THC intoxication, as well as prophylactic inhibition of the effect by CB_1-receptor antagonists.[18–20] For Part A of the study, a sample size of 20 per group was calculated to have a power of 0.986 to detect an inhibition of 50% of the VAS 'Feeling high', assuming a log normal distribution of VAS 'Feeling High', a coefficient of variation (CV%) of 55% (CV% defined as ratio of the standard deviation to the mean expressed as a percentage), and using a two-sample t-test with a 0.05 2-sided significance level. The emergent data from Part A were used for a sample size calculation for Part B of the study. Assuming a CV% of 55%, a sample size of 10 participants per treatment group had a power of 0.814 to detect an inhibition of VAS 'Feeling high' of 50%. For this reason, a treatment group size of N=10 was considered sufficient for Part B of the study.

Study staff and participants remained blinded until database lock. The randomization code was generated using SAS version 9.4 by a study-independent statistician. Blinded study staff assigned the randomization numbers to the participants sequentially after medical screening.

STATISTICAL ANALYSIS

To establish whether significant treatment effects could be detected, the endpoints were analyzed with a mixed effects model with treatment, time, and treatment by time as fixed factors and subject as random factor and the average baseline measurement as covariate. Post-dose measurements that were performed outside a 10% time window around the scheduled protocol time were excluded from analysis. For VAS 'Feeling High', a constant value of 2 mm was added to each measurement to allow log-transformation and satisfy the model's normality assumption for residuals; subsequently, the analysis results were back-transformed for reporting. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA). No adjustments for multiple comparisons were employed. The incidence of treatment-emergent AEs (TEAEs), defined as AEs that occurred or worsened after study treatments, was summarized.

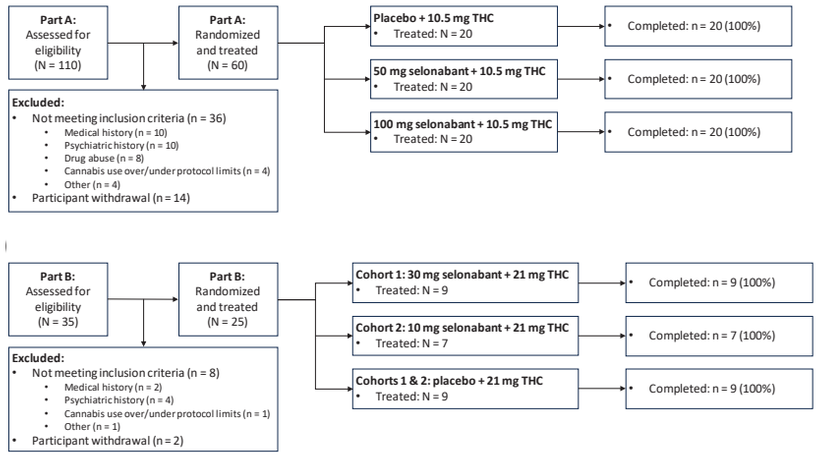
A pre-specified interim analysis of unblinded PK, PD and safety data was performed following completion of Part A. In Part B, each cohort was followed by a blinded review of PK, PD and safety data in a dose evaluation meeting.

Results

PARTICIPANTS AND DEMOGRAPHICS

The clinical phase of Part A, and Part B, cohorts 1 and 2 of the study, ran from December 2021 to September 2022. For Part A of the study, 110 participants were screened and 60 participants were dosed. For Part B, cohorts 1 and 2, 34 participants were screened and 25 participants were dosed, 5 less than the planned 15 participants per cohort due to recruitment challenges. There were no discontinuations and all dosed participants completed the study and were evaluated for pharmacodynamic, pharmacokinetic, and safety outcomes (**Figure 1**). The participant demographics are summarized in **Table S1**.

Figure 1 CONSORT study flow diagram.



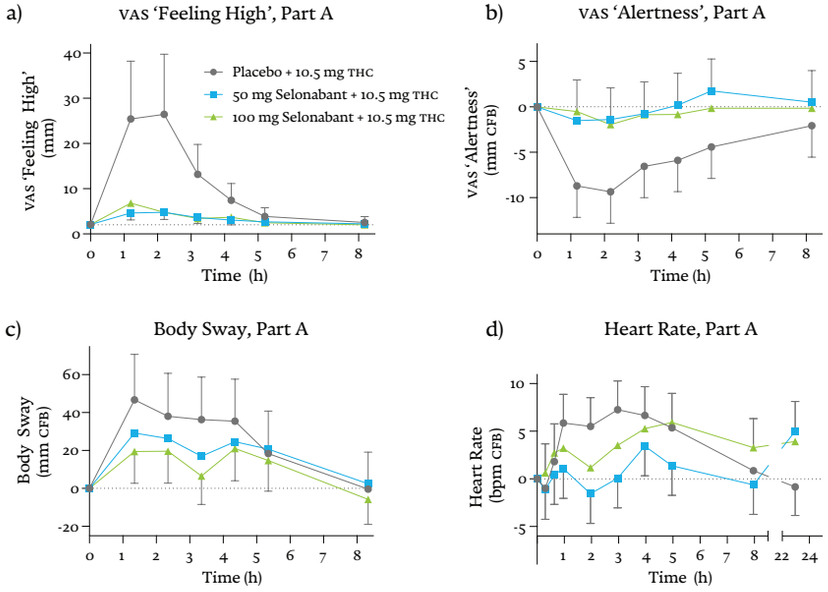
PHARMACODYNAMIC OUTCOMES

Primary:

The statistics of the primary pharmacodynamic results are summarized in **Table 1**. All measurements fell within the 10% time window around the planned timepoints and were included in the analysis.

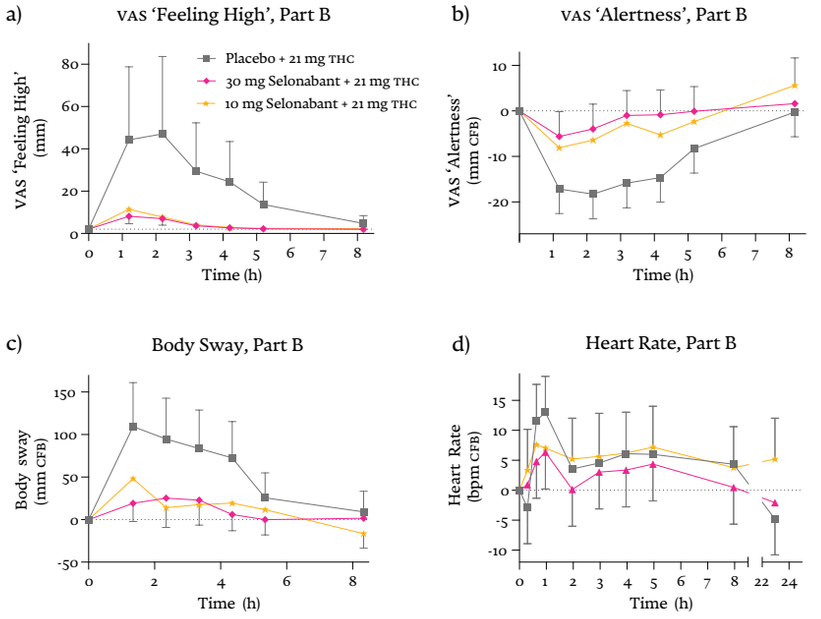
In Part A of the study, where a 10.5 mg THC was administered to all participants, selonabant significantly reduced mean VAS 'Feeling High' compared to placebo at both 50 mg (estimated difference (ED)): -63.7%, 95% confidence interval (CI): -77.3%, -41.8%, p<.0001) and 100 mg (ED: -61.8%, 95% CI: -75.8%, -39.4%, p<0.0001) dose levels (**Figure 2a**). The VAS 'Alertness' remained approximately at baseline throughout the study day for participants treated with selonabant, whereas a pronounced decrease in VAS 'Alertness' was observed in placebo participants; the difference from placebo treated participants was significant for both 50 mg (ED: 6.0 mm, 95% CI: 1.8, 10.1 mm, p=0.006 and 100 mg selonabant (ED: 5.4 mm, 95% CI: 1.3, 9.6 mm, p =0.011) treatment groups (**Figure 2b**). Selonabant did not significantly reduce the overall mean body sway or heart rate when compared to placebo in Part A of the study (**Figure 2c, 2d**).

Figure 2 Least Square Means of (A) VAS 'Feeling High' (absolute values in mm), (B) VAS 'Alertness' (change from baseline in mm), (C) Body sway (change from baseline in mm), (D) Heart rate (change from baseline in bpm), Part A of the study. Means are displayed with 95% confidence intervals.



In Part B of the study, where the THC dose was increased to 21 mg, the lower doses of 30 mg (ED: -82.8%, 95% CI: -91.0%, -67.2%, p<0.0001) and 10 mg of selonabant (ED: -80.4%, 95% CI: -90.5%, -59.5%, p<0.001) significantly reduced the mean VAS 'Feeling High' compared to placebo (**Figure 3a**). Similar to Part A, the VAS 'Alertness' remained close to baseline for the selonabant treatment groups in Part B of the study, whereas the placebo group had a notably decreased VAS 'Alertness', with the difference between selonabant and placebo being statistically significant for both 30 mg (ED: 10.8 mm, 95% CI: 4.7, 16.8 mm, p=0.001) and 10 mg (ED: 9.2 mm, 95% CI: 3.1, 15.3 mm, p=0.005) selonabant groups (**Figure 3b**). Both 30 mg (ED: -30.6%, 95% CI: -44.1%, -13.9%, p=0.002) and 10 mg (ED: -29.3%, 95% CI: -44.3%, -10.2%, p=0.007) selonabant treatments significantly reduced body sway compared to placebo in Part B of the study (**Figure 3c**). Selonabant did not significantly reduce the overall mean heart rate compared to placebo in Part B of the study (**Figure 3d**).

Figure 3 Least Square Means of (A) VAS 'Feeling High' (absolute values in mm), (B) VAS 'Alertness' (change from baseline in mm), (C) Body sway (change from baseline in mm), (D) Heart rate (change from baseline in bpm), Part B of the study. Means are displayed with 95% confidence intervals.



Secondary:

Selonabant significantly reduced VAS 'External Perception' compared to placebo at all dose levels, and VAS 'Internal Perception' in Part B only (**Table S2**). Selonabant significantly improved the adaptive tracking performance compared to placebo in Part B, but not Part A (**Table S2**). For the remaining secondary pharmacodynamic outcome measures (VAS 'Mood', VAS 'Calmness', STAI, saccadic and smooth pursuit eye movements, pupillometry and n-back), significant treatment effects were either absent, or identified at a single dose level only, and are not reported here further.

PHARMACOKINETICS

The concentration-time profiles of selonabant are shown in **Figure 4** and those of THC and 11-OH-THC in **Figure S3**. An overview of the pharmacokinetic parameters for all analytes is provided in **Table S4**.

Table 1 Overall treatment effects on main pharmacodynamic outcome measures (estimated mean differences with 95% CI and p-values).

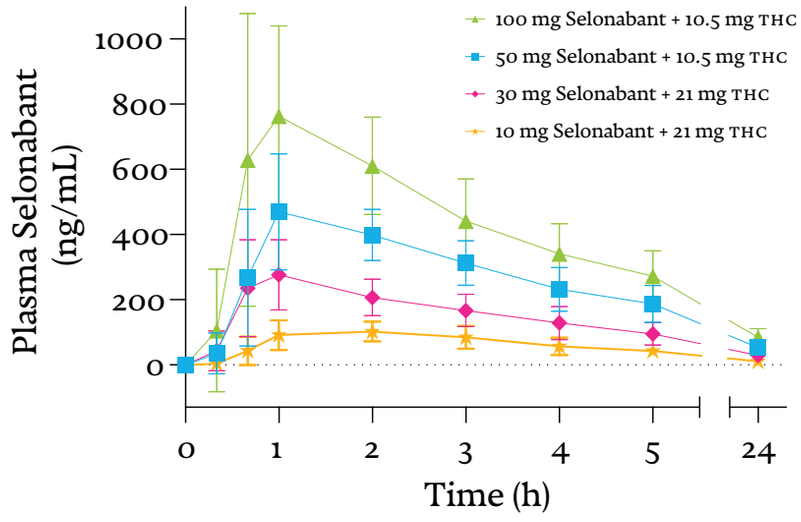
Study part	**Part A (10.5 mg THC)**		**Part B (21 mg THC)**	
Parameter	**100 mg Selonabant vs Placebo**	**50 mg Selonabant vs Placebo**	**30 mg Selonabant vs Placebo**	**10 mg Selonabant vs Placebo**
VAS 'Feeling High' (%)	**-61.8% (-75.8%, -39.4%) p<0.0001**	**-63.7% (-77.3%, -41.8%) p<0.0001**	**-82.8% (-91.0%, -67.2%) p<0.0001**	**-80.4% (-90.5%, -59.5%) p<0.001**
VAS 'Alertness' (mm)	**5.4 (1.3, 9.6) p=0.011**	**6.0 (1.8, 10.1) p=0.006**	**10.8 (4.7, 16.8) p=0.001**	**9.2 (3.1, 15.3) p=0.005**
Body sway (%)	-12.4% (-26.2%, 4.1%) p=0.129	-6.5% (-21.3%, 11.0%) p=0.436	**-30.6% (-44.1%, -13.9%) p=0.002**	**-29.3% (-44.3%, -10.2%) p=0.007**
Heart rate in supine position (bpm)	-0.2 (-3.0, 2.6) p=0.881	-2.6 (-5.3, 0.1) p=0.062	-2.2 (-8.6, 4.1) p=0.473	1.1 (-5.2, 7.4) p=0.726

The figures in bold indicate statistical significance. Abbreviations: bpm, beat per minute; CI, confidence interval; THC, Δ^9-tetrahydrocannabinol; VAS, visual analogue scale.

Peak concentrations of selonabant occurred at a median (min, max) t_{max} of 1 to 2 hours (0.67, 3) post-dose. The C_{max} increased approximately dose-proportionally, whereas the AUC_{last} was approximately dose proportional at 30 mg and higher and slightly greater than proportional at the 10 mg dose level. The terminal half-life of selonabant could not be calculated due to the limited plasma sampling time frame. Moderate variability was observed for the C_{max} and AUC_{last} of selonabant, with coefficient of variations (CV%) ranging from 27.8% to 35.5%.

Peak concentrations of THC occurred at a dose-independent median (min, max) t_{max} of 0.67 to 1 hours (0.33 to 3.0) post dose. Moderate to high variability was observed for the C_{max} and AUC_{last} of THC, with CV% ranging from 36.8% to 75.4%.

Figure 4 Concentration-time profiles of selonabant following oral administration, displayed as means with standard deviation.



SAFETY

No serious adverse events occurred during the study. All TEAEs were mild in severity, except for vomiting and nausea in one participant treated with 50 mg selonabant, and dizziness in two participants treated with placebo in Part B, all of which were considered moderate. An overview of TEAEs that were reported at least twice is provided in **Table 2** (full overview available in **Table S5**).

At the highest dose level of selonabant (100 mg), 40% of participants reported nausea and 30% reported vomiting, whereas only 10% of placebo-treated participants in Part A reported nausea and none reported vomiting. Incidence of nausea and vomiting were dose-dependent, and at the lowest dose level of selonabant (10 mg), no vomiting occurred, and the incidence of nausea (42.9%) was similar to the placebo group of Part B (44.4%). Furthermore, hyperhidrosis and feeling hot were reported by approximately 20-30% of participants treated with 30-100 mg selonabant, but were absent at the 10 mg selonabant dose level and in both placebo groups. TEAEs typically associated with THC, e.g. euphoric mood, dizziness, bradyphrenia, paresthesia, and dry mouth, generally occurred more frequently in the placebo groups compared to the selonabant groups, and more frequently in the placebo participants receiving 21 mg THC compared to 10.5 mg THC.

Events of depressed mood were infrequent (2 events in the 100 mg selonabant group and 1 event in the 50 mg selonabant group), transient and mild and were not considered clinically meaningful by the investigators. There was no suicidal ideation in any of the participants at any time during the study, as assessed by the Columbia Suicide Severity Rating Scale. Becks Depression Inventory scores were similar in the selonabant and placebo groups (**Figure S6**).

There were no clinically relevant group differences in blood pressure, hematology, biochemistry, urinalysis or ECG parameters between selonabant and placebo-treated participants. In one participant treated with 100 mg selonabant and 10.5 mg THC, a systolic blood pressure increase up to 32 mmHg from baseline occurred, peaking at 162 mmHg at 3 hours post-dose and returning to baseline at 24 hours post-dose.

Table 2 Summary of TEAEs by Treatment, SOC and PT (PTs with >1 event only).

	Part A (10.5 mg THC)						Part B, Cohorts 1 and 2 (21 mg THC)					
	50 mg Selonabant (n=20)		100 mg Selonabant (n=20)		Placebo (n=20)		30 mg Selonabant (n=9)		10 mg Selonabant (n=7)		Placebo (n=9)	
System Organ Class/ Preferred Term	Events n	Subjects n (%)	Events n	Subjects n (%)	Events n	Subjects n (%)	Events n	Subjects n (%)	Events n	Subjects n (%)	Events n	Subjects n (%)
ANY EVENTS	61	17 (85.0)	55	16 (80.0)	46	20 (100.0)	26	9 (100.0)	16	7 (100.0)	36	9 (100.0)
EYE DISORDERS	-	-	1	1 (5.0)	2	2 (10.0)	-	-	-	-	-	-
Dyschromatopsia	-	-	1	1 (5.0)	2	2 (10.0)	-	-	-	-	-	-
GASTROINTESTINAL DISORDERS	26	14 (70.0)	19	12 (60.0)	4	4 (20.0)	14	6 (66.7)	5	3 (42.9)	7	5 (55.6)
Abdominal discomfort	-	-	-	-	-	-	1	1 (11.1)	1	1 (14.3)	-	-
Diarrhoea	4	3 (15.0)	1	1 (5.0)	-	-	-	-	-	-	-	-
Dry mouth	-	-	-	-	2	2 (10.0)	-	-	1	1 (14.3)	3	3 (33.3)
Nausea	18	14 (70.0)	8	8 (40.0)	2	2 (10.0)	4	4 (44.4)	3	3 (42.9)	4	4 (44.4)
Retching	1	1 (5.0)	1	1 (5.0)	-	-	-	-	-	-	-	-
Vomiting	3	2 (10.0)	9	6 (30.0)	-	-	9	3 (33.3)	-	-	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	16	10 (50.0)	13	10 (50.0)	8	6 (30.0)	4	4 (44.4)	5	4 (57.1)	8	7 (77.8)
Asthenia	1	1 (5.0)	-	-	1	1 (5.0)	-	-	-	-	-	-
Fatigue	6	6 (30.0)	6	6 (30.0)	5	4 (20.0)	2	2 (22.2)	4	3 (42.9)	5	5 (55.6)
Feeling abnormal	1	1 (5.0)	1	1 (5.0)	1	1 (5.0)	-	-	1	1 (14.3)	-	-
Feeling cold	1	1 (5.0)	-	-	-	-	-	-	-	-	1	1 (11.1)
Feeling hot	7	6 (30.0)	4	4 (20.0)	-	-	2	2 (22.2)	-	-	-	-
Feeling of relaxation	-	-	2	2 (10.0)	1	1 (5.0)	-	-	-	-	-	-
Sluggishness	-	-	-	-	-	-	-	-	-	-	2	2 (22.2)
INFECTIONS AND INFESTATIONS	1	1 (5.0)	1	1 (5.0)	1	1 (5.0)	-	-	-	-	1	1 (11.1)
COVID-19	1	1 (5.0)	1	1 (5.0)	1	1 (5.0)	-	-	-	-	1	1 (11.1)
METABOLISM AND NUTRITION DISORDERS	1	1 (5.0)	2	2 (10.0)	-	-	-	-	-	-	-	-
Decreased appetite	1	1 (5.0)	2	2 (10.0)	-	-	-	-	-	-	-	-
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	-	-	-	-	1	1 (5.0)	-	-	1	1 (14.3)	-	-
Limb discomfort	-	-	-	-	1	1 (5.0)	-	-	1	1 (14.3)	-	-
NERVOUS SYSTEM DISORDERS	5	4 (20.0)	4	3 (15.0)	13	8 (40.0)	2	2 (22.2)	1	1 (14.3)	11	6 (66.7)
Dizziness	-	-	-	-	5	5 (25.0)	2	2 (22.2)	-	-	5	4 (44.4)
Headache	4	4 (20.0)	3	2 (10.0)	4	4 (20.0)	-	-	-	-	2	2 (22.2)
Paraesthesia	-	-	-	-	3	3 (15.0)	-	-	1	1 (14.3)	3	3 (33.3)
Presyncope	-	-	-	-	1	1 (5.0)	-	-	-	-	1	1 (11.1)
Tremor	1	1 (5.0)	1	1 (5.0)	-	-	-	-	-	-	-	-
PSYCHIATRIC DISORDERS	5	4 (20.0)	7	6 (30.0)	17	15 (75.0)	3	3 (33.3)	4	3 (42.9)	9	7 (77.8)
Bradyphrenia	-	-	1	1 (5.0)	4	4 (20.0)	1	1 (11.1)	1	1 (14.3)	2	2 (22.2)
Depressed mood	1	1 (5.0)	2	2 (10.0)	-	-	-	-	-	-	-	-
Euphoric mood	4	3 (15.0)	3	3 (15.0)	12	11 (55.0)	2	2 (22.2)	3	3 (42.9)	5	5 (55.6)
Inappropriate affect	-	-	-	-	-	-	-	-	-	-	2	2 (22.2)
Time perception altered	-	-	1	1 (5.0)	1	1 (5.0)	-	-	-	-	-	-
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1	1 (5.0)	1	1 (5.0)	-	-	-	-	-	-	-	-
Hiccups	1	1 (5.0)	1	1 (5.0)	-	-	-	-	-	-	-	-
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	6	6 (30.0)	7	6 (30.0)	-	-	3	3 (33.3)	-	-	-	-
Hyperhidrosis	6	6 (30.0)	7	6 (30.0)	-	-	3	3 (33.3)	-	-	-	-

Abbreviations: n, number; TEAE, treatment-emergent adverse event; PT, preferred term; SOC, system organ class; THC, Δ^9-Tetrahydrocannabinol.

Discussion

This study was designed to evaluate the potential of selonabant, a CB_1 receptor antagonist, to block the clinical effects of oral THC in healthy volunteers when administered simultaneously. Single doses of selonabant almost completely blocked the typical subjective effects of THC, i.e. VAS 'Feeling High' and VAS 'Alertness', and significantly reduced the impairment of postural stability caused by the administration of 21 mg THC. Selonabant had an approximately equal efficacy in blocking the THC effects across the entire dose range studied. Selonabant was generally safe; most adverse events were mild and transient and no SAEs occurred during the study. Nausea, vomiting and hyperhidrosis, the main adverse events related to selonabant in this study, were dose-dependent and were absent at the well-tolerated, but equally efficacious, 10 mg dose level. This study provides a successful proof of concept and supports future research into selonabant as a potential emergency treatment for cannabinoid-induced toxicity.

The duration of effect for single oral doses of selonabant was favorable; THC effects were blocked for the duration of the observation period. The actual effect duration of selonabant is likely even longer, but could not be established precisely due to wearing off of THC effects 5-8 hours post-dose at the THC doses used. Selonabant effectively blocked the effects of THC across the entire dose range tested, without a noticeable decline in efficacy at the lower dose levels, even while the THC dose was increased in Part B of the study. This confirms that selonabant is a potent CB_1 antagonist in vivo, and its efficacy was further evidenced by a lower incidence of typical THC-related adverse effects (e.g. euphoric mood, dizziness, paresthesia, bradyphrenia) in participants treated with selonabant compared to those treated with placebo.

Although selonabant had highly significant treatment effects on VAS 'Feeling High' and 'Alertness', this was not consistently observed for body sway and heart rate. For body sway, the difference between selonabant and placebo was significant in Part B, but not Part A, of the study. This is most readily explained by the relatively low THC dose (10.5 mg) administered in Part A, which had only modest effects on postural stability. When a higher THC dose of 21 mg was administered in Part B of the study, the effects on postural stability in placebo-treated participants were greater, allowing a significant treatment effect of selonabant versus placebo to be detected, despite administration of lower selonabant doses than in Part A. Co-administration of selonabant did not show a statistically significant effect of heart rate after co-administration with up to 21 mg of THC. A possible explanation lies in the modest and brief heart rate increases induced by the THC doses administered against a background of high intersubject variability. The study was not powered to detect treatment effects on heart rate, and a larger sample size might have been required to detect a significant difference versus placebo.

Selonabant was observed to be generally safe in the healthy participants in this study. Although selonabant-related adverse events at the higher dose levels included nausea, vomiting, and hyperhidrosis, the 10 mg dose level was well-tolerated and equally effective. Psychiatric symptoms associated with rimonabant, another CB_1 antagonist, notoriously led to its withdrawal from the market. In this study, however, only a minimal number of mood symptoms occurred. Three mild and transient events of depressed mood in selonabant-treated participants were not considered clinically significant by the investigator, and Beck's Depression Inventory scores were comparable between treatment groups. Indeed, it appears plausible that single doses of CB_1 antagonists administered in the context of ACI treatment should have a favorable safety profile compared to rimonabant, which was dosed chronically for weight loss. The safety of selonabant in patients presenting to the emergency department with ACI remains to be established.

Strengths of this study include the randomized and placebo-controlled design, adequate population size for the most sensitive outcome measure, the wide dose range of selonabant studied, and the use of pharmacodynamic measures that have been shown to be sensitive to both THC effects and mitigation of THC effects by prophylactic treatment with other CB_1 antagonists.[18–20,23,35] The main limitation of this study is the limited range of THC doses investigated. High THC doses capable of inducing severe neuropsychological symptoms were not administered in this study out of ethical considerations, but patients presenting to the emergency department with such symptoms may have higher THC blood concentrations than we observed in this study. Co-administration of selonabant with higher THC doses will be the subject of future publications. Although from a mechanistic point of view, a CB_1 receptor antagonist such as selonabant should be able to counteract the effects of any CB_1 receptor agonist, synthetic agonists that differ from THC in potency, receptor binding, and pharmacokinetics may require different selonabant doses and/or dosing regimens and warrant future investigation. It could be considered a limitation that this study lacked a treatment arm for selonabant placebo without the THC co-administration. However,

the main study aim was to evaluate the blocking of THC effects by selonabant, which did not require an arm without THC, and the effects of THC compared to placebo have been abundantly described by our institute and others.[23,35] Even though the two cohorts of Part B of the study were not fully enrolled to the targeted 15 participants per cohort due to recruitment difficulties, the achieved sample size was sufficient, as evidenced by the highly significant reductions of VAS 'Feeling High', VAS 'Alertness' and body sway.

The current study involved simultaneous co-administration of selonabant and THC. The results presented here supported subsequent clinical testing of delayed administration of selonabant, and the use of higher doses of THC, as the next steps in the development of selonabant for emergency treatment of patients suffering from ACI or related disorders.

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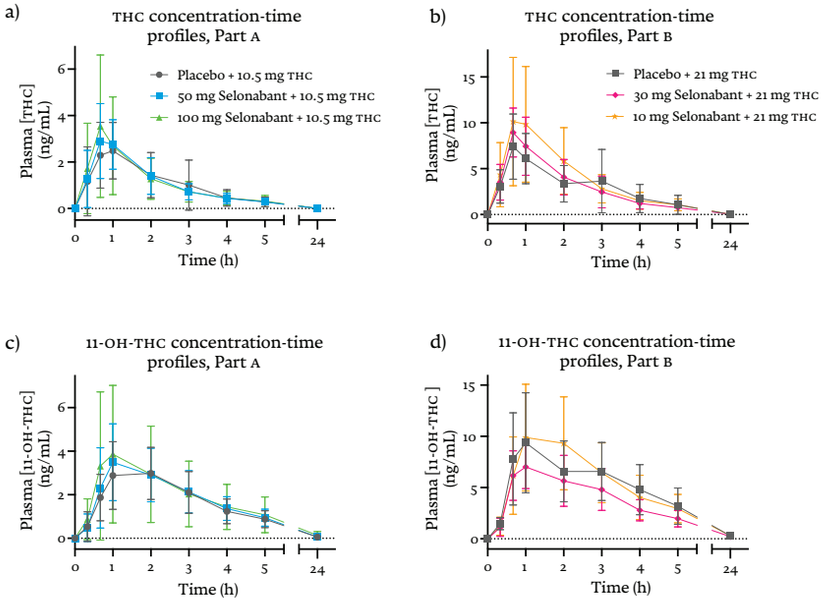
CHAPTER 6: SUPPLEMENTARY MATERIALS

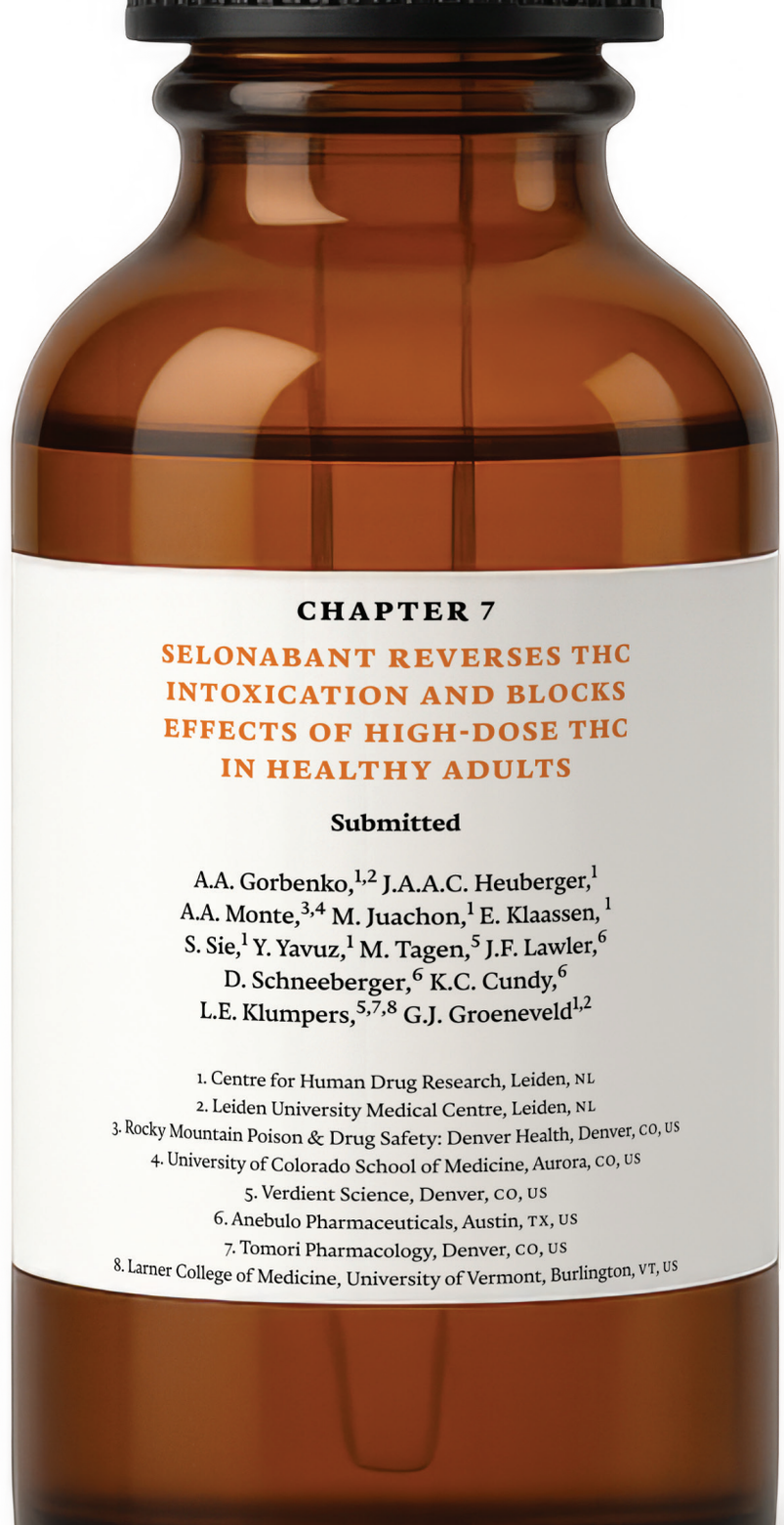
Supplementary materials available at the publisher's website:

https://tinyurl.com/yjnt3cev

Selected supplementary materials are provided below.

Figure S3 Concentration-time profiles of THC and 11-OH-THC following oral administration, displayed as means with standard deviation





CHAPTER 7

SELONABANT REVERSES THC INTOXICATION AND BLOCKS EFFECTS OF HIGH-DOSE THC IN HEALTHY ADULTS

Submitted

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Abstract

OBJECTIVE

Widespread use and increasing tetrahydrocannabinol (THC) concentration of cannabis products have increased emergency department visits due to acute cannabis-induced toxicity, including acute cannabinoid intoxication (ACI). This study assessed the potential of cannabinoid receptor type 1 (CB_1) antagonist selonabant for treatment of acute THC intoxication in healthy adults.

METHODS

This was a randomized, double-blind, placebo-controlled study of single oral doses of selonabant or placebo administered 1 hour after THC oral doses of 21, 30 or 40 mg, followed by an open-label phase, where selonabant was orally co-administered with 40 mg or 60 mg THC. Primary outcomes were THC-related effects on visual analogue scales (VAS) for feeling high and alertness, and objective measures of postural stability and heart rate, analyzed using a linear mixed effects model.

RESULTS

Forty-nine participants were enrolled in the delayed dosing phase and 20 were enrolled in the open-label co-administration phase. Delayed selonabant dosing effectively reversed THC effects on VAS 'Feeling High' (-80.2%, 95% CI: -89.4%, -63.0%, p<0.0001), body sway (-32.4%, 95% CI: -46.1%, -15.3%, p=0.001) and heart rate (-8.1 bpm, 95% CI: -14.4, -1.9 bpm, p=0.013), and increased VAS 'Alertness' (11.3 mm, 95% CI: 4.4, 18.3 mm, p=0.002) compared to placebo in participants dosed with 21 or 30 mg THC. Concomitant dosing of selonabant blocked the effects of 40 and 60 mg THC, demonstrating protection from THC-induced toxicity. in this clinical model of ACI.

CONCLUSIONS

Results of this study support further development of selonabant for treatment of acute cannabis-induced toxicity.

Introduction

BACKGROUND

Cannabis is the most widely used recreational drug in the world[1,2] and its use has further increased worldwide following legalization and decriminalization in many countries.[3] In parallel, the potency of cannabis has increased over time, with the average content of Δ^9-tetrahydrocannabinol (THC), its main psychoactive constituent, tripling between the years 1994 and 2014 in the US[4] and averaging 16% in the year 2022.[5] Consumers now also have access to potent concentrated products, such as cannabis resins[6] with an average THC concentration of 24.8%, cannabis extracts, typically containing more than triple the amount of THC compared to cannabis flower,[7] and various edible cannabis products, which are visually appealing to children and easily mistaken for regular foods.[8,9] Finally, potent synthetic cannabinoids have emerged as an unregulated alternative to botanical cannabis products and are associated with more frequent severe toxicities.[10] These factors have contributed to a growing incidence of emergency department visits related to cannabinoid-related toxicity.[9,11–13] As of 2018, there were approximately 1.7 million emergency department visits in the US associated with cannabis exposure.[14] Of these cannabis-related emergency department (ED) visits, approximately 25% are attributable to a direct effect of cannabis.[15] Cannabinoid-induced toxicities seen at the ED include acute cannabinoid intoxication (ACI) in adults, as well as unintentional cannabinoid exposure in children.

IMPORTANCE

Common adverse effects of acute cannabinoid intoxication (ACI) in adults include cognitive, motor impairment, anxiety, paranoia, tachycardia, and postural hypotension.[16] In adults presenting to emergency departments, adverse effects of high cannabis doses more commonly include gastrointestinal, cardiovascular, or neuropsychiatric symptoms, such as panic attacks, depersonalization, and acute psychosis.[16] In children, unintentional exposure to THC can result in severe complications, such as profound sedation, seizures, and respiratory depression.[16] These symptoms lead to prolonged lengths of stay and in 15-17% of cases, result in admission to inpatient care or supporting hospital units.[17] The toxic effects of THC are mediated by partial agonism at the cannabinoid receptor type 1 (CB_1), which is widely distributed in the CNS and regulates release of a wide range of neurotransmitters.[18]

Currently there are no approved therapies available for targeted treatment of cannabinoid-induced toxicity and management typically consists of supportive care.

GOALS OF THIS INVESTIGATION

Selonabant (ANEB-001) is a CB_1 receptor antagonist that was originally under development as a potential treatment for obesity. We conducted a multi-part Phase II study in healthy volunteers to explore the potential of selonabant as an emergency treatment for acute cannabis-induced toxicity. Previously, we showed that co-administration of selonabant was safe and efficacious in blocking the typical THC effects at oral THC doses up to 21 mg.[19] Here, we report on the subsequent stages of the study, where we investigated whether selonabant could *reverse* established THC effects when administrated after a delay, and the potential of selonabant to block the effects of higher oral THC doses up to 60 mg.

Methods

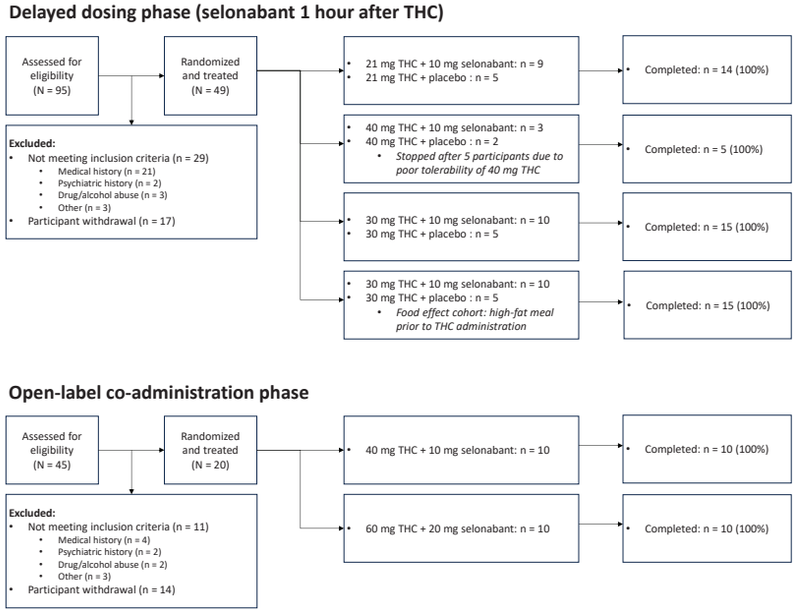
STUDY DESIGN

This Phase II study was conducted at the Centre for Human Drug Research in Leiden, the Netherlands between December 2021 and August 2023. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek and performed according to International Conference on Harmonization guidelines on Good Clinical Practice. Written informed consent was obtained from all participants before study participation. This study was registered prospectively with the IRCTN registry (ISRCTN45282100) and clinicaltrials.gov (NCT05282797). This study had two phases, a delayed selonabant dosing phase and an open-label selonabant and THC co-administration phase.

The delayed dosing phase of the study used a double-blind, randomized, placebo-controlled parallel-arm design. Participants in each sequential cohort were treated with 10 mg selonabant or placebo (target N=15 per cohort, 2:1 active/placebo) one hour after the administration of a single oral dose of THC (21 mg, 30 mg or 40 mg) (**Figure 1**).

The open-label co-administration phase consisted of two sequential cohorts of 10 participants, in which 40 mg THC was co-administered with 10 mg selonabant and 60 mg THC with 20 mg selonabant (**Figure 1**). The open-label co-administration design was used to assess high THC doses that were expected to elicit significant adverse effects, based on the findings of the delayed dosing phase. The administration of these high THC doses was only considered ethical in the presence of concomitant selonabant.

Figure 1 Participant flow diagram.



STUDY PARTICIPANTS

Healthy males and females, aged 18-45 years, were selected following medical screening according to protocol-specific inclusion and exclusion criteria (**Supplementary Material**). All included participants were occasional cannabis users (average cannabis use once per week maximum) who refrained from cannabis use from at least 3 weeks prior to dosing until the end of the study. Participants were excluded if they were regular users of sedatives, hypnotics, tranquilizers or any other addictive agent (other than the occasional use of cannabis), had a history of drug abuse, a positive drug screen result, a history of any psychotic disorder, clinically significant mood disorder, suicidal ideation in the past 5 years, or any life-time suicide attempts.

STUDY DRUGS

Participants received oral capsules containing 10 mg selonabant or matching placebo. THC was administered as oral tablets containing 1.5 mg THC per tablet (Namisol®, Echo Pharmaceuticals) to participants dosed with 21 mg THC, and for higher doses, a more practical formulation of oral capsules containing 10 mg THC per capsule (dronabinol, marketed by Ascend Laboratories, LLC) was used.

Selonabant and THC doses were chosen based on blinded review of safety and tolerability, PK, and PD data in dose evaluation meetings following each study cohort.

All treatments were administered following an overnight fast, except for the combination of 30 mg THC and 10 mg selonabant (or placebo), which was additionally repeated in participants receiving a high-fat meal (prepared according to FDA guidelines)[20] (**Figure 1**).

PHARMACODYNAMIC ASSESSMENTS

Pharmacodynamic assessments of THC effects were performed twice on the morning of the dosing day for baseline measurements and at 1, 2, 3, 4, 5, and 8 hours after the administration of selonabant or placebo. In the delayed dosing phase, primary pharmacodynamic measurements were additionally done 45 minutes after THC administration (corresponding to 15 minutes prior to the administration of selonabant or placebo).

Primary pharmacodynamic assessments included a visual analogue (VAS) scale for 'Feeling High', and for 'Alertness', postural stability and heart rate. 'Feeling High' was assessed on a scale of 0-100 mm using the Bowdle VAS, an instrument for evaluating subjective psychedelic effects.[21,22] 'Alertness' was calculated according to Bond and Lader, using a series of bi-polar visual analogue scales related to how a person feels, ranging 0-100, where 0 and 100 represented opposing subjective states and 50 represented the neutral state.[23] Postural stability was assessed by measurement of body sway, using a string attached from the waist to a device based on the Wright ataxiameter.[24] With the string attached, participants were asked to stand still with eyes closed for 2 minutes. All anteroposterior body movements over time were integrated and expressed as body sway in millimeters (mm). Heart rate was measured at the timepoints described above and additionally at 20 minutes, 40 minutes, and 22-24 hours following the administration of selonabant or placebo.

Various other, secondary, pharmacodynamic assessments were conducted, which are reported in the **Supplementary Material**.

PHARMACOKINETIC ASSESSMENTS

Venous blood samples were taken pre-dose, 20 min, 40 min, and 1, 2, 3, 4, 6, 8 and 24 hours following the administration of selonabant or placebo. In the delayed dosing phase, an additional blood sample was taken 45 minutes after THC administration (corresponding to 15 minutes prior to the administration of selonabant or placebo). Plasma selonabant, THC and 11-OH-THC concentrations were determined using a validated LC-MS/MS method.[19]

SAFETY ASSESSMENTS

Safety and tolerability were assessed by adverse event (AE) monitoring, clinical laboratory tests, vital signs, ECGs and physical and neurological examinations. The Beck Depression Inventory (BDI), second edition, Dutch version (BDI-II-NL) was used to measure depressive symptoms, and the Columbia Suicide Severity Rating Scale (C-SSRS) was performed throughout the study.

SAMPLE SIZE AND RANDOMIZATION

In the delayed dosing phase, a treatment group size of N=10 was calculated to have a power of 0.814 to detect an inhibition of VAS 'Feeling High' of 50% assuming a coefficient of variation percent (CV%) of 55% and was therefore considered sufficient.[19] For the co-administration phase, a treatment group size of N=10 was calculated to have a power of 0.80 to detect an overall change from baseline for VAS 'Feeling High' of 2.36 to 5.34 mm, based on an intra-subject variance estimate of 0.34 and 0.57 on log scale (based on data from previous study parts) and assuming a 2-sided alpha of 0.05 (further details in the **Supplementary Material**).

Study staff and participants remained blinded until database lock. The randomization code was generated using SAS v9.4 (SAS Institute, Inc., Cary, NC, USA) by a study-independent statistician. Blinded study staff assigned the randomization numbers to the participants sequentially after medical screening.

STATISTICAL ANALYSIS

To establish whether significant treatment effects could be detected, the endpoints were analyzed with a linear mixed effects model with treatment, time (as a categorical variable), and treatment by time as fixed factors, subject

as a random factor, and the average baseline measurement as covariate. For VAS 'Feeling High', a constant value of 2 mm was added to each measurement to handle zero values and to allow log-transformation and satisfy the model's normality assumption for residuals; subsequently, the analysis results were back-transformed for reporting. Participants receiving 21 and 30 mg THC in the delayed dosing phase were pooled together for statistical analysis. For the co-administration phase, a change from baseline analysis was performed, as well as a comparison to the pooled placebo participants receiving 21 and 30 mg THC in the delayed dosing phase. All calculations were performed using SAS v9.4. No adjustments for multiple comparisons were employed.[25]

Results

PARTICIPANT DISPOSITION

In the delayed dosing phase of the study, a total of 95 participants were screened and 49 were dosed across four study cohorts (**Figure 1**). The delayed dosing cohort where 40 mg THC was administered 1 hour prior to selonabant or placebo was stopped after 5 participants due to excessive and poorly tolerated THC effects. In the open label co-administration phase, 45 participants were screened and 20 were dosed across two study cohorts (**Figure 1**). All dosed participants completed the study and were evaluated for pharmacodynamic, pharmacokinetic, and safety outcomes, except for the delayed dosing cohort receiving 40 mg THC, in which pharmacodynamics were not analyzed statistically due to the low number of participants. The participant demographics are summarized in **Table 1**.

PHARMACODYNAMIC OUTCOMES

The results of statistical analysis of the primary pharmacodynamic outcomes are summarized in **Table 2**. In the pre-specified pooled analysis of participants dosed with 21/30 mg THC and 10 mg selonabant or placebo in the delayed dosing phase, selonabant significantly reduced VAS 'Feeling High' (estimate of difference (EOD): -80.2%, 95% confidence interval (CI): -89.4%, -63.0%, p<0.0001), body sway (EOD: -32.4%, 95% CI: -46.1%, -15.3%, p=0.001) and heart rate (EOD: -8.1 bpm, 95% CI: -14.4, -1.9 bpm, p=0.013), and increased VAS 'Alertness' (EOD: 11.3 mm, 95% CI: 4.4, 18.3 mm, p=0.002), compared to placebo (**Figure 2a-d**). In participants that received a high-fat breakfast prior to 30 mg THC, 10 mg selonabant significantly reduced VAS 'Feeling High' compared to placebo (EOD: -75.2%, 95% CI: -89.2%, -43.3%, p=0.003) but had no significant effects on the other primary outcome measures (**Table S1, Figure S1**).

Table 1 Participant demographics.

		Delayed administration		Co-administration
	All participants (n = 69)	**Selonabant participants (n = 32)**	**Placebo participants (n = 17)**	**(n = 20)**
AGE (YEARS)				
Mean (SD)	24.8 (4.3)	24.4 (5.0)	25.2 (3.9)	25.2 (3.7)
Median	24	23	25	26
Min, Max	18, 42	18, 42	20, 35	19, 34
HEIGHT (CM)				
Mean (SD)	177.7 (8.7)	177.5 (10.2)	176.9 (7.6)	178.9 (7.1)
Median	176.8	174.6	175.8	177.3
Min, Max	162.4, 198.4	162.4, 198.4	164.7, 189.0	163.5, 192.5
WEIGHT (KG)				
Mean (SD)	72.8 (9.8)	72.9 (9.8)	71.6 (11.4)	73.8 (8.6)
Median	72.5	71.8	69.9	73.9
Min, Max	56.3, 97.8	58.7, 97.8	56.4, 97.7	56.3, 91.4
BMI (KG/M^2)				
Mean (SD)	23.0 (2.3)	23.1 (2.3)	22.8 (2.7)	23.0 (2.0)
Median	22.9	23.0	22.1	23.1
Min, Max	19.2, 28.8	19.2, 28.2	19.2, 28.8	19.5, 28.0
SEX				
Female	33 (47.8%)	19 (59.4%)	7 (41.2%)	7 (35.0%)
Male	36 (52.2%)	13 (40.6%)	10 (58.8%)	13 (65.0%)
RACE				
Asian	3 (4.3%)	1 (3.1%)	2 (11.8%)	0 (0%)
Mixed	3 (4.3%)	2 (6.3%)	1 (5.9%)	0 (0%)
Other	1 (1.4%)	0 (0%)	0 (0%)	1 (5.0%)
White	62 (89.9%)	29 (90.6%)	14 (82.4%)	19 (95.0%)

Abbreviations: BMI, body mass index; max, maximum; min, minimum; n, number; SD, standard deviation.

In both cohorts of the open-label co-administration phase, selonabant significantly reduced VAS 'Feeling High' (up to -90.7%, 95% CI: -95.6%, -80.4%, p<0.0001 in participants receiving 60 mg THC and 20 mg selonabant), increased VAS 'Alertness' (up to 12.6 mm, 95% CI: 6.8, 18.4 mm, p<0.001 in participants receiving 60 mg THC and 20 mg selonabant), and decreased

heart rate (down to -11.2 bpm, 95% CI: -17.7, -4.7 bpm, p<0.001, in participants receiving 60 mg THC and 20 mg selonabant) compared to data for the pooled placebo participants receiving 21 and 30 mg THC in the delayed dosing phase. Body sway was initially analyzed without log-transformation and was significantly reduced compared to the pooled placebo participants (EOD: -93.7 mm, 95% CI: -183.3, -4.1 mm, p=0.041 in participants receiving 40 mg THC and 10 mg selonabant; EOD: -97.2 mm, 95% CI: -187.5, -6.9 mm, p=0.036 in participants dosed with 60 mg THC and 20 mg selonabant). In a later log-transformed re-analysis, done for consistency with previous study parts, the reductions showed a trend towards significance (down to -20.5%, 95% CI: -38.2%, 2.3%, p=0.073 in participants receiving 40 mg THC and 10 mg selonabant) (**Table 2, Figure 3**). The baseline versus post-dose analysis results are provided in **Table S2**. The results for the secondary pharmacodynamic outcome measures are summarized in **Tables S3-4**.

Figure 2 Primary pharmacodynamic outcome measures in the delayed administration phase, displayed as least square means with 95% confidence intervals.

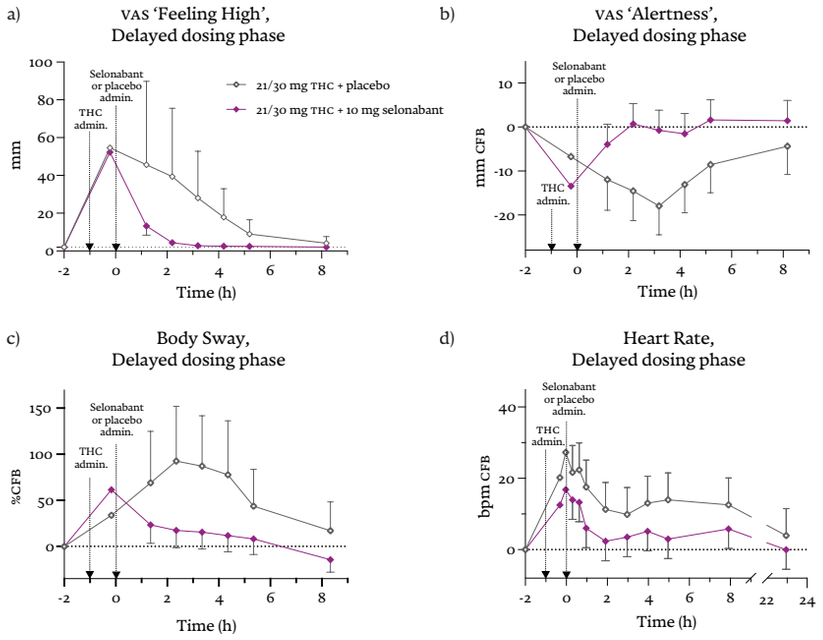


Table 2 Overall treatment effects on pharmacodynamic outcome measures (estimated mean differences with 95% CI and p-values).

	Delayed Dosing	Co-administration	
Treatment	**21-30 mg THC + 10 mg selonabant**	**40 mg THC + 10 mg selonabant**	**60 mg THC + 20 mg selonabant**
Control	**21-30 mg THC + placebo**	**21 or 30 mg THC + placebo***	**21 or 30 mg THC + placebo***
VAS 'Feeling High' (mm)	**-80.2% (-89.4%, -63.0%) p<0.0001**	**-87.7% (-94.1%, -74.1%) p<.0001**	**-90.7% (-95.6%, -80.4%) p<.0001**
VAS 'Alertness' (mm)	**11.3 (4.4, 18.3) p=0.002**	**12.3 (6.3, 18.3) p<.001**	**12.6 (6.8, 18.4) p<.001**
Body sway (mm)	**-32.4% (-46.1%, -15.3%) p=0.001**	**-20.5%** (-38.7%, 2.3%) p=0.073**	**-20.3%*** (-38.2%, 2.7%) p=0.077**
Heart rate in supine position (bpm)	**-8.1 (-14.4, -1.9) P=0.013**	-11.0 bpm (-17.4, -4.6 bpm) p=0.002	-11.2 bpm (-17.7, -4.7 bpm) p=0.002

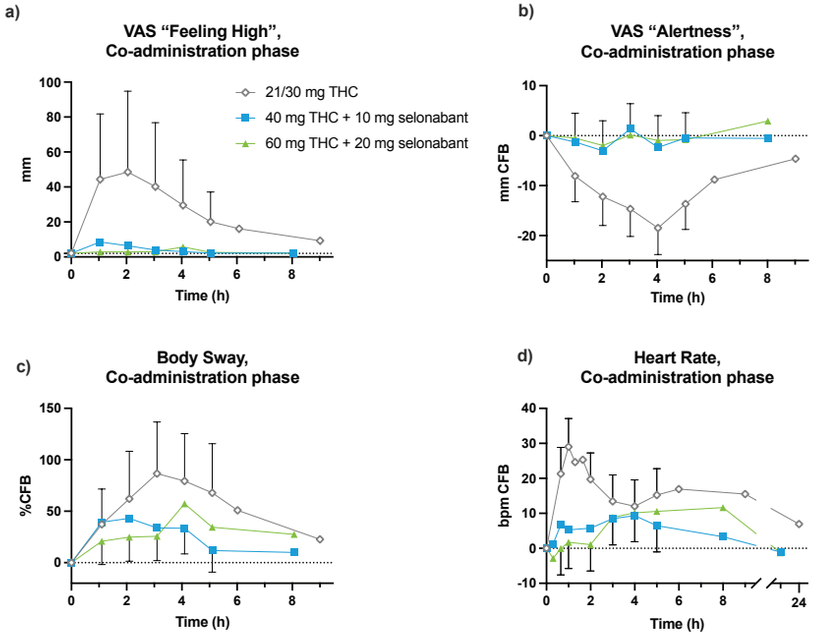
The figures in bold indicate statistical significance. Abbreviations: bpm, beat per minute; CI, confidence interval; THC, Δ^9-tetrahydrocannabinol; VAS, visual analogue scale.
**Placebo participants from the delayed administration part, receiving 21 or 30 mg THC.*
***Original analysis result (no log-transformation): estimated mean difference: -93.7 mm, 95% CI: -183.3, -4.1 mm, p=0.041.*
****Original analysis result (no log-transformation): estimated mean difference: -97.2 mm, 95% CI: -187.5, -6.9 mm, p=0.036.*

PHARMACOKINETICS

When 10 mg selonabant was administered in a fasted state, the mean±SD C_{max} was 109.3±31.7 ng/ml, the mean±SD AUC_{last} 1817.9±725.0 h*ng/mL, and the median (min-max) t_{max} 2 hours (0.67-4) (**Figure S2**). Administration of 10 mg selonabant following a high-fat meal resulted in delayed peak concentrations (median (min-max) t_{max} of 3 hours (1-24.1)) compared to fasted administration with a lower C_{max} (78.0±30.4 ng/mL), but a higher AUC_{last} (2285.1±682.6 h*ng/mL) (**Figure S2**). After the administration of 20 mg selonabant in a fasted state, the mean±SD C_{max} was 274.9±61.8 ng/ml, the mean±SD AUC_{last} 3269.3±854.4 h*ng/mL, and the median (min-max) t_{max} 0.67 hours (0.67-2) (**Figure S2**).

At the highest THC dose administered in this study (60 mg), a mean±SD C_{max} of 30.3±10.9 ng/mL and mean±SD AUC_{last} of 107.9 (38.6) h*ng/mL was reached for THC (**Figure S3**), and a mean±SD C_{max} of 30.1±12.3 ng/mL and mean±SD AUC_{last} of 151.2±48.8 h*ng/mL for 11-OH-THC (**Figure S4**).

Figure 3 Primary pharmacodynamic outcome measures in the co-administration phase, compared to the pooled placebo participants of the delayed dosing phase, displayed as least square means with 95% confidence intervals and aligned on THC dosing (t=0 is THC dosing in all groups). Non-overlapping timepoints displayed without confidence intervals and not included in the mixed effects model.



SAFETY

No serious adverse events occurred during the study. Fatigue and nausea were the most common AEs after the administration of selonabant or placebo, occurring in 33 (47.8%) and 27 (39.1%) participants respectively, and were reported at comparable rates in the active and placebo groups (**Table 3**). Euphoric mood was reported by 28 (57.1%) participants prior to the administration of selonabant or placebo in the delayed dosing phase (**Table S5**). All AEs were transient and mild, except for 18 events rated moderate in severity. Vomiting was the most frequent moderate AE, occurring in 6 (8.7%) participants; 3 (50%) treated with selonabant, and 3 (50%) treated with placebo. Of the 18 moderate AEs, 7 occurred in the 5 participants dosed with 40 mg THC one hour before 10 mg selonabant or placebo and included anxiety, agitation, emotional disorder, dizziness and vomiting, and were all considered probably or possibly related to THC, and unrelated or unlikely related to selonabant (**Table S6**).

Two mild events of depressed mood were reported following treatment with selonabant (one participant treated with 10 mg selonabant 1 hour after 21 mg THC, and one participant treated with 10 mg selonabant 1 hour after 30 mg of THC in fed condition). These symptoms resolved within hours to days after onset and were not considered clinically meaningful by the investigator. There were no clinically relevant group differences in BDI-II scores and there was no suicidal ideation in any participant during the study, as assessed by the C-SSRS.

There were no clinically relevant group differences in blood pressure, hematology, biochemistry, urinalysis or ECG parameters between participants treated with selonabant and placebo.

Table 3 Overview of the most frequently reported AEs following the administration of selonabant or placebo (displayed as number (%) of participants reporting an AE within the treatment group).

		Delayed dosing								Co-administration	
THC dose		21 mg		40 mg		30 mg		30 mg (fed)		40 mg	60 mg
Selonabant dose		10 mg	Placebo	10 mg	Placebo	10 mg	Placebo	10 mg (fed)	Placebo (fed)	10 mg	20 mg
N of pp	All pp (n=69)	n=9	n=5	n=3	n=2	n=10	n=5	n=10	n=5	n=10	n=10
Fatigue	33 (47.8)	6 (66.7)	2 (40.0)	3 (100.0)	1 (50.0)	5 (50.0)	3 (60.0)	4 (40.0)	3 (60.0)	2 (20.0)	4 (40.0)
Nausea	27 (39.1)	5 (55.6)	1 (20.0)	2 (66.7)	2 (100.0)	2 (20.0)	1 (20.0)	1 (10.0)	2 (40.0)	5 (50.0)	6 (60.0)
Headache	20 (29.0)	2 (22.2)	2 (40.0)	-	-	4 (40.0)	1 (20.0)	4 (40.0)	2 (40.0)	3 (30.0)	2 (20.0)
Euphoric mood	19 (27.5)	1 (11.1)	1 (20.0)	-	-	1 (10.0)	1 (20.0)	3 (30.0)	3 (60.0)	6 (60.0)	3 (30.0)
Postural dizziness	15 (21.7)	2 (22.2)	2 (40.0)	1 (33.3)	-	-	1 (20.0)	4 (40.0)	2 (40.0)	2 (20.0)	1 (10.0)
Dizziness	10 (14.5)	1 (11.1)	1 (20.0)	1 (33.3)	2 (100.0)	1 (10.0)	-	-	2 (40.0)	2 (20.0)	-
Vomiting	6 (8.7)	1 (11.1)	-	-	2 (100.0)	-	1 (20.0)	1 (10.0)	-	1 (10.0)	-

Abbreviations: pp, participants; n, number; AE, adverse event; THC, Δ^9-Tetrahydrocannabinol

Limitations

Participants receiving 21 and 30 mg THC in the delayed dosing phase were pooled in the pharmacodynamic analysis to achieve the calculated sample size of N=10 for placebo participants. Since the participants of the two cohorts received different doses of THC, this introduced additional variability into the analysis. However, the pharmacokinetic parameters and pharmacodynamic effects of the two dose levels were comparable and the pooled analysis was deemed broadly representative of the treatment effects of 10 mg selonabant on an intoxication with a moderate THC dose.

A total of 5 participants were dosed with placebo in the high-fat meal group, falling short of calculated sample size of 10 per group. However, this cohort primarily aimed to assess a food effect, to which end the pharmacokinetic findings in selonabant-treated participants were essential, whereas dosing additional placebo participants was not deemed informative.

Just 5 participants were dosed with 40 mg THC in the delayed dosing phase before the cohort was stopped due to the excessive adverse effects of the 40 mg THC dose, which precluded statistical analysis of pharmacodynamics.

Since the co-administration phase lacked its own control group for statistical analysis of pharmacodynamics, two alternative analyses were done instead. Although comparison of baseline versus post-dose data showed significant p-values, the magnitude of such changes was invariably small and clinically irrelevant (**Table S2**). Comparison of co-administration data to participants receiving 21 and 30 mg THC in the delayed dosing phase clearly showed that selonabant significantly reduced anticipated THC effects on three out of four primary outcome measures. This analysis almost certainly underestimated the treatment effect of selonabant, as THC doses of Part C (40-60 mg) are expected to produce stronger pharmacodynamic effects than the 21-30 mg THC administered in the delayed dosing phase.

This study was conducted in healthy adult volunteers and concomitant ingestion of other agents was not permitted. In contrast, ED patients frequently have ingested other agents, so the current study may not predict the efficacy of selonabant in such cases.

Discussion

Previously, we showed that single oral doses of selonabant blocked the effects of low to moderate oral doses of THC, when administered simultaneously in healthy participants.[19] The delayed dosing phase of the study expanded on previous findings by demonstrating that selonabant effectively reversed established THC intoxication, approximating a clinical treatment paradigm. Intoxication was established within 45 minutes after THC administration and subsequent selonabant administration returned the primary measures of intoxication close to baseline within 1 to 2 hours, while ongoing intoxication lasted for 5-8 hours in placebo-treated participants. The open label co-administration phase showed that selonabant effectively blocks the adverse effects of even higher THC doses up to 60 mg, which would be impractical or even unethical to administer in a placebo-controlled design due to their excessive intoxicating effects. Current findings highlight the potential of selonabant as a treatment for acute cannabis-induced toxicity in the ED and support its further clinical development.

The potential of selonabant as an antidote in the acute care setting was further underscored by its reversal of toxicity induced by the poorly tolerated THC dose of 40 mg in the delayed dosing phase. THC-induced anxiety, agitation, emotional disorder, and dizziness of moderate severity all resolved between 30 and 120 minutes following selonabant, while the placebo-treated participants experienced a notably slower resolution of their THC-induced AEs – by 3.5 to 5.5 hours following placebo.

The administration of a high-fat meal prior to dosing delayed the absorption of selonabant did not impact its bioavailability. The delayed absorption resulted in a diminished inhibition of THC effects, compared to the fasted participants. The significance of the food effect is likely to be dependent on the THC dose, the timing, and content of any prior meal, which may not be reliably known in the emergency department. A parenteral selonabant formulation may benefit cases where oral dosing may be impractical, such as THC-induced nausea, vomiting or reduced consciousness.

Selonabant did not cause clinically significant depressive symptoms or suicidality – adverse effects that were associated with rimonabant, another CB_1 receptor antagonist, and ultimately led to its withdrawal from the market.[26,27] These findings are in line with the expectation that selonabant is likely to be substantially safer when administered as a single low dose for

emergency treatment of acute cannabis-induced toxicity, compared to historical chronic daily use of other CB_1 antagonists for weight loss. Accordingly, the FDA briefing document for rimonabant shows that the increase in suicidality was only observed after chronic use, with on average at least 3 months of daily treatment.[28] Nevertheless, the safety of selonabant in ACI patients presenting to the ED, especially patients with prior psychiatric history, remains to be established in future research.

The key strength of this study is its randomized, placebo-controlled design and the use of established outcome measures for quantifying THC effects, leading to strong evidence of a beneficial effect of selonabant on reversal of THC toxicity following delayed administration of the antidote. Whereas it was previously established that selonabant can block THC effects from occurring when co-administered,[19] the effective reversal of established THC effects more closely resembles clinical practice in the emergency department, where cannabis toxicity precedes intervention. The open-label, uncontrolled design of the co-administration phase allowed for an ethical assessment of THC doses that would normally be sufficient to induce acute cannabis-toxicity that could plausibly warrant an emergency department visit.

In conclusion, this study demonstrated the potential of selonabant to reverse symptoms of acute THC intoxication, even at high THC doses. The results of this study support further development of selonabant as an emergency treatment for acute cannabis-induced toxicity.

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CHAPTER 7: SUPPLEMENTARY MATERIALS

SECONDARY PHARMACODYNAMIC ASSESSMENTS

Secondary pharmacodynamic assessments in the delayed administration part included VAS 'Internal Perception' and VAS 'External Perception' according to Bowdle,[1,2] VAS 'Mood' and VAS 'Calmness' according to Bond and Lader,[3] VAS 'Drug Effect', VAS 'Good Drug Effect' and VAS ' Bad Drug Effect' according to the Drug Effects Questionnaire (DEQ),[4] saccadic and smooth pursuit eye movements,[5,6] pupillometry,[7] state-trait anxiety inventory (STAI),[8] adaptive tracking[9,10] and the N-back,[11] performed as described elsewhere.

Secondary pharmacodynamic assessments in the open-label co-administration part included VAS 'Internal Perception' and VAS 'External Perception' according to Bowdle,[1,2] VAS 'Mood' and VAS 'Calmness' according to Bond and Lader,[3] VAS 'Drug Effect', VAS 'Good Drug Effect' and VAS ' Bad Drug Effect' according to the Drug Effects Questionnaire (DEQ),[4] the Symbol Digit Substitution Test,[12] Alternate Finger Tapping,[13] Timed Up and Go test,[14] the Basic Symptom Inventory,[15] the Brief Psychiatric Rating Scale[16] and the Visual-Verbal Learning Test (VVLT),[17] performed as described elsewhere.

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Sample size calculation for the open-label co-administration part

The intra-subject variance of VAS 'Feeling High' was estimated between 0.34 and 0.57 on log scale, based on data from previous parts of the study. This leads to a minimal detectable effect size of 2.36 to 5.34 mm for VAS 'Feeling High' (back-transformed to mm), assuming VAS 'Feeling High' has a baseline value of 0, N=10 subjects, alpha of 0.05 two sided and 80% power.

In other words, with a cohort of N=10, if the VAS 'Feeling High' overall change from baseline score stays under 2.5 mm, it is predicted to not significantly differ from zero – and thus we would not be able to conclude that there was a significant THC effect over time, implying that the selonabant dose inhibited the THC effect. This 2.5 mm change from baseline score is relevant, as previous cohorts treated with THC + selonabant showed similar change from baseline scores. For example, the overall change from baseline for VAS 'Feeling High' observed for 30 mg THC and 10 mg was estimated to be 1.14 mm. Moreover, this was shown to be significantly lower than the THC VAS 'Feeling High' effect in the THC + placebo comparator group, thus showing an inhibitory effect of selonabant. Finally, an increase of <2.5 mm VAS 'Feeling High' is not considered a clinically relevant change, further supporting that a sample size of N=10 in the delayed co-administration part of the study is sufficient to detect relevant changes in VAS 'Feeling High'.

Figure S1 Primary pharmacodynamic outcome measures the delayed administration, food effect cohort, displayed as least square means with 95% confidence intervals.

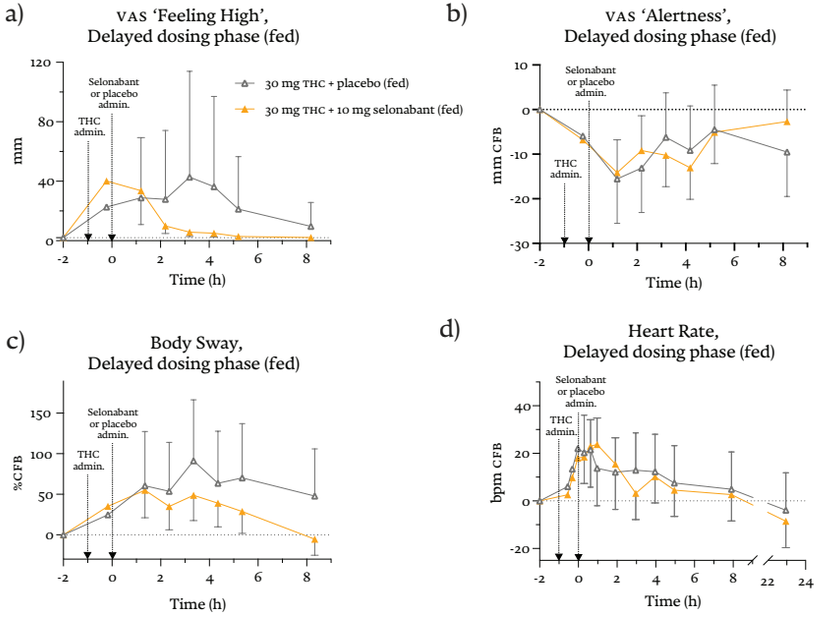


Figure S2 Concentration-time profiles of selonabant following oral administration, displayed as means with standard deviation.

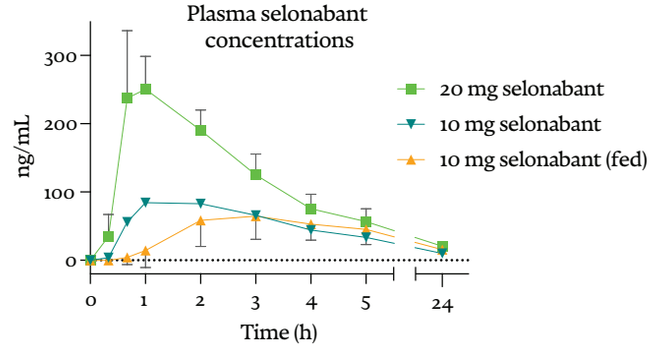


Figure S3 Concentration-time profiles of THC and 11-OH-THC following oral administration, displayed as means with standard deviation.

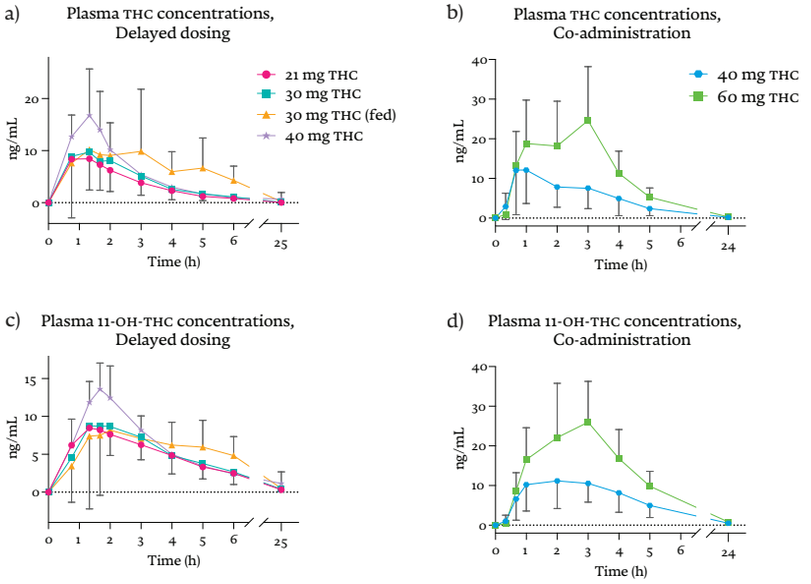


Table S1 Overall treatment effects on main pharmacodynamic outcome measures in the delayed administration, food effect cohort (estimated mean differences with 95% CI and p-values).

Treatment	**30 mg THC (-1h) + 10 mg selonabant**
Control	**30 mg THC (-1h) + placebo**
VAS 'Feeling High' (mm)	**-75.2% (-89.2%, -43.3%) P=0.003**
VAS 'Alertness' (mm)	0.6 (-7.3, 8.5) P=0.868
Body sway (mm)	-19.5% (-43.2%, 14.1%) P=0.200
Heart rate in supine position (bpm)	-0.9 (-13.5, 11.6) P=0.872

The figures in bold indicate statistical significance. Abbreviations: bpm, beat per minute; CI, confidence interval; THC, Δ^9-tetrahydrocannabinol; VAS, visual analogue scale.

Table S2 Baseline versus post-dose comparison of primary pharmacodynamic outcomes, open-label co-administration.

			95% CI	
Effect/Contrast	**P-value**	**LSM Estimate change from baseline**	**Lower**	**Upper**
VAS 'FEELING HIGH'				
40 mg THC + 10 mg selonabant	**0.037**	87.8%	5.2%	235.1%
60 mg THC + 20 mg selonabant	**0.014**	47.7%	11.0%	96.4%
VAS 'ALERTNESS'				
40 mg THC + 10 mg selonabant	0.298	-2.1	-6.3	2.2
60 mg THC + 20 mg selonabant	0.133	-0.6	-1.3	0.2
BODY SWAY				
40 mg THC + 10 mg selonabant	**0.023**	68.5	12.3	124.6
60 mg THC + 20 mg selonabant	**0.012**	71.0	20.7	121.3
HEART RATE				
40 mg THC + 10 mg selonabant	0.051	5.0	-0.0	10.0
60 mg THC + 20 mg selonabant	**0.002**	5.8	3.0	8.7

Abbreviations: CI, confidence interval; LSM, least square mean; THC, Δ^9-Tetrahydrocannabinol; VAS, visual analogue scale.

Table S3 Overall treatment effects on secondary outcome measures, delayed dosing (estimated mean differences with 95% CI and p-values).

Treatment	21/30 mg THC (-1h) + 10 mg selonabant (n = 19)	30 mg THC (-1h) + 10 mg selonabant (fed) (n = 10)
Control	**21/30 mg THC (-1h) + placebo (n = 10)**	**30 mg THC (-1h) + placebo (fed) (n = 5)**
VAS 'BOND AND LADER'		
VAS 'Calmness' (mm)	**-8.5 (-15.8, -1.2) p=0.024**	n.s.
VAS 'Mood' (mm)	n.s.	n.s.
VAS 'BOWDLE'		
VAS 'External Perception' (log(mm))	**-0.37 (-0.52, -0.21) p<0.0001**	**-0.33 (-0.55, -0.10) p=0.009**
VAS 'Internal Perception' (log(mm))	**-0.12 (-0.20, -0.04) p=0.004**	n.s.
VAS 'DRUG EFFECTS'		
VAS 'Feel Drug' (log(mm))	**-0.7 (-1.0, -0.3) p=0.0003**	n.s.
VAS 'Like Drug' (log(mm))	**-0.6 (-1.0, -0.2) p=0.002**	n.s.
VAS 'Dislike Drug' (log(mm))	**-0.4 (-0.7, -0.0) p=0.027**	n.s.
State-Trait Anxiety Inventory	n.s.	n.s.
Saccadic Peak Velocity (deg/s)	n.s.	n.s.
Smooth pursuit (%)	n.s.	n.s.
Adaptive tracking (%)	n.s.	n.s.
Pupil size (mm)	n.s.	n.s.
N-back (proportion correct 2-back)	n.s.	n.s.

The figures in bold indicate statistical significance. Abbreviations: CI, confidence interval; THC, Δ^9-Tetrahydrocannabinol; VAS, visual analogue scale.

Table S4 Overall treatment effects on secondary outcome measures, open-label co-administration (estimated change from baseline with 95% CI and p-values).

Treatment	40 mg THC + 10 mg selonabant (n = 10)	60 mg THC + 10 mg selonabant (n = 10)
VAS 'BOND AND LADER'		
VAS 'Calmness' (mm)	n.s.	n.s.
VAS 'Mood' (mm)	n.s.	n.s.
VAS 'BOWDLE'		
VAS 'External Perception' (log(mm))	n.s.	n.s.
VAS 'Internal Perception' (log(mm))*	n/a	n.s.
VAS 'DRUG EFFECTS'		
VAS 'Feel Drug' (log(mm))	**0.45 (0.10, 0.80) P=0.02**	**0.32 (0.18, 0.47) p=0.0008**
VAS 'Like Drug' (log(mm))	**0.71 (0.30, 1.12) P=0.004**	n.s.
VAS 'Dislike Drug' (log(mm))	**0.28 (0.04, 0.53) p=0.03**	n.s.
State-Trait Anxiety Inventory	n.s.	**3.3 (1.0, 5.6) p=0.01**
Symbol-Digit Substitution Test		
Overall average reaction time	n.s.	n.s.
Total number of correct responses	n.s.	n.s.
Total number of incorrect responses	**1.5 (0.5, 2.6) P=0.01**	**2.4 (1.0, 3.8) P=0.005**
VVLT		
Immediate word recall number correct	n.s.	n.s.
Delayed word recall number correct	**-4.0 (-7.4, -0.6) P=0.03**	**-6.1 (-7.8, -4.4) P<0.0001**
Delayed word recognition averagereaction time correct	n.s.	n.s.
Delayed word recognition number correct	**-2.7 (-4.0, -1.4) p=0.001**	**-4.3 (-6.4, -2.2) p=0.001**
Timed Up and Go test	n.s.	n.s.
ALTERNATE FINGER TAPPING: ACCURACY		
Number of correct taps	**12.2 (6.3, 18.0) p=0.001**	n.s.
Mean spatial error (mm)	**2.1 (1.2, 2.9) p=0.0002**	**1.8 (0.8, 2.8) p=0.0031**
Average ratio correct:incorrect	n.s.	n.s.
ALTERNATE FINGER TAPPING: FATIGUE		
Inter-tap interval change (ms/min)	n.s.	**74.0 (23.7, 124.4) p=0.01**
Spatial error change (mm/min)	**4.2 (2.5, 5.9) p=0.001**	n.s.
ALTERNATE FINGER TAPPING: RHYTHM		
Inter-tap interval SD (ms)**	n/a	n.s.
Spatial error SD (mm)	**1.5 (0.7, 2.3) p=0.002**	**1.2 (0.3, 2.1) p=0.015**
ALTERNATE FINGER TAPPING: SPEED		
Total number of taps	**19.2 (8.1, 30.4) p=0.0041**	**10.6 (4.3, 16.9) p=0.0046**
Inter-tap interval (ms)	**-64.0 (-87.9, -40.2) p<0.001**	**-32.5 (-56.5, -8.5) p=0.0142**

The figures in bold indicate statistical significance. Abbreviations: CI, confidence interval; n, number; THC, Δ^9-Tetrahydrocannabinol; VAS, visual analogue scale.

Table S5 Overview of the most frequently reported AEs after administration of THC and before selonabant or placebo in the delayed dosing part of the study (displayed as number (%) of participants reporting an AE within the treatment group).

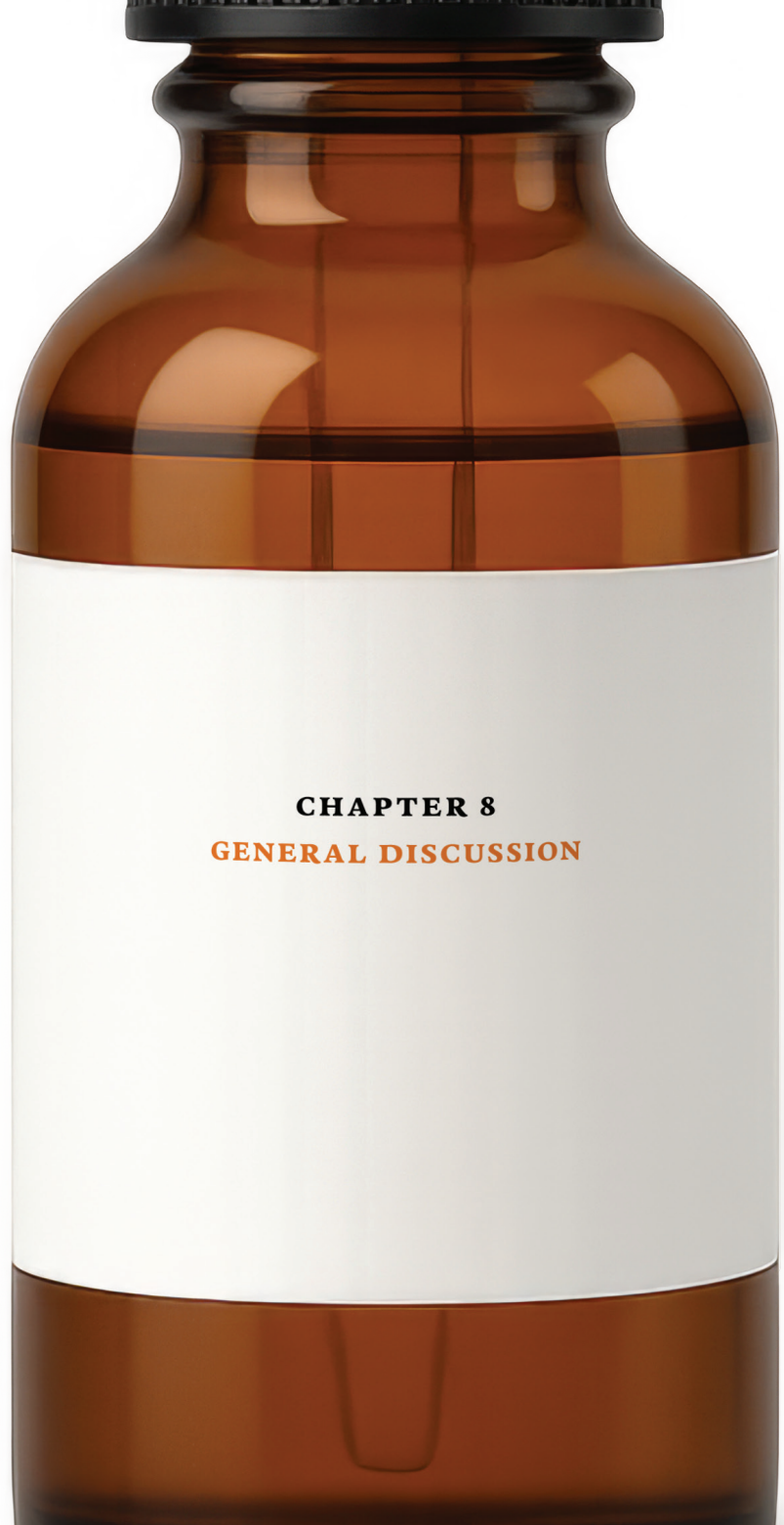
THC dose		21 mg		40 mg		30 mg		30 mg (fed)	
Selonabant dose		10 mg	Placebo	10 mg	Placebo	10 mg	Placebo	10 mg (fed)	Placebo (fed)
	All delayed dosing pp (n=49)	n=9	n=5	n=3	n=2	n=10	n=5	n=10	n=5
Euphoric mood	28 (57.1)	6 (66.7)	2 (40.0)	3 (100.0)	2 (100.0)	7 (70.0)	4 (80.0)	3 (30.0)	1 (20.0)
Fatigue	9 (18.4)	1 (11.1)	3 (60.0)	-	-	-	2 (40.0)	2 (20.0)	1 (20.0)
Postural dizziness	8 (16.3)	3 (33.3)	2 (40.0)	1 (33.3)	1 (50.0)	-	1 (20.0)	-	-
Paresthesia	7 (14.3)	2 (22.2)	1 (20.0)	-	1 (50.0)	-	-	1 (10.0)	2 (40.0)
Nausea	7 (14.3)	2 (22.2)	-	-	-	1 (10.0)	2 (40.0)	2 (20.0)	-
Dizziness	7 (14.3)	4 (44.4)	-	-	-	1 (10.0)	-	2 (20.0)	-
Presyncope	7 (14.3)	1 (11.1)	3 (60.0)	1 (33.3)	-	-	2 (40.0)	-	-

Abbreviations: pp, participants; n, number; AE, adverse event; THC, Δ^9-Tetrahydrocannabinol.

Table S6 Overview of AEs of moderate severity.

Treatment	pp	Event	Onset (relative to selonabant or placebo admin.)	Duration	Treatment	Selonabant relatedness	THC relatedness
21 mg THC (-1h) + 10 mg selonabant	3003	Fall	-8 min	-	-	Unrelated	Probable
	3009	Vomiting	2 h 22 min	3 min	-	Possible	Possible
40 mg THC (-1h) + 10 mg s elonabant	4002	Anxiety	2 min	30 min	-	Unrelated	Probable
	4003	Agitation	24 min	55 min	1 mg oral lorazepam	Unlikely	Probable
	4003	Emotional disorder	19 min	1 h	-	Unlikely	Probable
	4005	Dizziness	1 h 21 min	47 min	-	Unlikely	Probable
40 mg THC (-1h) + placebo	4001	Vomiting	1 h 7 min	2 min	-	Unrelated	Possible
	4001	Euphoric mood	-30 min	5 h 35 min	-	Unrelated	Probable
	4004	Vomiting	1 h 43 min	3 short instances; last at 3 h 36 min	-	Unrelated	Possible
30 mg THC (-1h) + 10 mg selonabant	5006	Emotional disorder	5 min	1 h 15 min	-	Unrelated	Probable
30 mg THC (-1h) + placebo	5003	Euphoric mood	51 min	2 h 5 min	-	Unrelated	Probable
	5013	Nausea	41 min	3 h 23 min	-	Unrelated	Possible
	5013	Vomiting	1 h 13 m	3 short instances; last at 1 h 24 min	-	Unrelated	Possible
	5014	Euphoric mood	-16 min	7 h 7 min	-	Unrelated	Probable
30 mg THC (-1h) + 10 mg selonabant (fed)	6007	Vomiting	1 h 6 min	2 min	-	Possible	Possible
	6008	Nausea	-10 min	2 h 40 min	-	Unrelated	Probable
30 mg THC (-1h) + placebo (fed)	6009	Euphoric mood	4 min	8 h 30 m	-	Unrelated	Probable
40 mg THC + 10 mg selonabant	7009	Vomiting	2 h 26 min	1 min	-	Possible	Possible

Abbreviations: pp, participant; AE, adverse event; THC, Δ^9-Tetrahydrocannabinol.



CHAPTER 8
GENERAL DISCUSSION

The discovery and characterization of the human endocannabinoid system at the end of last century has caused a surge of medical and scientific interest in cannabinoids. Various therapeutic uses of cannabinoids have been identified since, including the treatment of pain and spasticity in multiple sclerosis, chronic neuropathic pain, chemotherapy-induced nausea and vomiting and appetite stimulation. Epidiolex®, a purified CBD formulation, has been approved for treatment of three rare epileptic syndromes. In many other therapeutic areas, studies failed to identify clinically relevant treatment effects.

However, even for the indications where cannabinoid therapeutics look most promising, current understanding of their pharmacology still contains crucial gaps. In the case of neuropathic pain, health authorities generally refuse to reimburse cannabinoids due to the heterogeneity and methodological limitations of the evidence base and insufficient clarity with regards to safety, interactions and the preferred formulation. CBD-rich varieties are widely claimed to cause fewer side effects, and potentially stronger analgesia, due to a purported beneficial interaction of CBD with THC. The evidence for such interaction, however, is limited and contradictory, and as a result, clinicians and patients are guided more by folklore than clinical pharmacology. In the case of treatment of seizures with CBD, where there is high quality evidence from RCTs, it is still uncertain to what degree CBD truly possesses anti-epileptic properties – or whether it merely inhibits the metabolism of clobazam, another anti-seizure medicine.

The rapid expansion of the recreational cannabinoid space also presented new challenges. Over-the-counter CBD products are ubiquitous, with claims of wide-ranging benefits (despite absent scientific evidence) and little consideration for potential adverse effects or drug interactions. A steady rise in THC content of cannabis, the emergence of novel, concentrated products and the availability of edibles, which are easily mistaken for regular foods and appealing to children, have coalesced to increase the incidence of cannabinoid toxicity. Its clinical management, however, has seen little progress.

In this thesis, we attempted to contribute to the development of cannabinoids into safe, effective, widely adopted and reimbursed medicines. We performed clinical trials aimed at addressing key gaps in the current understanding of cannabinoid pharmacology. First, we focused on the questions pertaining to the use of cannabis in the treatment of neuropathic pain. We conducted clinical trials designed to untangle the pharmacology of the interaction between THC and CBD and to assess the risks of interactions between CBD and other analgesics. Second, we performed a clinical study to investigate the extent of the intrinsic anti-epileptic properties of CBD, hoping to shed light on its mechanism of action in the treatment of seizures. Finally, we completed a dose-finding mechanistic study of the CB_1 receptor antagonist selonabant in humans, evaluating its potential as an emergency treatment for cannabinoid intoxication.

The *'Summary'* section below provides an overview of the main findings and their interpretation, whereas broader perspectives on cannabinoid pharmacology are discussed under *'Implications and future work'.*

Summary

Chapter 2 provided clinical evidence that contradicts the widely touted belief that CBD reduces the psychotropic effects of THC. Not only did CBD fail to reduce the pharmacological effects of THC in this clinical trial – it did the exact opposite, increasing the psychotropic effects along with plasma THC concentrations. This is highly suggestive of a pharmacokinetic interaction, and CBD-induced inhibition of CYP2C19 and CYP3A4 (the main enzymes responsible for THC metabolism) is the most likely explanation. Meanwhile, analgesia remained similar in all participants who received THC, regardless of CBD co-administration.

Although clinically relevant CBD-induced drug interactions had been reported previously, they typically occurred in patients who were treated for rare epileptic syndromes.[1–3] Daily CBD doses administered to such patients ranged from 10 to 50 mg *per kilogram bodyweight*. An important novel finding of **Chapter 2** was that CBD already significantly inhibited CYP450-mediated metabolism at a dose as low as 30 mg (*total*), an order of magnitude lower than the doses used to treat seizures.

This new finding suggests that the risk of CBD-induced drug interactions may not be limited to patients treated with high doses of CBD for rare epileptic syndromes. Patients who use medicinal cannabis for a variety of indications, including pain, may plausibly reach CBD intake that is sufficient to cause CYP-enzyme inhibition; same is true for individuals who use CBD as an OTC health supplement. **Chapter 3** describes a clinical study in healthy volunteers

that evaluated the potential interaction between CBD (administered at a low dose of 30 mg) and amitriptyline and tramadol, two analgesics commonly prescribed for treatment of chronic neuropathic pain and metabolized by cytochrome P450 enzymes. Increases in concentrations of amitriptyline, tramadol and their active metabolites were found (although only statistically significant for amitriptyline), indicative of the presence of an interaction. However, the magnitude of this interaction was limited; it may go unnoticed by some patients and would certainly not cause any additional toxicity. The study concluded that CBD was safe to use with amitriptyline and tramadol at the achieved CBD exposures – although higher CBD exposures, and thus greater interactions, could occur in patients due to repeated dosing, prandial status and impaired organ function.

Perhaps the most clinically important CBD-induced drug interaction is with clobazam, a commonly prescribed anti-seizure medication. CBD increases the concentrations of the active metabolite of clobazam by 2.6 to 6-fold, with substantially better seizure control and increased incidence of sedation observed in CBD-treated patients on clobazam, compared to the no clobazam group. Provided that in the registration trials of CBD for Dravet syndrome and Lennox-Gastaut syndrome over half of all patients used clobazam concomitantly, there is grounds to suspect that CBD may not have much inherent anti-seizure activity, if at all, and that most of its effectiveness arises from the pharmacokinetic interaction with clobazam. This consideration is also reflected in the decision of the European Medicines Agency to only approve CBD for these syndromes in combination with clobazam.

Chapter 4 investigated intrinsic anti-seizure activity of CBD. We performed a double-blind, placebo-controlled, three-way crossover trial in healthy volunteers, in which we administered therapeutically relevant (single) doses of CBD. Cortical excitability, a proxy measure for anti-seizure drug effects, was assessed using transcranial magnetic stimulation (TMS) coupled with electromyography (TMS-EMG) and electroencephalography (TMS-EEG). Additionally, sensitive tests of vigilance and sedation were performed. Contrary to expectations for an anti-seizure drug, CBD produced no changes on any of the parameters measured by TMS-EMG. Some significant treatment effects were found using TMS-EEG, but these lacked a clear physiological or clinical interpretation. CBD left vigilance and memory entirely unaffected and produced no sedation. Taken together, these results imply that CBD does not meaningfully reduce cortical excitability in humans and therefore may lack intrinsic anti-epileptic effects, strengthening the hypothesis that the interaction with clobazam is responsible for much, if not all, of the anti-seizure activity of CBD instead.

Chapter 5 provided a commentary on a publication by another research group, which reported on an open-label clinical trial, in which symptoms associated with long COVID were treated with a CBD-dominant cannabis-based medicinal product. We argued that the administration of a diverse herbal composition to a heterogeneous population without a well-defined pathological basis in an open-label design was unlikely to yield useful conclusions and advocated for closer adherence to basic principles of clinical pharmacology in cannabinoid research.

The growing prominence of therapeutic cannabinoids has been matched – if not outpaced – by the expansion of their recreational use, which has been legalized in large parts of the world. Coupled with increasingly potent cannabinoid products, this has led to a rising incidence of emergency room visits due to cannabinoid intoxication. **Chapters 6 and 7** explored the potential of selonabant, a novel CB_1 receptor antagonist, as an emergency treatment for cannabinoid intoxication. **Chapter 6** focused on the simultaneous administration of oral selonabant (or placebo) with moderate doses of THC (administered as oral tablets) and found that selonabant largely blocked the effects of up to 21 mg THC. **Chapter 7** described the delayed administration of selonabant, i.e. selonabant (or placebo) administered 1 hour after moderate to high THC doses (21-40 mg), thus closer mimicking the intended therapeutic use. Delayed administration of selonabant was able to reverse the effects of THC intoxication and associated adverse effects. In the final, open-label part of the study (**Chapter 7**), selonabant was administered simultaneously with high doses of THC (40-60 mg). Strikingly few THC effects were observed post-dose, whereas pronounced toxicity would be expected at such a high THC dose otherwise. Overall, we concluded that selonabant was efficacious in treating THC intoxication and was safe and well-tolerated at therapeutic doses. The next steps in the clinical development of selonabant could include late-phase clinical trials in patients presenting to the emergency department with cannabinoid intoxication and development of a parenteral formulation, which could be preferable in an emergency setting due to quicker onset of action and its usability in states of reduced consciousness.

Implications and future work

THE ENTOURAGE EFFECT HYPOTHESIS IS UNTENABLE

The 'entourage effect' hypothesis posits that multiple constituents of the cannabis plant synergize to yield superior clinical outcomes compared with isolated compounds. Specifically, it may concern therapeutic effects, e.g. the hypothesis that THC has better analgesic effects when administered as a cannabis plant extract, instead of an isolated pharmaceutical formulation. Alternatively, it may concern adverse effects, e.g. the hypothesis that cannabis of botanical origin is better tolerated than pharmaceutical formulations containing isolated THC; this is the most common form of the hypothesis, with the purported interaction between CBD and THC often at the center of the mechanistic rationale. What is constant in the entourage effect hypothesis, however, is that administration methods that utilize unprocessed cannabis (e.g., vaporizing cannabis flower) are preferred to formulations with isolated cannabinoids. If pharmaceutical formulations are to be used, then 'whole-plant' extracts (i.e., containing all cannabinoids and terpenes that occur naturally in a plant) are deemed superior to isolated cannabinoids. The hypothesis has resulted in a quest to cultivate cannabis strains with chemical compositions that are as beneficial to humans as possible.

Chapter 2, along with other recent high-quality trials,[4–6] provides clinical evidence that contradicts specific claims within the wider entourage effect hypothesis. However, we argue that the credibility of the hypothesis is most of all undermined by its pharmacologically unsound theoretical basis. While interactions between various pharmacologically active compounds are indeed common, the entourage effect theory is completely unjustified in assuming that such interactions between constituents of the cannabis plant must be beneficial to humans.[7] Most interactions between medicines are harmful and something to be avoided; polypharmacy generally constitutes a problem to address, not a goal to pursue.[7,8] The unjustified belief in the benign nature of the cannabinoid polypharmacy constitutes a form of naturalistic fallacy, which is a logical error of concluding that because something is 'natural', it must therefore also be good. The naturalistic fallacy is an unsuitable basis for a scientific hypothesis.

There is little historical precedent for superiority of medicinal plants over pharmaceutical formulations containing their purified active ingredients. A post-operative patient may receive morphine but is unlikely to be prescribed poppy juice; digoxin tablets appear to have outcompeted the foxglove plant leaves; willow tree bark is a rare sight in modern pharmacies – in contrast to bottles of aspirin. Pharmaceutical formulations offer great advantages in dose standardization, stability, and convenience and have tended to make plants obsolete in medical practice.

Proponents of the entourage effect hypothesis tend to be overly optimistic about the interpretation of supporting evidence. For example, in a seminal publication in support of the hypothesis,[9] a randomized controlled trial is cited, in which a whole-plant extract containing CBD and THC significantly reduced intractable cancer-related pain compared to placebo, whereas a THC-predominant cannabis extract failed to do so.[10] Absent from the interpretation is any mention of effect size: pain, on a 10-point numerical rating scale, decreased by 0.69 in the placebo group, 1.37 with the whole-plant extract, and 1.01 in the THC-predominant extract. A more grounded interpretation of the findings is that both kinds of cannabis extract provided no clinically relevant analgesia compared to placebo, and that the small difference between the extracts may well be explained by chance. Another cited study found that a full-spectrum CBD extract improved the dose-response curve of analgesia in an animal pain model compared to pure CBD.[11] This finding is impertinent, however, given that CBD is not yet known to be an effective analgesic drug in humans.[12] Perhaps the most intriguing result comes from a study that found that a botanical drug preparation outperformed a pure THC formulation on antitumor effects in preclinical breast cancer models.[13] However, the simplest interpretation of this finding is that some constituents of the botanical preparation may have antitumor properties; this is still a long way removed from a general 'entourage effect'.

On the epistemological level, the vagueness of the entourage effect hypothesis renders it practically unfalsifiable. Although some of its specific claims can be tested and falsified (as shown in **Chapter 2**), proponents of the hypothesis can always incorporate empirical setbacks by shifting the focus to a different combination of cannabinoids or a different measure of benefit. With hundreds of naturally occurring molecules and countless outcome measures to choose from, there is always another promising combination to investigate, should the previous one fail to meet the expectations.

In short, the cannabinoid entourage effect hypothesis is not based on sound pharmacological principles, has no analogies within pharmacology,

fails to show up in randomized controlled trials designed to find it, is rationalized using overly optimistic interpretations of research and is vague to the point of being unfalsifiable. At the core of the hypothesis, we do not find a solid theoretical rationale or compelling empirical evidence. Instead, we find a belief in the superiority of the plant over pharmaceuticals, a conviction that 'the plant does it better', to quote the closing remark of the previously mentioned, highly cited paper.[10]

The pursuit of such insufficiently substantiated theories is wasteful and potentially harmful. A considerable amount of funding and effort has gone into research on the entourage effect hypothesis, that could have been used more fruitfully for funding state-of-the-art clinical pharmacology studies testing plausible hypotheses. Another detrimental aspect of this fallacy is that the entourage effect hypothesis is often regarded as scientific truth by cannabis users and even prescribers, which drives the demand for CBD-rich cannabis varieties. The CBD content of such cannabis can put individuals at risk of harmful pharmacokinetic interactions. The scientific community should re-examine the notion of a cannabinoid entourage effect and the supposed superiority of the whole plant.

CBD-INDUCED DRUG INTERACTIONS ARE A REASON FOR CONCERN

This thesis contends that potential beneficial interactions of cannabinoids have received too much scientific and popular attention; in contrast, their adverse interactions have arguably received too little. The findings of **Chapter 2** and **Chapter 3** highlight that drug-drug interactions are a concern even at low CBD doses and that further investigation is required.

A matter of first concern is the characterization of the dose-response curve of the CBD-induced CYP inhibition for each relevant enzyme. Clinical studies utilizing validated CYP-substrate cocktails and a dose range of CBD would generate data that allows for accurate prediction of the risk of CBD-induced drug interactions for any given CBD exposure. Such a design has an important advantage over studies of specific CBD-drug combinations, as its results could be easily extrapolated to any substrate drug with sufficiently well-characterized metabolism.

Second, it is critical to gain insight into real-world patterns of CBD use. The overall impact of CBD-induced drug interactions on public health depends on prevalence of its use, specific formulations and administration schedules involved, patient motivation, history and comedications, all of which are insufficiently understood at this moment. Clarifying the reasons for CBD self-medication could help identify more effective treatment options, as CBD currently lacks proven efficacy for most indications. Potentially, we might even discover that some of the effects that users ascribe to CBD stem from CBD-induced changes in blood concentration of concomitant medications, instead.

We hypothesize that CBD-induced drug interactions may be more common than expected. CBD inhibits CYP2C19, 2C9, 2B6, and 3A4; numerous commonly used medications are metabolized by one or more of these enzymes. Many of the individuals who use cannabis for medical or recreational reasons could have sufficient CBD intake to cause drug interactions. Possibly even more individuals consume CBD in the form of over-the-counter health supplements, judging by the substantial selection of such supplements available for sale at any Dutch drug store. The CBD content of such supplements is sufficient to cause interactions, with typical servings containing from 5 to 50 mg CBD,[14] while CBD doses up to 70 mg daily can be recommended by vendors.[15]

The interaction with clopidogrel, a common antiplatelet drug and a CYP2C19 substrate, could pose a particularly serious public health concern. Clopidogrel is a prodrug that requires conversion to its active H4 metabolite to exert its therapeutic effects. Genetic variations and comedication that reduce CYP2C19 activity are known to diminish clopidogrel's antiplatelet activity. CBD-induced CYP2C19 inhibition is thus expected to impair the therapeutic efficacy of clopidogrel, exposing patients to a higher risk of acute ischemic events. This interaction is not only serious, but potentially also common, with almost half a million clopidogrel users in the Netherlands, and little awareness of this risk.

To conclude, it is imperative that future research characterizes the full dose-response curve of CBD-induced CYP-enzyme inhibition in humans. In parallel, it is necessary to acquire a thorough understanding of the populations that use CBD. Only then can the substantial risks of CBD-induced drug interactions be adequately assessed and managed.

THE CHALLENGE OF (DIS)PROVING THAT CBD IS AN ANTI-SEIZURE MEDICATION

It remains challenging to draw definitive conclusions on the origins of the anti-seizure effects of CBD. The results presented in **Chapter 4** make the hypothesis that a pharmacokinetic interaction with clobazam is the main

mechanism of anti-seizure action of CBD more likely, even if it is inherently limited by the use of a proxy measure (cortical excitability) as opposed to the actual outcome of interest (seizures). Conversely, two recent retrospective studies in monogenic epilepsies lend additional (circumstantial) support to the existence of an intrinsic anti-seizure effect of CBD: both found no difference in seizure outcomes between patients who were and were not receiving clobazam.[16,17]

And yet, it is important that the mechanism by which CBD reduces seizure frequency is clarified. Should the benefit of CBD prove to stem chiefly from CYP2C19 inhibition, more economical and better-characterized inhibitors could theoretically replicate its effect with fewer safety concerns. The mean cost of treatment with Epidiolex® is almost €74.69 per day,[18] whereas omeprazole, a proton-pump inhibitor, has similar effects on CYP2C19 at just €0.04 per day.[19] More potent alternatives would be less likely to cause liver enzyme elevations, as risk of drug-induced liver injury for a drug is correlated with therapeutic dose.[20] Potentially, even simply prescribing higher doses of clobazam could prove sufficient.

If CBD is, in fact, an anti-seizure medication with intrinsic pharmacodynamic effects, then it is even more crucial to unravel its mode of action. As **Chapter 4** has shown, CBD does not appear to share a mode of action with the main types of anti-seizure medications and would therefore represent a new class of anti-seizure drug. The identification of this novel pathway would open the door to the development of new and improved drugs, and further our understanding of the physiology underlying seizures.

The issue could be definitively resolved by a clinical study that compared seizure rate between patients on clobazam + CBD, clobazam + an equipotent dose of omeprazole, and clobazam without CBD or omeprazole. However, it is hard to imagine the manufacturer of Epidiolex® voluntarily sponsoring such a study, and government funders are likely more interested in funding research intended to find new therapies, instead of refuting existing ones. Additionally, patients may not be eager to participate. A potential solution could lie in the ability of regulatory bodies like the FDA and EMA to request additional trials from the drug manufacturer. Perhaps mechanistic studies such as **Chapter 4** could inspire them to do so – if not within the therapeutic indication for which Epidiolex® is already approved, then maybe when considering the extension of approval to additional epileptic syndromes.

THE GREAT PARADOX OF CANNABIDIOL

Scientists have evaluated CBD for a strikingly wide range of therapeutic indications. Besides the epileptic syndromes, clinical studies have evaluated CBD for treatment of anxiety, psychosis, schizophrenia, post-traumatic stress disorder, substance abuse, improvement of sleep quality, Parkinson's disease, autism, smoking cessation, graft-versus-host-disease, cancer, Huntington's disease, type 2 diabetes, and indeed, long COVID (**Chapter 5**).[21]

This exceptional scientific interest in treating virtually any disease with CBD appears to be, in part, a consequence of the large number of molecular mechanisms it has been found to affect. One publication lists over 20 pathways that are known to be modulated by CBD in vitro.[22] Many of the identified pathways have established roles in (patho)physiological processes and have been fruitful targets for previous drug development. In some cases, e.g. epilepsy, the therapeutic effects of CBD may even appear overdetermined, with at least 5 different pathways identified that could plausibly explain an anti-seizure drug effect – excluding the interaction with clobazam (**Chapter 4**).

And yet, CBD has failed to materialize as an effective treatment for any condition, except for certain epileptic syndromes, and even its anti-seizure effects are suspect for reasons discussed in **Chapter 4**. This apparent gap between the many proposed targets and the lack of clinical efficacy across almost all indications has been aptly dubbed 'the great paradox' of CBD by chemist Peter Cogan.[23] The logical conclusion is that CBD may not actually act upon all identified molecular targets when administered in humans – a point made even more clear by the remarkably mild (CNS) side effect profile of CBD. The main adverse effects observed in the epilepsy trials were liver enzyme elevations, which is unsurprising for a drug with daily dosage that can reach grams and is a strong inhibitor of multiple CYP iso-enzymes. Additionally, sedation, which might well be explained by interactions with other CNS drugs, was completely absent when we administered CBD alone to healthy volunteers in **Chapter 4**. If CBD indeed activated >20 different molecular targets in humans, one would expect it to cause a wide array of side effects – akin to a patient treated with 20 different drugs – which is clearly not the case.

'The great CBD paradox' currently lacks a proper explanation. Perhaps, some of the in vitro experiment results were chance findings, or incorrect, e.g. if performed in animal species with relevant differences in target protein structure, or simply at CBD concentrations far exceeding those achievable in

humans; may be, CBD fails to reach the site of action in humans in relevant concentrations; some of the experiments might have used plant extracts contaminated with THC and other cannabinoids. Artifactual and false-positive *in vitro* assay results are oftentimes observed in highly lipophilic drugs, of which CBD is one.[23] Whatever the explanation will turn out to be, it appears premature to pursue development of CBD as a therapeutic agent, until it is better understood why its in vitro pharmacology has translated to humans so poorly to date.

WE CAN TREAT CANNABINOID INTOXICATION — DOES IT MEAN WE SHOULD?

Chapters 6 and 7 provided ample evidence of the effectiveness of selonabant as an antidote for THC intoxication. The bigger challenge will likely lie in convincing regulatory agencies and physicians that cannabinoid intoxication is an emergency worth treating. Although cannabinoid intoxication can cause highly unpleasant side effects, it is seldom (if ever) lethal, a stark contrast with opioid intoxication, which causes around 80,000 deaths yearly in the USA alone[24].

Additionally, concerns about safety may impede the approval and adoption of selonabant. Rimonabant, another CB_1 antagonist, was previously withdrawn from the market after post-approval studies linked it to depression and suicidality. However, it appears unlikely that CB_1 antagonism would cause such severe adverse effects, when used for treatment of cannabinoid intoxication. Whereas chronic rimonabant dosing was necessary for weight loss, single and low doses of selonabant should suffice for treating intoxication. Still, future clinical trials will need to confirm its safety, especially in patients with prior psychiatric disease.

Nevertheless, in a world where the prevalence of cannabis use is rising alongside the potency of the products, demand for a drug like selonabant can be expected to increase. The strongest case for treatment of cannabinoid intoxication may be in the pediatric population, where the consequences of intoxication are typically more severe. Availability of a parenteral formulation that is immediately effective, e.g. intravenous or a nasal spray, should boost the attractiveness of selonabant in the emergency room. Ultimately, only time will tell whether physicians will prefer to treat cannabinoid intoxications with selonabant over the current gold standard therapy of 'wait and see'.

Conclusion

Taken together, the chapters of this thesis further the understanding of cannabinoid pharmacology. They show that whole-plant mystique cannot substitute for actual pharmacological evidence; that the apparently benign CBD is a CYP-enzyme inhibitor at everyday doses; that its anti-seizure efficacy likely stems less from an intrinsic anti-epileptic effect, than from concomitant clobazam use; and that cannabinoid toxicity can be effectively addressed by targeted pharmacotherapy. The pathway forward is therefore one of precision: treat cannabinoids as discrete entities, define their exposure-response curves, map their interactions, and develop selective emergency treatments. If researchers, clinicians and regulators adopt this pharmacology-driven ethos, the field can finally move beyond slogans about plants and into an era where cannabinoid-based therapies deliver reproducible benefit with predictable risk, thereby fulfilling the therapeutic potential of *Cannabis sativa.*

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NEDERLANDSE SAMENVATTING

Medicinaal gebruik van de *Cannabis sativa* plant vindt al duizenden jaren plaats, maar het is pas in de afgelopen decennia dat de effectiviteit bij verschillende aandoeningen ook wetenschappelijk wordt onderzocht. Recente onderzoeken tonen aan dat cannabis kan helpen tegen neuropathische pijn ('zenuwpijn'), pijn en spasticiteit bij multipele sclerose (MS) en misselijkheid die veroorzaakt wordt door chemotherapie. Tetrahydrocannabinol (THC) is geïdentificeerd als de belangrijkste actieve stof in cannabis. THC zorgt voor het prettige 'high' gevoel en veranderde waarneming, maar kan ook bijwerkingen als angst en verwardheid veroorzaken. Daarnaast is cannabidiol (CBD), een bestanddeel dat geen duidelijke roes veroorzaakt, goedgekeurd voor behandeling van bepaalde zeldzame epileptische aandoeningen. In Nederland mogen artsen medicinale cannabis voorschrijven, en zijn er diverse CBD-producten vrij verkrijgbaar.

Het enthousiasme voor medicinale toepassingen van cannabis loopt echter flink voor op de beschikbare wetenschappelijke kennis, en essentiële vragen blijven nog onbeantwoord. Het mechanisme van de pijnstillende werking is nog niet goed begrepen. Het is bekend dat THC onmisbaar is voor het pijnstillend effect, maar de bijdrage van CBD is onduidelijk. Bij zowel artsen, patiënten en onderzoekers leeft de gedachte dat CBD de werking van THC verbetert en de bijwerkingen ervan neutraliseert, terwijl een overtuigende farmacologische onderbouwing hiervoor ontbreekt. Er heerst onduidelijkheid over de effectiviteit en bijwerkingen van de verschillende cannabissoorten en -preparaten, artsen zijn vaak terughoudend met voorschrijven en de behandeling wordt doorgaans niet vergoed. Hoewel CBD met succes wordt toegepast voor behandeling van epilepsie, zijn er ook redenen om te twijfelen of het anti-epileptische effect echt aan CBD zelf te danken is. En terwijl het gebruik van CBD als zelfzorgmiddel wijdverspreid is geworden, is er nog nagenoeg geen wetenschappelijk bewijs dat dit effectief of veilig is.

Dit proefschrift heeft daarom als doel om de werking van de belangrijkste cannabisbestanddelen systematisch te bestuderen bij mensen. Niet door te kijken naar de cannabisplant als geheel, maar juist door afzonderlijke bestandsdelen als THC en CBD (de zogenaamde 'cannabinoïden') en hun mogelijke interacties te onderzoeken. Daarbij staat de klinische farmacologie centraal: wat gebeurt er in het lichaam met deze stoffen, hoe beïnvloeden ze elkaar, welke effecten veroorzaken ze en welke risico's brengt dat met zich mee? Het beantwoorden van deze vragen is essentieel voor een veilige en effectieve medicinale toepassing van cannabinoïden.

In **Hoofdstuk 2** toetsen wij de wijdverspreide overtuiging dat CBD de bijwerkingen van THC vermindert. Wij hebben een gecontroleerd klinisch onderzoek uitgevoerd in gezonde vrijwilligers. Zij kregen op verschillende dagen THC alleen of THC gecombineerd met verschillende doseringen CBD. Vervolgens werden subjectieve effecten (zoals het gevoel 'high' te zijn), cognitieve prestaties en motorische coördinatie gemeten en werden er bloedspiegels van THC, CBD en metabolieten bepaald. Tevens hebben wij pijnprikkels toegediend en pijnperceptie gemeten, om de afzonderlijke bijdrage van de twee cannabinoïden aan de pijnstillende werking van cannabis te beoordelen.

De uitkomst was duidelijk: CBD vermindert de ongewenste effecten van THC niet. Sterker nog, bij hoge dosering CBD nam het benevelende effect juist toe. Deelnemers voelden zich sterker onder invloed en hun reactiesnelheid en balans verslechterden meer dan bij THC alleen. Tegelijkertijd werd het pijnstillende effect van THC niet beter met toevoeging van CBD. In het bloed zagen we dat de concentraties THC en vooral de actieve metaboliet 11-OH-THC sterk verhoogd waren wanneer CBD werd toegevoegd. Dit komt hoogstwaarschijnlijk doordat CBD de leverenzymen (zogenaamde 'CYP-enzymen') remt die THC normaal afbreken. Met andere woorden: CBD beschermt helemaal niet tegen de bijwerkingen van THC, maar verhoogt juist de blootstelling aan THC (zonder de pijnstilling te verbeteren). Onze resultaten zijn dus tegenovergesteld aan de veelgehoorde aanname dat cannabis milder wordt van CBD.

Deze bevinding leidde tot een tweede onderzoeksvraag: als CBD de afbraak van THC remt, doet het dat mogelijk ook bij andere geneesmiddelen. Daarom onderzoeken wij in **Hoofdstuk 3** de interactie tussen CBD en twee veelgebruikte pijnstillers voor neuropathische pijn: amitriptyline en tramadol. Hierbij gaven wij een lage dosis CBD, die makkelijk binnen te krijgen is bij gebruik van een medicinale cannabisolie op recept of via de supplementen bij de drogist. We zagen een duidelijke stijging van concentraties amitriptyline in het bloed. Deze bevinding bevestigt verder dat CBD functioneert als een CYP-enzym remmer en zelfs in lage dosissen interacties kan veroorzaken met andere geneesmiddelen. Hierdoor kunnen bijwerkingen ontstaan of kan de effectiviteit van andere geneesmiddelen veranderen.

In **Hoofdstuk 4** richten we ons vervolgens op epilepsie. CBD is geregistreerd voor enkele zeldzame epilepsiesyndromen, maar het werkingsmechanisme is onduidelijk. Veel patiënten gebruiken naast CBD een ander

anti-epilepticum (clobazam), waarvan bekend is dat CBD de bloedspiegel sterk verhoogt via CYP-enzym inhibitie. In eerdere onderzoeken bleek dat patiënten die al clobazam gebruikten, veel meer baat hadden bij CBD, dan patiënten die geen clobazam gebruikten – en ook meer bijwerkingen hadden die typisch zijn voor clobazam, zoals slaperigheid. Dit doet vermoeden dat de effectiviteit van CBD op z'n minst gedeeltelijk berust op het verhogen van clobazamspiegels, in plaat van een eigen anti-epileptisch effect.

Wij weten uit eerder onderzoek wat de effecten van reguliere anti-epileptica zijn in gezonde vrijwilligers. Anti-epileptica verlagen doorgaans de prikkelbaarheid van de hersenen, dat wil zeggen dat hersencellen minder makkelijk 'vuren'; zo wordt een epileptische aanval voorkomen. In **Hoofdstuk 4** onderzoeken wij of CBD (zonder clobazam) op een vergelijkbare wijze de prikkelbaarheid zou verlagen. Wij hebben een hoge en een lage dosering CBD toegediend aan gezonde vrijwilligers en de prikkelbaarheid van hun hersenen bepaald met (non-invasieve) neurofysiologische meetapparatuur. Wij vonden geen aanwijzingen dat CBD op zichzelf de prikkelbaarheid van hersenen vermindert of slaperigheid veroorzaakt. Dit ondersteunt de hypothese dat het anti-epileptische effect van CBD voor een belangrijk deel berust op het verhogen van de clobazamspiegels – hoewel dit alleen definitief bevestigd zou kunnen worden in een onderzoek in patiënten met epilepsie.

In **Hoofdstuk 5** leveren wij commentaar op een onderzoek dat door een andere groep wetenschappers is uitgevoerd. Daarin werd de behandeling van symptomen van long COVID met een cannabisextract onderzocht. Dit onderzoek heeft een aantal tekortkomingen in de onderzoeksmethodiek die het lastig maken om betrouwbare conclusies te trekken. Zo was er geen placebogroep, werd een cannabisextract gebruikt dat meerdere potentieel actieve stoffen bevatte, en werden patiënten met zeer uiteenlopende symptomen geïncludeerd. In dit hoofdstuk pleiten wij voor een striktere hantering van de basisprincipes van klinische farmacologie in onderzoek met cannabinoïden, zodat de onderzoeksresultaten informatiever en betrouwbaarder worden.

Hoofdstukken 6 en 7 van het proefschrift richten zich op behandeling van acute cannabisintoxicatie. Door toenemende THC-concentratie in recreatieve cannabis en de populariteit van eetbare vormen (*'edibles'*) en andere THC-houdende producten neemt het aantal acute overdoseringen toe. We onderzochten *selonabant*, een middel dat het aangrijpingspunt van THC blokkeert, als mogelijke behandeling. In gezonde vrijwilligers bleek selonabant de effecten van THC te kunnen voorkomen (**Hoofdstuk 6**) en omkeren nadat intoxicatie al was opgetreden (**Hoofdstuk 7**). Dit suggereert dat gerichte farmacologische behandeling van cannabisintoxicatie met selonabant mogelijk is, vergelijkbaar met antidota bij andere geneesmiddelen.

Gezamenlijk dragen de hoofdstukken van dit proefschrift bij aan een beter begrip van de farmacologie van cannabinoïden. Ze weerspreken het wijdverspreide idee dat cannabis beter werkt en minder bijwerkingen heeft doordat THC wordt gecombineerd met CBD. Tegelijkertijd brengen ze de potentieel risicovolle interacties tussen CBD en andere geneesmiddelen aan het licht. Verder maken ze het aannemelijker dat de anti-epileptische werking van CBD grotendeels op een interactie met andere geneesmiddelen berust, en beschrijven ze een nieuwe manier om THC-intoxicatie gericht te behandelen met een antidotum.

Concluderend, de uitkomsten van dit proefschrift dragen bij aan verdere ontwikkeling van cannabinoïden als medicijn met dezelfde benadering als ieder ander geneesmiddel: namelijk het opdoen van kennis over effectiviteit, bijwerkingen en interacties middels *lege artis* klinisch-farmacologisch onderzoek. Daarbij staat niet de plant zelf centraal, maar de afzonderlijke werkzame stoffen. Alleen door cannabinoïden op deze manier te bestuderen, zullen mythes en marketingclaims vervangen kunnen worden door een onderbouwde medische toepassing en zal het volledige therapeutische potentieel van cannabinoïden benut kunnen worden.

PHD PORTFOLIO

PhD training	Year
MANDATORY ACTIVITIES	
Workshop Scientific Conduct for PhDs	2022
Responsible Research	2025
Basic Methods and Reasoning in Biostatistics	2025
Leiden University Onboarding Programme Inform and Connect	2025
SCIENTIFIC COURSES, WORKSHOPS AND OTHER TRAINING ACTIVITIES	
Paul Janssen Future Lab Clinical Development Course	2021
PK/PD Modeling Course	2021
EU CTR Expert in Nederland	2022
Teach the Teacher	2024
CONFERENCE ATTENDANCE	
EFIC, Dublin (poster)	2022
BPS, Liverpool (attendance)	2022
ICRS, Toronto (plenary presentation)	2023
EFIC, Budapest (poster)	2023
Spierziektecongres, Veldhoven (oral presentation to patient audience)	2023
ICRS, Salamnaca (poster)	2024
EACPT, Rotterdam (poster)	2024
LACDR Spring Symposium, Leiden (oral presentation)	2025
NVGO, Elspeet (oral presentation)	2025
FIGON, Dutch Medicine Days, Leiden (poster)	2025
Dutch Parkinson Days, Leiden (attendance)	2025
TEACHING ACTIVITIES	
Work Groups Pharmacology (BSc of Medicine 2nd year course)	2020-2022
Supervising student research project (MSc Biomedical Sciences)	2021-2023
Supervising honours student research project (BSc Neuroscience)	2022
AWARDS AND PRIZES	
Awarded prof. Van Os prize by NVGO	2025
OTHER	
Project leadership (including design, regulatory submission, set-up, clinical execution and reporting) of a first-in-human trial for a disease-modifying drug for Parkinson's disease	2019-2020
Peer review for British Journal of Clinical Pharmacology	2021-2025
Project leadership (including design, regulatory submission, set-up and clinical execution) in a clinical trial aimed at phenotyping responders to treatment of chronic neuropathic pain with THC oil	2022-2025
Clinical Pharmacology Internship (Internal Medicine)	2024
Clinical Pharmacology Internship (Psychiatry)	2025

CURRICULUM VITAE

Andriy Andriyovych Gorbenko was born on 24 April 1993 in Vinnytsya, Ukraine. In 2004 he moved to Leiden, the Netherlands, where he attended the Stedelijk Gymnasium Leiden. He enrolled in the Medicine program at Leiden University in 2012, developing an interest in the central nervous system and completed the half minor Translational Neuroscience during the bachelor's (BSc) program. Next, he pursued a semester in Philosophy before continuing with the medical master's (MSc) program. During the master's, he completed a research internship investigating the effect of frailty on outcomes in elderly psychiatric patients and took elective internships in psychiatry and geriatrics.

Soon after obtaining his medical degree in April 2019, Andriy started working as a research physician at the Centre for Human Drug Research (CHDR) in Leiden under the supervision of prof. dr. G.J. Groeneveld. His work initially focused on a first-in-human trial of a disease-modifying therapy for Parkinson's disease, after which he transitioned to investigating the pharmacology of cannabinoids and their potential antidotes. In addition to the research described in this thesis, he was involved in several other projects during his PhD, such as an ongoing trial of cannabis oil for treatment of neuropathic pain. Furthermore, he is currently finalizing his clinical pharmacologist certification.

In 2025 Andriy assumed the position of Senior Clinical Scientist at CHDR, where he provides daily supervision to PhD students investigating novel therapies for neurodegenerative and neuromuscular disorders. Andriy lives in Leiden with his partner Daphne.

LIST OF PUBLICATIONS

Gorbenko AA, Groeneveld GJ, Heuberger JAAC. Medicinale cannabis in de neurologie. TNN-jaargang 122, nummer 7, november 2021. (*Tijdschr Neurol Neurochir*. 2021;122(7):316–26)

Gorbenko AA, Heuberger JAAC, Klumpers LE, de Kam ML, Strugala PK, de Visser SJ and Groeneveld GJ. Cannabidiol increases psychotropic effects and plasma concentrations of Δ^9-tetrahydrocannabinol without improving its analgesic properties. *Clin Pharmacol Ther*. 2024;116(5). doi:https://doi.org/10.1002/cpt.3381

Gorbenko AA, Cohen AA. On the use of open-label studies for the evaluation of cannabis-based products for the treatment of long COVID. *Br J Clin Pharmacol*. 2024;1. doi:https://doi.org/10.1111/bcp.16169

Gorbenko AA, Heuberger JAAC, Juachon M, Klaassen E, Tagen M, Lawler JF, Schneeberger D, Cundy KC, Klumpers LE and Groeneveld GJ. CB_1 receptor antagonist selonabant (ANEB-001) blocks acute THC effects in healthy volunteers: a phase II randomized controlled trial. *Clin Pharmacol Ther*. 2025;117(5). doi:https://doi.org/10.1002/cpt.3581

Gorbenko AA, de Cuba CMKE, de Goede AA, Post TE, Bohoslavsky R, Strugala PK, Heuberger JAAC and Groeneveld GJ. Cannabidiol lacks direct effect on cortical excitability: a randomized, double blind, placebo controlled, 3-way crossover trial. *Clin Pharmacol Ther*. 2025;119(1). doi:https://doi.org/10.1002/cpt.70038

Eijsvogel PPNM, **Gorbenko AA**, Tardiff DF, Skupien M, Rhodes K, Scannevin RH, Yavuz Y, van Brummelen EMJ, Robertson B, Kremer PHC, Groeneveld GJ. Fatty acids as potential biomarkers of stearoyl-CoA desaturase inhibition: variation in healthy subjects and Parkinson's disease patients. *Biomark Neuropsychiatry*. Published online 2025:100132. doi:https://doi.org/10.1016/j.bionps.2025.100132

Gorbenko AA, Post TE, Strugala PK, Klaassen ES, Klumpers LE, de Visser SJ, Sempio C, Klawitter J, Heuberger JAAC, Groeneveld GJ. Low-dose cannabidiol increases plasma concentrations of amitriptyline: A clinical drug-drug interaction study. *Br J Clin Pharmacol*. Published online December 2025. doi:10.1002/bcp.70415

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