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Pharmacological quest for gold: pursuing safer opioid analgesia

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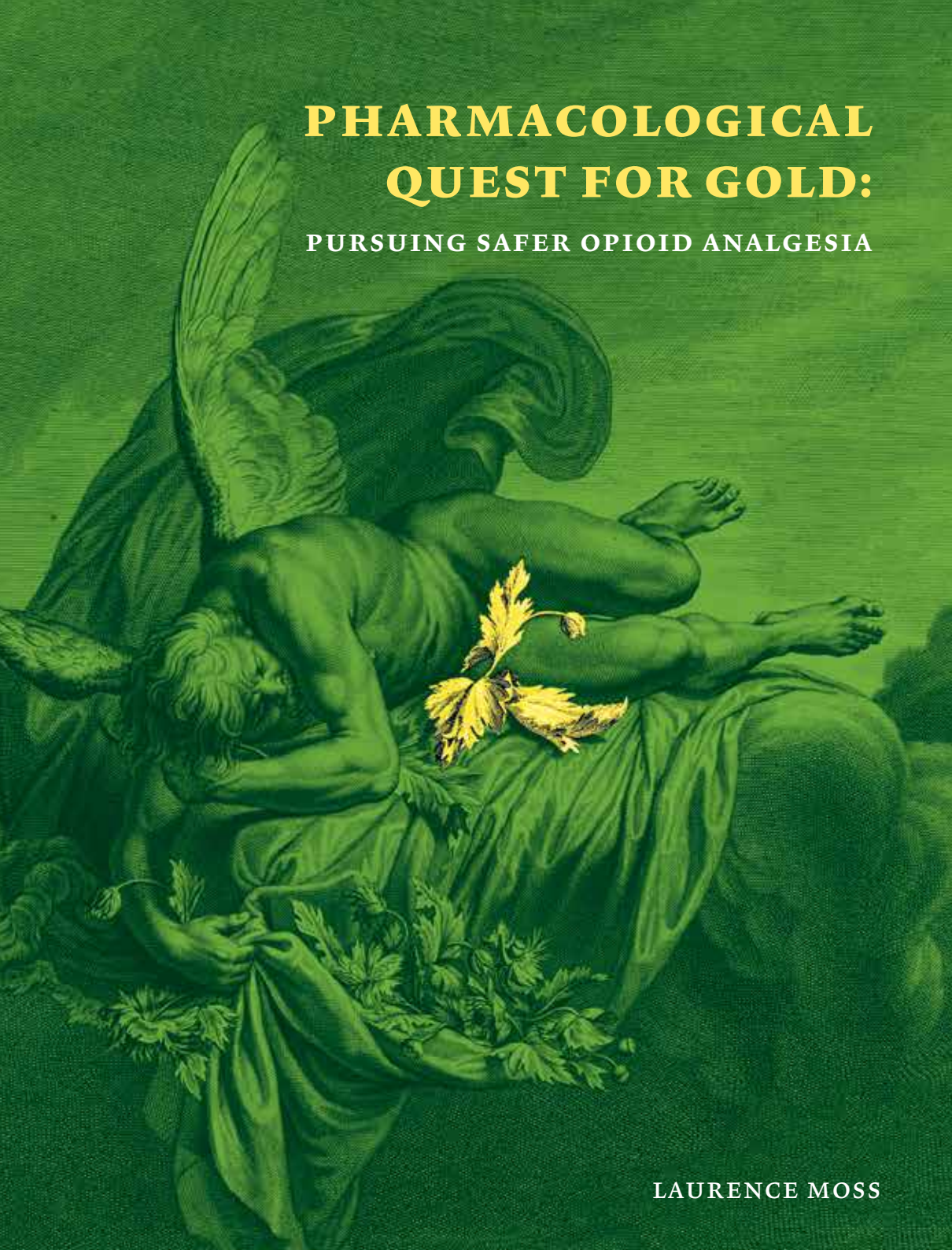

PHARMACOLOGICAL QUEST FOR GOLD:

PURSUING SAFER OPIOID ANALGESIA

LAURENCE MOSS

PHARMACOLOGICAL QUEST FOR GOLD: PURSUING SAFER OPIOID ANALGESIA

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PHARMACOLOGICAL QUEST FOR GOLD: PURSUING SAFER OPIOID ANALGESIA

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op woensdag 5 november 2025
klokke 11:30 uur

door
Laurence Maxim Moss
geboren te Amsterdam
in 1990

FOR MY TITIA AND OUR DEAR CHILDREN

LIMITE
Nu sunt ce am vrut!
Oricui îi lipseste
O împlinire.
Căci nu-i posibil să fii
Și lance și sabie și scut.
- Bobi (opa) Armeanu -

GRENZEN
Ik ben niet wat ik had willen zijn
iedereen mist een vervulling
Omdat het nu eenmaal onmogelijk is
én speer én sabel én schild te zijn.

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CHAPTER 1

INTRODUCTION

Preventing opioid misuse and overdose: a review of the pharmacological armamentarium

To this day, opioid analgesics continue to be a cornerstone in the management of acute and chronic pain due to their unparalleled potency. Despite their significant drawbacks, ranging from manageable disadvantages like nausea to severe, potentially fatal side effects such as respiratory depression, drugs targeting our body's opioid system remain extensively prescribed in clinical practice. This thesis will evaluate several pharmacological strategies aimed at improving the benefit-to-harm ratio of drugs involving the opioid system.

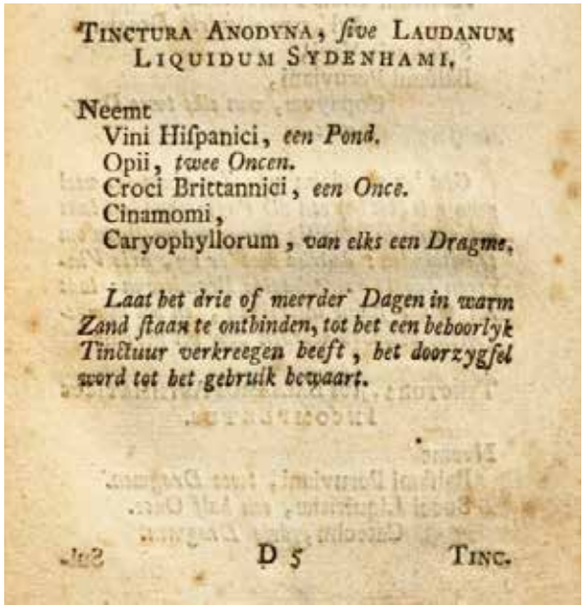
How we got here

The opium poppy, scientifically known as *Papaver somniferum*, is an ancient source of alkaloids, each with significant pharmacological properties.[1] Opium has long been valued for its potent analgesic and euphoric effects, with documented cultivation of the poppy dating back to early civilizations: from agricultural practices in Western Europe dating back to 5000 BCE[2] to the Ancient Near East during the Late Bronze Age.[3,4] Taking a leap through history, we see the administration of opium for pain starting to take on a modern, semi-standardised form with the introduction of laudanum (opium tinctura) in the 16th century (Figure 1). However, it was the isolation of the active ingredient in opium by Prussian pharmacist Friedrich Sertürner in 1805 that revolutionised the field of pain management. He named the substance after *Morpheus*, the Greek god of sleep and dreams, and morphine has been the gold standard of analgesics ever since. From that point onwards, scientific advances further transformed the landscape of opioid pharmacotherapy with the synthesis of diacetylmorphine, or 'heroin' (introduced, ironically, as a non-addictive substitute for morphine),[5] the discovery of fully synthetic opioids such as fentanyl (with even more potent analogues being illicitly produced), and more recently developed molecules based on new pharmacological insights.

Importantly, opioid abuse is not a modern phenomenon either and associated health risks have been reported throughout history. For instance, recreational opium use flourished in 18th and early 19th century China, largely facilitated by British trade, which led to significant societal impact and the Opium Wars. Over the past 100 years, the number of marketed opioids has grown exponentially, and administration of prescription opioids gradually increased with it. This trend was accompanied by an escalating pattern of opioid abuse and surge in opioid-related fatalities, particularly pronounced in the United States of America (US). It is estimated that close to 110.000 drug overdose deaths occurred in the US in 2022 alone, and ~75% involved opioids.[6] Due to its extent, the US Centres for Disease Control termed this public health crisis as the 'opioid crisis'.

Research into new therapies, as outlined in this thesis, is therefore essential for developing safer, more effective treatments involving opioids and mitigating the ongoing opioid epidemic.

FIGURE 1 Preparation instructions for Laudanum from the Amsterdam Pharmacopoeia of 1767.[7]



TINCTURA ANODYNA, *sive* LAUDANUM LIQUIDUM SYDENHAMI.

Neemt
Vini Hiſpanici, *een Pond.*
Opii, *twee Oncen.*
Croci Brittannici, *een Once.*
Cinamomi,
Caryophyllorum, *van elks een Dragme.*

Laat het drie of meerder Dagen in warm Zand ſtaan te ontbinden, tot het een behoorlyk Tinctuur verkreegen heeft, het doorzygſel word tot het gebruik bewaart.

D 5 TINC.

Mechanisms of action

Opioids exert their effects through a family of G protein coupled receptors (GPCRs) that currently consists of the delta-opioid (DOP), kappa-opioid (KOP), mu-opioid (MOP) and nociceptin/orphanin FQ receptors [NOP]).[8] The NOP receptor is an atypical receptor as it, in contrast to the other opioid receptors, has no affinity for the opioid-receptor

antagonist naloxone. Although all four receptor-types can contribute to analgesia, most therapeutic and side effects of clinically relevant opioids are mediated by MOP.[9,10] Therefore, this section focuses on treatment strategies involving agonists that interact with this receptor in particular: partial agonism, functional selectivity, endogenous opioids and multifunctional ligands.

Partial agonism

Opioid-induced respiratory depression (OIRD) is the most dangerous side effect of opioid (ab)use, driven by MOP mediated inhibition of neurons in brainstem respiratory networks.[11] Full MOP agonists induce a receptor conformation that triggers the maximum effect, leading to both strong analgesia and, potentially, cessation of breathing. In contrast, partial agonists cause a limited response. This may offer a respiratory safety benefit, theoretically, due to an observed 'ceiling effect' (i.e., no complete OIRD occurs), even if all MOPs are saturated. However, whether this ceiling effect also applies to analgesia and other side effects remains debated.[12] When partial agonists are co-administered with full agonists, their effects are additive at low doses. At higher doses, the partial agonist competes with the full agonist for receptor binding, limiting the overall effect (i.e., relative antagonistic). Partial MOP agonists could therefore be useful in limiting respiratory depression caused by full MOP agonists.[13]

A prime example is buprenorphine, a mixed partial MOP/NOP agonist and DOP/KOP antagonist,[14] that binds to the MOP with high affinity where it exerts its main effects. Despite its partial agonism, buprenorphine is associated with common opioidergic gastrointestinal side effects and has been reported to cause apnoea in some cases.[15] However, it can displace full MOP agonists like fentanyl and exhibits slow dissociation from MOP receptors. Studies showed that high-dose buprenorphine (plasma concentrations >2 ng/ml) can prevent lethal OIRD from fentanyl and reverse methadone-induced respiratory depression in patients presented to the emergency department. Researchers even concluded that treatment with buprenorphine was superior to naloxone in terms of duration of effect and opioid withdrawal, as well as outcomes including need for intubation and death.[16] Another example is the MOP partial agonist dezocine, which showed a ceiling effect on respiratory depression in a small clinical trial and recent findings suggest it may limit sufentanil-induced respiratory depression in patients undergoing awake intubation.[17–19] Dezocine is no longer available in Europe and US but is widely used in Asia, where it has a large share of the opioid analgesic market.[20,21]

These data demonstrate the potential of partial agonism as a safer opioid treatment strategy and warrant further studies to confirm the clinical benefit in a large population.

Functional selectivity

After ligand binding to the MOP receptor, intracellular signalling cascades are initiated resulting in a certain pharmacodynamic (PD) drug profile.[22,23] There are multiple mechanisms that can cause a functionally selective response, commonly referred to as 'biased signalling', including receptor-, ligand-, and system bias.[24] Here we focus on ligand bias: relative to a reference non-biased agonist (i.e., balanced; often [D-Ala^2, *N*-MePhe^4, Gly-ol^5]-enkephalin (DAMGO) in the case of MOP engagement), binding results in a different receptor conformation thereby triggering a distinct downstream signalling pathway. Currently, the MOP downstream functional signalling is divided into two pathways: G-protein coupling and β-arrestin 2 scaffolding. Studies showing enhanced analgesic response to morphine and reduced side effects like constipation and respiratory depression in β-arrestin 2 knockout mice led to the pharmacological paradigm of 'biased opioids'.[25–27] This refers to MOP agonists preferentially activating G-proteins over β-arrestin 2, which can be expected to be potentially safer opioids. Multiple studies performed over the last 20 years support the hypothesis that β-arrestin 2 recruitment is to a large extent responsible for opioid side effects, although the concept remains debated with recent studies proposing that low intrinsic efficacy might play a major role in the improved safety profile and not any reduced β-arrestin 2 recruitment. While more research may shed light on the underlying mechanism, 'biased' opioids do show promise both in animal experiments as well as humans. Given this apparent successful approach in pain pharmacotherapy, many researchers are investigating novel compounds of this type, of which I will discuss several promising candidates.

Oliceridine (Trevena), the first US Food and Drug Administration (FDA) approved biased MOP agonist (2020), demonstrated a safer profile compared to existing opioids in multiple studies, leading to less respiratory depression in particular. Moreover, a recent study in healthy volunteers suggested oliceridine had less impact on neurocognitive

functions than morphine. TRV734, another biased MOP agonist being developed by Trevena, showed promising safety and analgesic potential compared to oxycodone in early phase clinical drug trials.[28]

PZM21, a biased MOP ligand recently discovered through structure-based docking studies and optimization, showed antinociception in non-human primates without rewarding properties.[29,30] However, it was recently reported to induce tolerance, withdrawal, and respiratory depression similar to morphine so it remains to be seen whether this drug will endure through clinical trial testing.[31]

Several other promising biased MOP ligands include SHR9352, MP1207, and MP1208, which all exhibit reduced β-arrestin signalling and demonstrated analgesic properties without traditional opioid side effects in vivo.[32,33] However, results from clinical trials are yet to follow.

Throughout history, humans investigated the medicinal properties of derivatives from natural sources. As such, naturally occurring biased opioids are also currently under investigation. Mitragyna speciosa (kratom) contains opioidergic alkaloids like mitragynine, which exhibited antinociceptive effects in preclinical studies that could be inhibited by naloxone administration but have shown not to be devoid of reinforcing properties.[34–36] A second example regards herkinorin, derived from Salvia divinorum, which demonstrated antinociceptive effects in rats but a lower tolerance liability than morphine.[37–39] Remarkably, findings suggested that the analgesia was peripherally mediated (restricted to the site of injection). Similarly, corydine and corydaline, alkaloids from Corydalis (family of the opium poppy) produced analgesic effects without significant opioid-like side effects in preclinical models.[40]

Endogenous opioids

Endogenous opioids such as enkephalins and β-endorphin play a crucial role in pain regulation.[41] This innate system may be used to induce analgesia without the administration of exogenous opioids, thereby hypothetically keeping away from unwanted opioid side effects. Endogenous opioids themselves can be made druggable, but inhibition of enzymatic degradation of physiologically produced endogenous opioids is an alternative strategy.

Using endogenous opioids has limitations due to short half-lives ($t_{½}$), poor bioavailability and blood-brain barrier (BBB) penetration. Various compounds have been chemically modified (e.g., modification of the peptide to make it amphipathic) to improve their pharmacological properties and demonstrated promising analgesia/safety profiles in preclinical studies.[42–45] CYT-1010 (CYTOgel Pharma, US), an endomorphin 1 analogue, has shown promising results in early clinical trials with a favourable safety profile.[46,47]

Endogenous opioids are degraded by the enzymes enkephalinase (ENK), neutral endopeptidase (Neprilysin, NEP), and aminopeptidase N (APN), making these enzymes targets for analgesic treatments. Thiorphan was the first inhibitor to be developed shortly after ENK was discovered in 1978. However, antinociceptive activity in animal models was inconsistent, discouraging further development as an analgesic. Instead, the drug ended up being developed as an antidiarrheal (racecadotril, its prodrug) thanks to its ability to effectively reduce water and electrolytes secretion into the intestinal lumen.[48–51]

Dual ENK inhibitors (DENKIS) exert their effect by inhibiting two endogenous opioid degrading enzymes (i.e., ENK, NEP or APN).[52] PL37 and PL265 (Pharmaleads, France), both DENKI pro-drugs, are going through clinical trials, with early results reportedly indicating favourable safety/tolerability profiles, and PL37 showing preliminary analgesic efficacy in patients with peripheral neuropathic pain of diabetic origin.[53–56] STR-324 (Alaxia, France), the chemically stable analogue of the naturally occurring opiorphin, is the latest DENKI being evaluated in clinical trials. Preclinical studies indicated analgesic effects while high-dose STR-324 was devoid of respiratory depression or hemodynamic effects.[57,58] No respiratory effects were recorded in the first in human (FIH) trial either, although no analgesic potential was observed in a wide range of human evoked pain models.[59] However, a recent study in patients who underwent laparoscopic surgery found a noteworthy decrease in pain score meeting pre-defined responder-criteria in 35% of the patients treated with STR-324 for 20-min post-operatively, versus 46% in the comparator group who received intravenous (IV) morphine.[60]

Multifunctional

Recent advancements in opioid pharmacology have focused on (low selectivity) multifunctional mixed ligands, recognizing significant interactions between opioid receptor subtypes. For example, preclinical findings indicate that combining MOP agonism with DOP antagonism produced anti-nociception without tolerance,[61] while co-administration of MOP and NOP ligands at low doses suggests synergistic analgesic

effects.[62] Additionally, NOP activation may stimulate breathing and reduce abuse potential, improving the risk-benefit profile when co-administered with a MOP agonist.[63–65] Many opioid analgesics are multifunctional ligands to some extent and several examples are provided below (excluding those mentioned in previous sections).

Levorphanol (developed in the 1940s) is a MOP agonist with activity at N-methyl-D-aspartate (NMDA) receptors and inhibits norepinephrine (NRI) and serotonin (SRI) reuptake, offering central pain modulation.[66] However, it is less potent than other opioids while the risk of respiratory depression remains high. Tramadol, a synthetic opioid acting on μ-, κ- and δ- receptors with SRI/NRI effects, is also not as potent as other available opioids but widely used in clinical practice thanks to its relatively good safety profile.[67] This led to the development of Tapentadol (Grünenthal) which combines MOP agonism with selective norepinephrine reuptake inhibition (and limited SRI), providing an improved safety profile with reduced respiratory depression compared to classic opioids.[68–70] New mixed ligands in development include MMP-2200 (MOP/DOP agonist), which showed reduced tolerance and self-administration in preclinical studies,[71] and BU08028, a buprenorphine analogue with greater NOP affinity, offering analgesic efficacy in primates without addiction potential.[72,73]

Non-opioid receptors can also modulate opioid side effects. Combined MOP engagement with neuropeptide FF receptors 1 and 2 antagonism (e.g., drug candidates DP32 and DP50) has been demonstrated to suppress opioid-induced adverse effects, including opioid-induced apnoea, opioid-induced hyperalgesia (OIH) and associated antinociceptive tolerance in animal models.[74–76] Cholecystokinin (CCK), an 'anti-analgesic' peptide, is upregulated in neuropathic pain, and novel bifunctional compounds like RSA 504 and RSA 601 target neuropathic pain by combining MOP/DOP agonism (K_i values δ<μ) with CCK antagonism.[77–79] Lastly, neurokinin receptors (NK1, and possibly NK3) have been suggested to drive opioid-mediated reward and tolerance in the ventral tegmental area, as well as OIH and tolerance in other circuits. and multifunctional MOP agonist/NK1 antagonists are being explored for analgesia with reduced tolerance and minimal reward pathways.[79–81]

OPIOID RECEPTOR HETERODIMERS

Receptors, including the MOP, can form heterodimers which can display distinct binding properties and signalling behaviours compared to individual receptors. Multifunctional drugs designed to selectively target specific receptor heterodimers hold potential for improved pain relief with lower addiction and tolerance risks, and several promising candidates are currently in development.[82]

MMG22 is a bivalent ligand targeting MOP-mGluR5 (metabotropic glutamate receptor 5) heterodimers, combining MOP agonism with mGluR5 antagonism, aiming to deliver analgesia with fewer side effects for inflammatory and neuropathic pain.[83] Other compounds like NNTA (N-naphthoyl-β-naltrexamine) and TY027, target MOP-KOP and MOP-DOP heterodimers, respectively, and demonstrated effective analgesia with reduced tolerance and withdrawal symptoms, and fewer gastrointestinal side effects in preclinical studies.[84,85] Lastly, MCC22 targets MOP-CCR5 (C-C chemokine-receptor type 5) heterodimers and has been reported to effectively treat inflammatory arthritis pain in mice without inducing tolerance.[86]

Splice variants

Opioid receptors are G protein structures encoded by DNA within genes like OPRM1, which codes for the MOP. Clinical variability in opioid response has sparked interest in OPRM1 splice variants, which produce receptor forms with different amino acid sequences and potentially unique biological effects.[87,88] MOP splice variants are thought to influence patient-specific opioid responses, with three primary forms: full-length 7 transmembrane (7TM), truncated 6TM, and single TM.[89] The 6TM variant, which has been associated with an improved safety profile, is of particular interest. IBNtxA, a ligand selectively targeting 6TM, has demonstrated promising preclinical results consisting of analgesia with fewer side effects such as respiratory depression compared to traditional opioids like morphine.[90,91] However, the exact mechanisms and clinical relevance of these splice variants remain unclear, requiring further research into MOP signalling pathways and selective ligand efficacy to fully explore their therapeutic potential.

Concurrent medications

Concurrent medications aimed at preventing and/or treating overdose have an important place in opioid pharmacotherapy. MOP antagonists for rapid reversal of OIRD act by displacing opioids from receptors. Other strategies have been gaining attention, including immuno-pharmacotherapy and ligand sequestration, respiratory stimulants that have the ability to counteract OIRD without compromising analgesia and multimodal analgesia. All of these will be discussed below.

Antagonism

The definition of an antagonist is that binding of the drug results in zero downstream activity. In emergency settings acute reversal of OIRD is required, and loss of any desired analgesic effect as a result of the antagonism would be accepted. Importantly, abrupt reversal of opioid intoxication can also precipitate withdrawal with sympathetic hyperactivity causing nausea/vomiting and cardiovascular symptoms such as tachycardia, hypertension, or even cardiac arrest.[92,93] Ironically, in. rare instances, reversal of OIRD with opioid antagonists has even been reported to result in pulmonary oedema and subsequent hypoxic respiratory failure, highlighting the complexity of this treatment strategy.

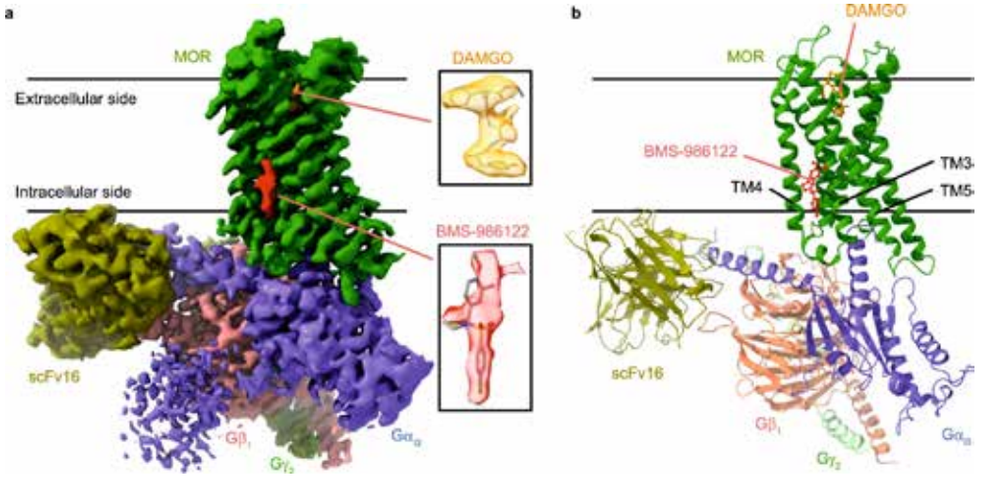
Naloxone may be the most prominent example of an opioid receptor antagonist, given that opioid overdose emergency kits containing naloxone inhalators or injectors are currently about as common in the US as automated external defibrillators. Acting as a competitive, pure antagonist to the MOP, naloxone is a good candidate to reverse OIRD. However, because of its relative short $t_{½}$ (32 min), repeated administration is sometimes required to prevent renarcotisation (i.e., onset of OIRD) when opioids with a longer $t_{½}$ have been used.[93–96] In particular, naloxone might fall short when counteracting high potency, long-acting synthetic opioids like fentanyl. Therefore, there is a need for formulations of opioid antagonists with a significantly longer $t_{½}$.[97,98]

Nalmefene and naltrexone are examples of longer-acting MOP antagonists but neither is available in IV formulation needed for acute rescue of OIRD. Although studies have demonstrated nalmefene's effectiveness in both preventing and reversing OIRD when administered IV,[99,100] the injection formulation (REVEX) has been discontinued for commercial reasons. However, a formulation suited for intranasal administration has been approved in the US in 2023 (OPVEE, Indivior) and we might soon see a rise in its clinical use.[101,102] Although naltrexone is available as extended-release formulations and used in the treatment of opioid use disorder (OUD), it is not marketed as antidote in acute/emergency situation.[103,104] Another MOP antagonist that is currently still in development is methocinnamox, which has shown an extended duration of action of up to 2 weeks in preclinical studies.[105–108] This drug is currently being evaluated in phase 1 trials and the results can be expected to follow soon. Finally, nanoparticle-based delivery systems are being developed that covalently link naloxone to a polymer, thereby creating a formulation leading to controlled release of the antagonist which completely blocked morphine-induced analgesia for over 26 hours in animal studies.[109]

Allosteric modulation

Whereas antagonists bind at extracellular orthosteric sites of the MOP and have overlapping properties regarding downstream signalling pathways, modulators bind to allosteric pockets of the MOP enabling selective tuning of receptor signalling (e.g., induce biased signalling).[110] Allosteric modulators can exert their effect without competing with endogenous or exogenous opioids, potentially resulting in enhanced therapeutic specificity with reduced side effects. BMS-986122 (**Figure 2**), for example, is a positive allosteric modulator of MOP capable of enhancing the response to endogenous opioids.[111,112] This presents an innovative path to safer opioid therapies and advancing treatment options in pain management with a possible reduction in liability for tolerance, dependence, and overdose.

FIGURE 2 Allosteric modulator BMS-986122 interacting with the MOP.
(A) cryogenic electron microscopy density map of the MOP(DAMGO)-Gi-scFv16 complex in the presence of BMS-986122; (B) overall structure of the MOP(DAMGO)-Gi-scFv16 complex in the presence of BMS-986122.[111]



Drug sequestration

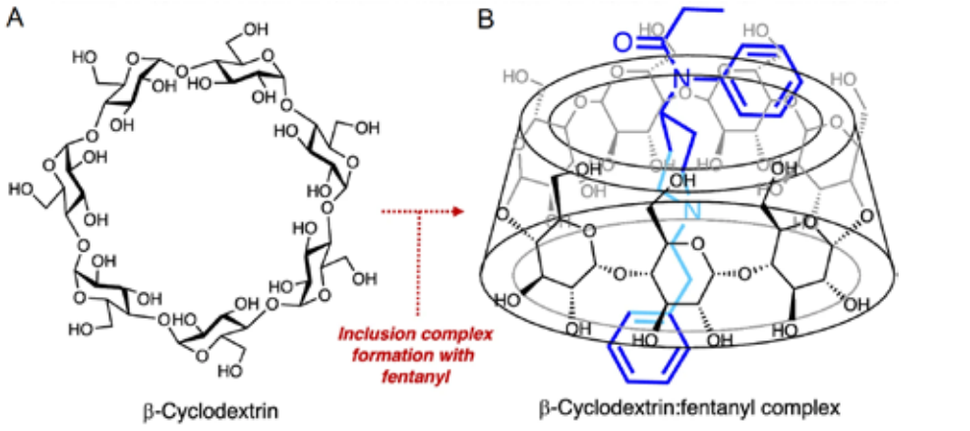
Another pharmacotherapeutic approach aimed at reducing agonist engagement with the MOP, consists of opioid sequestration in the blood. This prevents opioids from reaching the effect site (i.e., central nervous compartment) or redistributing them back into the systemic circulation. There are two main methods to achieve this: administering molecules that bind opioids and through immuno-pharmacotherapy, where antibodies neutralize opioids. In contrast to overdose treatment, immuno-pharmacotherapy has the potential to prevent (fatal) overdoses, for example in OUD patients who relapse or in case of individuals that use other drugs (such as MDMA) that are often laced with potent opioids and consequently might inadvertently overdose. Many companies are developing these types of drugs, and below a few promising developments are discussed.

CONTAINER MOLECULES

As mentioned, sequestration can result from administration of container molecules that neutralize opioids in the bloodstream, thereby reducing their central nervous system (CNS) effects. One example is Calabadion 1 (CLB1), an acyclic cucurbit[n]uril, which has shown pre-clinical efficacy in reversing fentanyl-induced respiratory depression by encapsulating the drug and facilitating renal clearance.[113] Similarly, promising preclinical efficacy and phase 1 safety data suggests that CS-1103, a first-in-class injectable small molecule sequestrant, can dose-dependently reverse fentanyl-induced OIRD in less than 10 minutes and is safe in humans.[114] NarcoBond is another interesting candidate; a biomimetic nanosponge that mimics the lipid bilayer of cell membranes.[115] Its surface contains human proteins from erythrocytes, neurons, and MOP's, which lead to opioid binding and thus reduction of circulating drug. Preclinical studies demonstrated reversal of fentanyl-induced antinociception, respiratory depression, and bradycardia, complementing naloxone's action by serving as an opioid 'buffer' in the systemic circulation.

Cyclodextrins are cyclic structures with a hydrophobic interior, able to effectively capture circulating unbound hydrophobic drugs like fentanyl (**Figure 3**).[116,117] In anaesthesia, sugammadex is a cyclodextrin widely used to counteract neuromuscular blocking drugs like rocuronium. However, cytotoxicity and haemolytic activity limit successful development to sequester opioids at this time. If these can be overcome, then this may also hold promise as a countermeasure for opioid overdose. Still, studies so far show that these containers lack specific selectivity for just opioids. These techniques currently focus on lowering plasma concentrations of opioid molecules in such a state that they can't pass the BBB and therefore indirectly reduce central opioid effects through peripheral sequestration. Although these drugs act in a different compartment than where opioids exert their effect, studies demonstrated a relative short time to E_{MAX} (i.e., reversing opioid effects), suggesting they could play a valuable role in clinical practice.

FIGURE 3 Molecular entrapment of fentanyl by the container molecule β-Cyclodextrin.
(A) Structure of β-cyclodextrin showing the seven glucose units linked in an α1,4-fashion giving rise to a conical structure (frustrum) open at both ends with a hydrophobic interior and a hydrophilic exterior; (B) representation of a hypothetical inclusion complex formed between β-CD and fentanyl.[116]



IMMUNO-PHARMACOTHERAPY

Immuno-pharmacotherapy involves the use of antibodies, raised passively or actively, to sequester specific drugs in plasma.[118,119] While passive immunization (i.e., administration of monoclonal antibodies) works directly after commencing treatment, active immunization (vaccination) takes longer to reach the intended effect. Although opioid immuno-pharmacotherapy has been researched as early as the 1970s,[120,121] no such treatment is currently available to clinicians.[122] Since the opioid epidemic, this strategy has regained interest, and multiple efforts are being undertaken to develop therapies. Promising preclinical efficacy data has recently emerged for vaccines targeting fentanyl, oxycodone, hydrocodone, and heroin.[123–133]

Passive immunisation consists of the administration of antibodies that target opioids. CSX-1004 is a fentanyl-specific monoclonal antibody which has successfully undergone testing in non-human primates (NHP).[134] Preclinically, reversal of fentanyl-induced antinociception and carfentanil-induced respiratory depression was observed. In NHP's, protection from repeated fentanyl challenges was reported for a duration of 3-4 weeks. Other researchers derived monoclonal antibodies (mAbs) against opioids from mice vaccinated with a fentanyl conjugate vaccine.[135] These mAbs hold promise by reversing OIRD in rodent models, and a significantly improved $t_{½}$ of ~6 days compared to e.g., naloxone.

Although research data seem promising, one major drawback of immuno-pharmacotherapy is that it generally targets specific opioids, whereas overdose often may occur as a result of poly-substance abuse. Still, reduction of the opioid part of OIRD will improve the likelihood of survival.

Respiratory stimulants

Several locations in the brainstem control our body's respiratory function.[136,137] The respiratory networks involved in rhythmogenesis can be engaged via many different routes and thus types of ligands; both drugs acting directly in the brainstem as well as others modulating the input signals to those networks are being researched as treatments for OIRD. The strategy of using respiratory stimulants to treat respiratory depression (regardless of cause) pre-dates modern medicine but has received increased attention over recent years because of the potential benefit to reduce adverse respiratory effects while preserving the analgesic function of opioids (i.e., as opposed to antagonizing all effects).[138–141] Because these drugs don't act through the MOP receptor, they've been dubbed 'agnostic respiratory stimulants'. The major advantage lies in the fact that these drugs can stimulate breathing, even in situations where patients took other (unknown) CNS depressants together with an opioid (which is often the case). Given the complex interplay of receptors that influence respiratory function, there are many different drug targets of this type that are being pursued. Below several examples are briefly discussed.

Serotonin (5-hydroxytryptamine, 5HT) receptors play a significant role in respiratory control (hypothesized to act in the brainstem's pre-Bötzinger complex).[142] Consequently, one approach to restore OIRD is through the serotonergic system and involves enhancing activity in respiratory neurons using 5HT receptor ligands. Multiple attempts at developing agonists for 5HT subtypes (e.g., $5HT^{1a}$, $5HT^{4a}$ etc.) have been made and examples like buspirone and mosapride have been shown to reverse OIRD without affecting pain relief in animals.[143] However, these drugs have thus far failed to demonstrate translation of respiratory stimulation from preclinical studies to humans at the doses tested, and higher dosing was limited due to side effects.[144,145]

Ampakines are drugs that bind as positive allosteric modulators to the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) thereby enhancing glutamate mediated excitation, including in pre-Bötzinger neurons.[146] CX717 and CX1739 are two ampakines currently in clinical development stages after having shown promising results by reversing OIRD in animal models, and preliminary human data on safety and respiratory function in coadministration with opioids appear promising.[147–155]

Located within the carotid bodies (located just above the bifurcation of the carotid arteries), there are receptors that respond to changes in blood chemistry including oxygen levels and pH. It is thought that this signalling is mediated through potassium channels expressed on type 1 carotid body cells. When the chemoreceptors detect hypoxia or acidosis, the body responds by increasing ventilation aimed at correction of these abnormalities.[156–160] Doxapram is an older drug that stimulates breathing by blocking those potassium channels in the carotid body cells and is used off-label to treat apnoea of prematurity.[161,162] Although effective reversal of OIRD has been observed in animals, it is associated with dose limiting analeptic side effects (e.g., hypertension and severe anxiety) in humans. Another example in this drug class is ENA-001, an analogue of the discontinued respiratory stimulant almitrine, which acts on large-conductance calcium-activated potassium channels (BK_{Ca2+}).[163–165] Mimicking hypoxia at the receptor, ENA-001 has shown to stimulate breathing and thereby attenuating OIRD and reducing the blunting effect of propofol on the respiratory response to acute hypoxia in healthy volunteers.

The potential of certain hormones to reverse OIRD has been suggested based on animal data. Thyrotropin-releasing hormone (TRH) and oxytocin, both naturally produced in the hypothalamus, have demonstrated beneficial effects in rodents and nonhuman primates with respect to stimulating respiration when co-administered with opioids.[166,167] Preclinical studies found that TRH produced a dose-dependent

excitatory effect on breathing and could mitigate OIRD by significantly increasing respiratory rate (although precipitating or worsening acidosis in all studies).[168,169] However, these findings could not be replicated in a small human trial where IV administration of up to TRH 0.1 mg/kg failed to reverse remifentanil-induced respiratory depression, possibly due to differences in TRH receptor types involved in respiratory stimulation between mice and man.[170] Taltirelin, a more potent TRH analogue with superior pharmacokinetic (PK) properties including increased BBB penetration, has been proposed as potential drug candidate in the context of OIRD. Until now, studies in rodents have not demonstrated a favourable pharmacological profile (e.g., rapid shallow breathing and lactic acidosis without normalising arterial oxygen concentration) and human respiratory data is not yet available.

Oxytocin has been shown to increase respiratory rate in patients with obstructive sleep apnoea[171] and a recent study evaluating the respiratory effects of oxytocin observed a bell-shaped dose-response curve with maximal reversal of fentanyl-induced respiratory depression at low doses but no effect at higher doses as measured by phrenic nerve activity in anaesthetised rats.[167] This limited effect was attributed to cross-activation of vasopressin receptors at high oxytocin levels and reversal of respiratory depression could be regained by co-administration of a vasopressin antagonist.

Lastly, other, diverse strategies may lead to reversal of OIRD, such as, ketamine (through NMDA antagonism, but also via enhancing monoaminergic neurotransmission and possibly AMPAR agonism),[172–174] amphetamine and cocaine (both psychostimulants affecting monoamine neurotransmission)[175,176] and cannabinoid type 2 receptor agonists (multiple drugs showed potential in reversing OIRD in animal models)[177–179]. However, despite possible beneficial effects on respiratory function of each specific drug type, clinical utility may be limited especially in an outpatient setting given that none demonstrated complete prevention or reversal of respiratory depression induced by high dosed opioids, and each have a significant potential for abuse.

Multimodal analgesia

The idea of combining opioids with non-opioid pharmacological treatments to increase analgesia, thereby reducing the need for the opioid, has also gained attention in recent years and is worth mentioning in this section. This 'opioid-sparing' pharmacotherapy ranges from over-the-counter medicine such as paracetamol[180] and non-steroidal anti-inflammatory drugs (NSAID's)[181] to combinations requiring specialist supervision including $\alpha_2\delta_1$ calcium channel blocker (e.g., gabapentin),[182] alpha-2 agonists (e.g., clonidine and dexmedetomidine),[183] NMDA antagonists (e.g., ketamine),[184,185] sodium channel blockers (e.g., lidocaine),[186,187] glucocorticoids (e.g., dexamethasone),[188] magnesium[189,190] and many more.[191–194] A detailed discussion of these therapy combinations is outside the scope of this review.

Formulation & pharmacokinetics

From a pharmacological perspective, inhaling, snorting and injecting (illicit) drugs are regarded excellent routes of administration because these result in high bioavailability and rapid onset of action in the CNS. Orally prescribed opioids are often misused through these alternative routes, frequently leading to overdose. Armed with this knowledge, scientists have focused their efforts on the development of new opioid formulations over the last decades in order to curb prescription opioid abuse.

Pharmaceutical formulation is defined as the process of combining active pharmaceutical ingredients with other components to produce a final medicinal product. In pharmacotherapy, the formulation of compounds is a key determinant in their PK and PD profile, thereby having significant impact on the balance between efficacy and safety. When reviewing safety in the context of opioids, we need to look beyond adverse drug reactions after drug administration and include also the potential for abuse of the drug as a whole.

Abuse-deterrent formulations (ADFS)

Development of ADFS is based on the pharmacological paradigm that likelihood of opioid abuse increases with (I) higher peak plasma concentrations, or C_{max}, which is related to the intensity of psychoactive effects (notably euphoria); and (II) the speed at which these concentrations are reached (t_{max}), which reinforces the association between substance use and the subjective experience (i.e., the subsequent dopamine surge in the mesolimbic pathway). Simultaneously, these parameters are related to adverse opioidergic effects with respiratory depression being most lethal (mainly C_{max} driven). In addition to concerns about abuse and overdose, non-oral routes of administration increase the risk for contracting infectious diseases such as hepatitis C and for deep vein

thrombosis (IV abuse) or tissue necrosis with septum/palatal perforation (intranasal abuse).[195] Therefore, it is only fair that the FDA considers the development of these new formulations a high public health priority.

By designing physicochemical barriers in formulations, making opioids tamper-resistant, the risk of manipulation of the medicinal product can be reduced.[196] This hampers administration of the opioid through a different route then intended. In parallel, formulations can deter abuse by decreasing the desirability of manipulating the drug by combining the opioid with other ingredients (i.e., antagonist or aversive agent) or through the use of an inactive form of the parent compound (i.e., prodrug). Lastly, the FDA considers certain drug release designs or methods of drug delivery as ADF.

ADF labelling requires extensive FDA-mandated pre-market abuse deterrence studies in addition to standard drug development trials: in-vitro evaluation of manipulation and extraction, PK studies (e.g., intact and manipulated drug comparison) and human abuse-potential studies (assessing e.g., drug liking). Lastly, post-marketing epidemiological studies need to be performed.[197,198] As a consequence, not all opioids marketed as ADF's survive.

TAMPER-RESISTANT FORMULATIONS

OxyContin extended release (Purdue Pharma) is a notorious example of a drug initially marketed as abuse-resistant, but widely misused due to its bypassable extended-release mechanism (e.g., crushing/chewing). After undeniable abuse and the drug patent nearing its expiration, the FDA approved reformulated OxyContin in 2010 with a new matrix to resist physical compromise, and solvent extraction by forming a viscous gel in water. As of 2024, three other extended release ADF's are currently available, each using particular tamper-resistant technologies: Hysingla (hydrocodone, Purdue Pharma) and its generic version (Alvogen Inc), and Xtampza (oxycodone, Collegium Pharm Inc). Additionally, one immediate release ADF named RoxyBond is available (oxycodone, Daiichi Sankyo).[199] Nucynta (tapentadol with a physicochemical barrier, Collegium) is the only available opioid with tamper-resistant properties without the ADF[200] labelling since Zohydro (hydrocodone, UCB) was removed from the market[201] and the manufacturer (Mallinckrodt plc) of Exalgo (hydromorphone) and Xartemis (oxycodone/acetaminophen) succumbed to litigation resulting from false abuse-deterrent claims.[202]

Over the past decade, the FDA has withdrawn multiple opioids due to abuse risks.[203] OxyContin remains the only ADF opioid on the market with available post-market safety studies claiming reduced abuse and overdose fatalities, although recent research indicates no overall effect on overdose rates.[204–206]

Despite their design to deter abuse through non-oral routes, ADF's still permit the most common form of opioid misuse: oral ingestion of doses higher than intended, thereby increasing plasma- and subsequently effect site concentration.[207] Additionally, these formulations often fail to account for the PK/PD hysteresis, focusing on time to peak plasma concentration (C_{max}) rather than the time to maximum effect (Emax) and neglect the area under the effect curve, thereby overlooking the duration of euphoria (which is why extended-release formulations are reportedly preferred by some abusers).[208]

ADDITION OF ANTAGONISTS

Combining an opioid with a narcotic antagonist that is poorly absorbed after oral intake, is a strategy to prevent parenteral abuse. Talwin NX, the opioid pentazocine combined with the antagonist naloxone (Winthrop Laboratories), was the first of its kind and FDA approved in 1982. It regarded the reformulation of pentazocine, prompted by the widespread abuse of this predecessor (in particular taken together with tripelennamine, an antihistamine).[209,210] Similarly, formulations combining naloxone with buprenorphine are available (e.g., Suboxone, Reckitt Benckiser Pharmaceuticals Inc.).[211,212]

To prevent oral bioavailability of the antagonist (more relevant when using naltrexone), it can also be sequestered in the formulation so that it is only released when the drug product is manipulated. Formulations including oxycodon/naloxone (Targiniq and Troxyca, Purdue Pharma and Pfizer, resp.) and morphine/naltrexone (Embeda, Pfizer) were previously available but have been discontinued in recent years.[213,214]

AVERSIVE AGENTS

The earliest report of an aversive agent in an opioid formulation regards Lomotil, which combines diphenoxylate (opioid) with atropine (anticholinergic).[215,216] The development originated from US CIA funded research in the 1950s, seeking a nonaddictive substitute for codeine,[216] but eventually found its way to the public as an anti-diarrheal. Atropine's subtherapeutic dose only induces somatic aversive side

effects, such as tachycardia, when overdosed. The list of potential aversive agents is long (e.g., capsaicin),[196,217] but this strategy is not widely applied. One example includes oxycodone with two aversive agents: subtherapeutic niacin (vitamin B3),[218] causing peripheral vasodilatory symptoms (e.g., flushing) when taken in excess, and sodium lauryl sulfate, causing nasal irritation when snorted., although the FDA approved the drug after niacin was excluded (available as Oxaydo, Egalet Ltd).[196]

PRODRUGS

Prodrugs require in vivo enzymatic activation for conversion from parent to active drug. These formulations can be considered abuse-deterrent because diversion from intended oral use may negatively impact PK (i.e., less rewarding C_{max} or t_{max}), and oral ingestion after manipulation (e.g., crushing) wouldn't yield a more rewarding PK profile. An additional strategy related to prodrugs focusses on saturation of specific enzymes (e.g., isoforms of CYTochrome P450) to prevent the conversion of a particular opioid prodrug into its active form and thereby prevent overdosing.

Codeine and tramadol are both commonly prescribed opioid prodrugs that are metabolized into their active form morphine and O-desmethyltramadol, respectively.[219] However, ADF designation requires reduced abuse potential to be demonstrated and at present no such studies are being conducted nor are new formulations e.g., including added abuse-deterring mechanisms in development.

Several opioid prodrugs that are converted to their active form by enzymes in the gastrointestinal tract are being developed. KP511 (Zevra Therapeutics), for example, is a hydromorphone prodrug and preclinical studies indicate that its initial metabolism saturates, suggesting that excessive ingestion would not lead to overdose[220]. Another interesting drug candidate regards PF614, which undergoes activation by trypsin followed by non-enzymatic cyclization and cleavage producing free oxycodone. Human abuse potential studies for this drug are currently underway.[221,222] Similarly, research aimed at utilizing pH-dependent release mechanisms is being performed.[223,224] The ingestion of an excessive number of tablets would change the stomach's pH, thereby preventing the release of the drug. However, individual human variability and the possibility to bypass the mechanism through consumption of e.g., acidic beverages pose significant challenges for these approaches.

DELIVERY SYSTEMS

A final ADF strategy regards drug delivery systems that are even more difficult to manipulate. This includes injectable depot formulations, implants and patches, container molecules (in particular liposomes) and parenteral infusion systems (i.e., designed for ambulant use).

In recent years, several injectable sustained-release formulations of buprenorphine have been approved for clinical use.[225,226] The first one was Sublocade (Indivior), which is a non-aqueous solution in a solvent that rapidly solidifies upon monthly subcutaneous administration. Buvidal/Brixadi (Camurus/Braeburn Pharmaceuticals) is administered every 1- or 4-weeks and involves a liquid depot that restructures when interstitial fluids cause solvent diffusion, forming a shell around the depot and allowing steady-state drug diffusion until system degradation. Additionally, a subdermal implant system providing continuous buprenorphine release over six months is available as Sixmo/Probuphine (Titan Pharmaceuticals). However, the continuous dose is also the drawback of implants, as additional oral analgesics might be required for breakthrough pain.

Transdermal delivery has been used in modern medicine for nearly half a century, after introduction of the anticholinergic scopolamine patch to treat motion sickness.[227] Current opioid patches apply either reservoir (rate-controlling membrane between the drug reservoir and the skin) or matrix (continuous release from adhesive polymer matrix) technologies. Two opioids are available as patch formulations: fentanyl (e.g., Duragesic by Janssen and generic version), and buprenorphine (e.g., BuTrans by Mundipharma and generic version), which are applied every couple of days. Additionally, a patient controlled iontophoretic transdermal system has been developed, which uses an electric current to release ionized fentanyl from the patch (IONSYS®).[228] However, several case reports discuss the abuse of transdermal opioid patches, such as by chewing on the patches, or heating the patches to speed-up delivery.

In a previous section, I discussed container molecules in the context of reversing respiration depression by capturing the drug in plasma (e.g., forming calabadion–fentanyl complexes). Conversely, this approach may also be used to encapsulate opioids in order to improve their therapeutic efficacy and safety profile by enhancing solubility, preventing degradation, controlling release, and engineering them to target delivery to specific tissues. This approach primarily focuses on lipid-based drug

delivery systems, as these facilitate passage through the BBB, allowing better access to the CNS, the target effect compartment. An example that has been used clinically was DepoDur, an extended-release formulation of morphine in multivesicular liposomes (Endo Pharmaceuticals),[229,230] but this has been discontinued for commercial reasons.

Lastly, opioids can also be administered directly into the cerebrospinal fluid via intrathecal drug infusion systems. Although technically not an ADF, this method does not allow abuse and is therefore included briefly in this section. Examples include the SynchroMed (Medtronic) which was the first of its kind (introduced in the 1980s), and the Prometra II (Flowonix) which has an innovative design that enhances precision and safety in drug delivery, along with extended refill intervals.[231] Intrathecal drug delivery pumps eliminate systemic opioid exposure and may have a reduced abuse potential, but the complexity compared to oral formulations restricts their use.

Pharmacokinetics

To conclude this chapter, we will review PK properties of opioids relevant to improving safety in clinical use. PK, often described as '*what the body does to the drug*,' examines how a drug moves through the body via absorption, distribution, metabolism (or biotransformation), and excretion (ADME). For opioids, as for many drugs, these processes are key to determining both therapeutic effects and risks, such as side effects or toxicity. Understanding the opioid's PK is essential for optimizing efficacy while minimizing harm. While the previously discussed drug formulation primarily influences absorption (incl. bioavailability), this section focuses on distribution, biotransformation, and excretion.

Opioids move from the systemic circulation (central compartment) to their effect site, with PK typically modeled using multi-compartment systems. Key parameters include the volume of distribution (V_d), which reflects drug disposition into tissues, and keo, the rate constant for drug transfer out of the effect compartment.[232,233] The half-life of k_{eo} ($t_{½}k_{eo}$) is clinically relevant, as it correlates with the onset and offset of effect. For example, remifentanil has a short $t_{½}k_{eo}$ and acts quickly, while morphine (long $t_{½}k_{eo}$) has a slower onset and offset, although receptor kinetics play an important role as well in the onset/offset of effect. It has been suggested that opioids with longer $t_{½}k_{eo}$ may cause less respiratory depression, as gradual onset will allow the rise and accumulation of tissue pCO_2 to counteract large respiratory changes.[234] However, opioids like morphine can still cause complete cessation of breathing at high doses, regardless of $t_{½}k_{eo}$.

Aforementioned parameters are influenced by various drug properties. Drug distribution depends on blood flow, with highly perfused organs like the brain, liver, and kidneys receiving more drug initially, while less perfused tissues (e.g., fat) act as reservoirs for lipophilic drugs like opioids during prolonged/high dose exposure.[235] Once in the systemic circulation, drugs bind to plasma proteins or lipids, reducing the active, free fraction, of the drug. Ionization, governed by a drug's pKa and local pH, also affects membrane diffusion, with only unbound and non-ionized molecules crossing barriers like the blood-brain-barrier. These properties are important to consider when determining the right dose; for example, hypoalbuminemia (e.g., in critically ill patients) could increase free drug concentrations, amplifying effects of normally highly bound opioids. Drug diffusion into tissues is further influenced by solubility differences between plasma and tissue. Drugs bound predominantly in the plasma compartment diffuse more slowly into tissues, limiting their equilibration. Lipophilicity, expressed as the octanol-water partition coefficient (logP), is particularly important for crossing lipid membranes like the BBB. Together with ionisation, the logP determines (for a great part) how effectively an opioid crosses the blood-brain-barrier. Most opioids are weak bases with a pKa near 8, meaning that they exist as a mix of ionized (charged) and non-ionized forms at physiological pH (~7.4).[236] Only the non-ionized fraction can passively diffuse across the blood-brain-barrier. Fentanyl is an example of a drug where the extremely high lipophilicity compensates for the fact that only ~9% is non-ionized at pH 7.4 due to its pKa of 8.4, enabling it to easily cross the blood-brain-barrier. Alfentanil, on the other hand, is far less lipophilic than fentanyl, but its higher non-ionized fraction at physiological pH and smaller V_d result in a greater free fraction available to cross the blood-brain-barrier and therefore to be active in the CNS, leading to a faster onset of effect but shorter duration of action.[237] Molecular size also impacts CNS penetration, with smaller drugs (< 400 Da) crossing the blood-brain-barrier more readily. While molecular size may be challenging to modify, it remains an important consideration in the design of safer opioids. Alvimopan, a MOP antagonist, illustrates this principle: its large molecular weight (460.6 Da) and low lipophilicity prevent blood-brain-barrier penetration, limiting its effects to peripheral tissues c.q., the gastrointestinal tract.[238] Lastly, the body's

active defences against CNS drug penetration, such as efflux transporters, also impact opioid PK. Transporters such as P-glycoprotein (P-gp) further restrict CNS penetration by actively removing their substrates, such as morphine, from the brain.[239,240] This reduces therapeutic effects and has been proposed to contribute to tolerance. Genetic polymorphisms in genes encoding efflux pumps may also affect an individual's response to a drug by influencing the PK profile. When designing novel opioids, interactions with for example P-gp should be considered to enhance therapeutic efficacy and minimize tolerance.

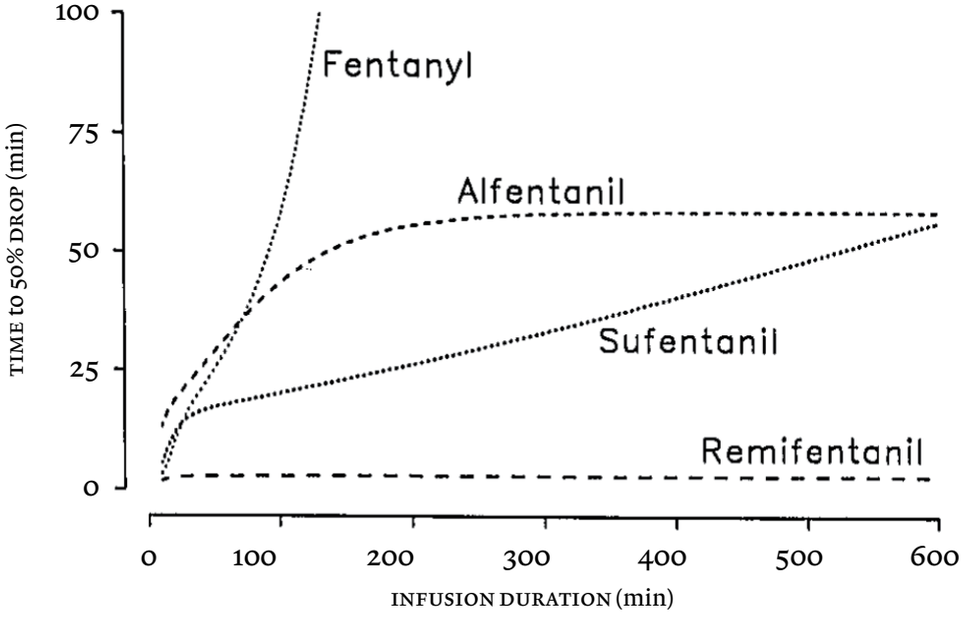
To bypass limitations imposed by the blood-brain-barrier, opioids can be administered closer to their target in the CNS. Fentanyl, for example, is sometimes added in very low dose to a spinal local anesthetic a to enhance intrathecal analgesia. However, not all formulations are suitable for intrathecal or epidural use due to excipients (e.g., remifentanil's neurotoxic glycine buffer). Moreover, these invasive techniques are impractical for most ambulant patients requiring analgesia. Alternatively, novel delivery systems like nanoparticles or liposomes, as discussed in the previous section, offer promising solutions for CNS drug delivery and may see wider application in the coming years.[229]

Redistribution of lipophilic opioids from peripheral tissues into to the central compartment have an important influence on plasma concentration and drug clearance. This phenomenon initially reduces drug availability at the effect site but eventually extends the duration of effect, particular when the opioid is administered for long time periods. Standard half-life metrics ($t_{½}$) are insufficient for predicting emergence time, so context-sensitive half-times and decrement times, as function of infusion duration, have been developed. Remifentanil has predictable emergence times as an opioid minimally affected by infusion duration due to both limited peripheral distribution and rapid intravascular esterase metabolism (**Figure 4**).[241] In contrast, due to peripheral accumulation, fentanyl has an increasing context-sensitive half time, with 50% reduction in plasma concentration lasting > 2 h after just a 2-h infusion.

Most opioids undergo hepatic metabolism via CYP enzymes or conjugation (except remifentanil). Some, like hydrocodone, become more active metabolites (i.e., hydromorphone), while prodrugs like codeine are converted to their active forms (i.e., morphine). Genetic polymorphisms and CYP interactions (e.g., CYP3A4 for buprenorphine) significantly influence PK. Chemical modifications can slow metabolism, as seen with deuterated buprenorphine in preclinical studies, whereas rapid clearance – as with remifentanil – is sometimes advantageous. Stereoselective metabolism may further affect opioid behavior; for instance, CYP2B6 preferentially metabolizes S-methadone (less potent than R-methadone).[242,243] Hepatic clearance depends on extraction ratios: high-extraction opioids like morphine are more blood flow-limited, whereas low-extraction drugs like alfentanil are enzyme-dependent. Excretion occurs via renal or biliary routes, sometimes leading to enterohepatic recirculation, as seen with morphine glucuronides, prolonging drug exposure. Renal elimination depends on plasma protein binding and ionization, with unbound drugs filtered by glomeruli and non-ionized forms subject to tubular reabsorption, influenced by urinary pH.

In conclusion, clinicians must consider individual patients' characteristics such as the required intensity and duration of pain-relief (e.g., quick and short versus slow and prolonged), habitus, genetic polymorphisms and liver/kidney function when selecting opioids and determining dosing regimens.

Figure 4 Simulation of the time necessary to achieve a 50% decrease in drug concentration in the blood (or plasma) after variable-length intravenous infusions.[241]



Thesis outline

In this introduction (Chapter 1), the pharmacological armamentarium that may include a way out of the contemporary opioid crisis is reviewed. The subsequent chapters present clinical studies evaluating different therapeutic approaches: in Chapters 2 and 3 the effects of the partial MOP agonist buprenorphine on respiratory function when co-administered with fentanyl are evaluated; Chapter 4 discusses the safety, tolerability, PK, and PD of STR-324, a DENKI, as assessed in a FIH trial; in Chapters 5 and 6 the effects of the biased MOP agonist Oliceridine on CNS functioning are evaluated and compared to morphine; Chapter 7 presents findings from the FIH trial involving ALKS 6610, another biased MOP agonist; the study discussed in Chapter 8 investigated the effects of the respiratory stimulant ENA-001 (which is being developed to reverse OIRD) on ventilation both when co-administered with propofol and without. Lastly, a general discussion and conclusions are presented in the final chapter (Chapter 9).

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CHAPTER 2

EFFECT OF SUSTAINED HIGH BUPRENORPHINE PLASMA CONCENTRATIONS ON FENTANYL-INDUCED RESPIRATORY DEPRESSION: A PLACEBO-CONTROLLED CROSSOVER STUDY IN HEALTHY VOLUNTEERS AND OPIOID-TOLERANT PATIENTS

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Abstract

BACKGROUND Opioid-induced respiratory depression driven by ligand binding to mu-opioid receptors is a leading cause of opioid-related fatalities. Buprenorphine, a partial agonist, binds with high affinity to mu-opioid receptors but displays partial respiratory depression effects. The authors examined whether sustained buprenorphine plasma concentrations similar to those achieved with some extended-release injections used to treat opioid use disorder could reduce the frequency and magnitude of fentanyl-induced respiratory depression.

METHODS In this two-period crossover, single-centre study, 14 healthy volunteers (single-blind, randomised) and eight opioid-tolerant patients taking daily opioid doses ⩾90 mg oral morphine equivalents (open-label) received continuous intravenous buprenorphine or placebo for 360 minutes, targeting buprenorphine plasma concentrations of 0.2 or 0.5 ng/mL in healthy volunteers and 1.0, 2.0 or 5.0 ng/mL in opioid-tolerant patients. Upon reaching target concentrations, participants received up to four escalating intravenous doses of fentanyl. The primary endpoint was change in isohypercapnic minute ventilation (V_E). Additionally, occurrence of apnoea was recorded.

RESULTS Fentanyl-induced changes in V_E were smaller at higher buprenorphine plasma concentrations. In healthy volunteers, at target buprenorphine concentration of 0.5 ng/mL, the first and second fentanyl boluses reduced V_E by [least squares mean (95% confidence interval (CI))] 26% (13–40%) and 47% (37–59%) compared to 51% (38–64%) and 79% (69–89%) during placebo infusion ($p = 0.001$ and $< .001$, respectively). Discontinuations for apnoea limited treatment comparisons beyond the second fentanyl injection. In opioid-tolerant patients, fentanyl reduced V_E up to 49% (21–76%) during buprenorphine infusion (all concentration groups combined) versus up to 100% (68–132%) during placebo infusion ($p = 0.006$). In opioid-tolerant patients, the risk of experiencing apnoea requiring verbal stimulation following fentanyl boluses was lower with buprenorphine than with placebo (odds ratio: 0.07; 95% CI: 0.0 to 0.3; $p = 0.001$).

INTERPRETATION Results from this proof-of-principle study provide the first clinical evidence that high sustained plasma concentrations of buprenorphine may protect against respiratory depression induced by potent opioids like fentanyl.

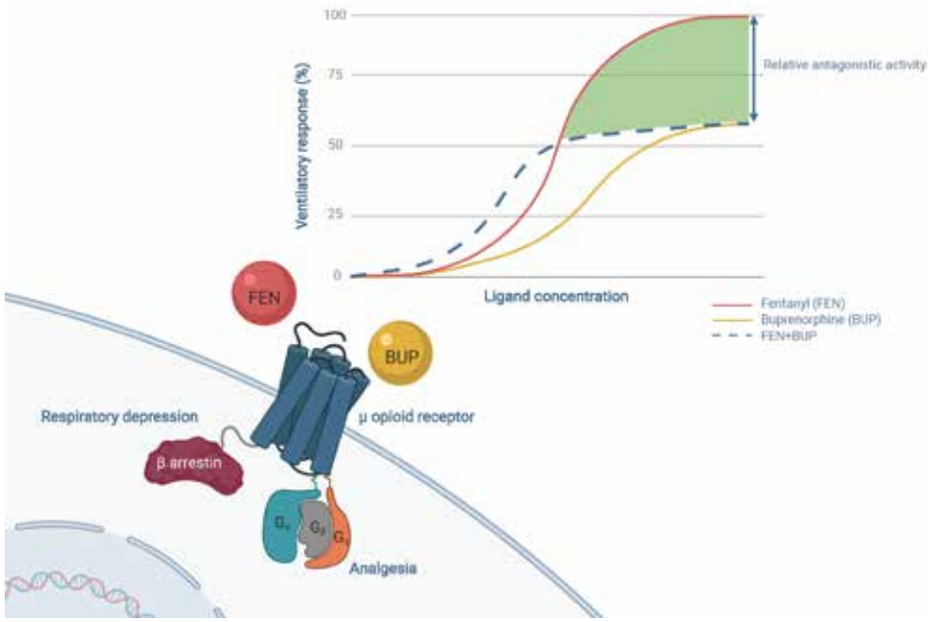
Introduction

Opioid use disorder (OUD) is a major source of morbidity and mortality.[1] The opioid epidemic has been fuelled in recent years by increasingly widespread prescription and illicit opioid consumption for many indications,[2–5] including the treatment of non-cancer pain.[6] Fatalities attributable to opioid misuse and overdose in the USA increased six-fold between 1999 and 2017 to an estimated 47,600.[7] The alarming increase in mortality has been observed in other countries and is largely driven by the increasing use of fentanyl and fentanyl analogues, often surreptitiously mixed with heroin.[8–10]

Potentially fatal respiratory depression is the main hazard associated with opioid use and abuse.[11] Opioid-induced respiratory depression (OIRD) is driven by ligand binding to mu-opioid receptors (MOP's) expressed on neurons in brainstem respiratory centres.[12] Binding to MOP's induces complex changes in respiratory regulation that result in increased arterial carbon dioxide concentrations and reduced tidal volume and minute ventilation.[13]Breathing slows and becomes irregular, potentially culminating in fatal apnoea, the major cause of death in opioid overdose.[14] As an additional complication, development of tolerance to opioid analgesic/euphoric effects often precedes the development of tolerance to OIRD, which may lead to dangerous self-regulated dose escalation.[15]

Buprenorphine has been proven as an effective medication for the treatment of OUD.[16] Buprenorphine is a semi-synthetic MOP partial agonist that binds to MOP's with high affinity and slowly dissociates from the receptors, enabling it to displace MOP full agonists such as fentanyl and mitigate their physiological effects.[17,18] Buprenorphine itself is associated with OIRD, but a study in healthy volunteers at intravenous bolus doses ranging from 0.05 to 0.60 mg/70 kg demonstrated an apparent maximum, or ceiling, effect on respiratory depression.[19,20] Based on a pharmacokinetic-pharmacodynamic model of OIRD reversal, the authors previously proposed that at maximum buprenorphine MOP occupancy, the effect of fentanyl on respiration would be limited, even at high fentanyl doses (Figure 1).[12,21] The present study aimed to provide proof of principle for this hypothesis. The results of this study confirm that high sustained buprenorphine plasma concentrations can reduce the respiratory depression caused by injection of a potent, short-acting MOP full agonist such as fentanyl.

FIGURE 1 Schematic representation of competitive binding at the MOP by fentanyl and buprenorphine, resulting in a ventilatory response.



Methods

Trial design

This was a two-part, placebo-controlled crossover study. Both Parts A and B included two study periods, during which participants received continuous intravenous infusion of buprenorphine or placebo co-administered with up to four escalating fentanyl doses. For healthy volunteers in Part A, treatment sequence was randomly assigned so participants received placebo or buprenorphine infusion during Period 1 and the alternate infusion during Period 2. Because tolerance to opioid effects is poorly characterised in patients receiving long-term opioids, opioid-tolerant patients in Part B had a fixed treatment sequence, receiving placebo infusion plus fentanyl challenges in Period 1 to optimize the fentanyl dose escalation before buprenorphine and fentanyl were co-administered in Period 2.

There were no major changes to trial design after commencement of each study part, other than an amendment of the eligibility criteria for Part B to enable recruitment of a broader group of patients. Changes regarded concurrent use of central nervous system depressants (e.g. benzodiazepines), inclusion of smokers (measurements not affected), and exclusion of patients with clinically significant risks of Torsades de Pointes instead of a history of risk factors. There were no changes to trial endpoints after the trial commenced.

Participants

The study enrolled healthy volunteers (Part A) and opioid-tolerant patients (Part B). All participants provided written informed consent prior to any study-related procedure and screening was completed within 30 days of the first study drug administration. In Part A, male and female healthy volunteers, aged 18 to 45 years with a body mass index of 18 to 30 kg/m2, were eligible. Exclusion criteria included history of any clinically relevant medical, psychiatric, or neurologic condition; positive pregnancy test; history of current substance use disorder according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th edition;[22] smoking or having smoked in the last 6 months; alcohol consumption >20 units/week (men) or >13 units/week (women); use of any medication within 14 days or 5 half-lives before dosing; opioid use (including opioid antagonists) within 30 days before dosing; use of medication that induces/inhibits relevant cytochrome P450 enzymes; history of suicidal ideation within 30 days or suicide attempt within 6 months prior to informed consent; or any other condition that, in the opinion of the investigators, could interfere with the ability to participate in the study.

For Part B, male and female opioid-tolerant patients, aged 18 to 55 years, with a body mass index of 18 to 32 kg/m^2 using daily doses of opioids ⩾ 90 mg oral morphine equivalents,[23] and who were in stable condition based on their medical evaluation were eligible. All exclusion criteria were similar to Part A, except for modified alcohol consumption limits to >27 units/week (men) or >20 units/week (women); broadened nicotine permissions to no smoking on dosing days; and no use of buprenorphine within 10 days of the first study drug administration. Opioid-tolerant patients were recruited through national advertisements, out-patient clinics with expertise in the treatment of pain, and in collaboration with specialised opioid-abuse treatment clinics.
All eligibility criteria are provided in the study protocol, which is available as a supplementary file.

SETTING AND LOCATION OF DATA COLLECTION

This study was conducted in Leiden, The Netherlands. Dosing day procedures were performed at the department of anaesthesiology of the Leiden University Medical Centre (LUMC) and all other activities regarding trial execution were performed at the Centre for Human Drug Research (CHDR). The study was conducted in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH GCP), and ethical principles as referenced in EU Directive 2001/20/EC. The protocol (EudraCT 2017-004858-42) was approved by the Medical Review and Ethics Committee of the BEBO foundation (Assen, The Netherlands).

Interventions

Healthy volunteers in Part A were admitted the day prior to the experiment for each study period, with a washout of two weeks between periods. Opioid-tolerant patients in Part B were admitted to the clinic 2–5 days before the first study period and remained in the clinic until completion of both study periods. To ensure washout of each patient's usual opioids, tailored substitution schedules with oxycodone began a minimum of 48 hours before Period 1, and the last dose of oxycodone was administered at least 15 hours before study drug administration. Due to the short half-life of fentanyl, Period 2 was separated from Period 1 by 40 hours. During this washout period, patients again received oxycodone for opioid substitution.

On the morning of each study period, an intravenous line was placed for administration of study medication and an arterial line was placed for blood sampling in the opposite arm. Isohypercapnic ventilation was measured during buprenorphine/placebo infusion for approximately 6 hours using the dynamic end-tidal forcing technique, as described elsewhere,[20,21] allowing the investigator to direct ventilation towards pre-defined end-tidal pCO_2 (7 kPa) and pO_2 (14.5 kPa) values. A combination of oxygen, carbon dioxide, and nitrogen was delivered to the participants through a face mask and inspired minute ventilation was measured by pneumotachography. A finger probe with pulse oximeter was used for continuous surveillance of arterial oxygen saturation (SpO_2). These ventilation parameters were captured as one-minute breath-to-breath averages.
Intravenous infusion with buprenorphine (Indivior UK Ltd., UK) or placebo started once baseline minute ventilation (V_E) had stabilised at 20 ± 2 L/min (about 4-fold above normal resting V_E). In healthy volunteers, an infusion rate of 0.02 or 0.05 mg/70 kg/h buprenorphine was selected to target plasma concentrations of 0.2 or 0.5 ng/mL, respectively. In opioid-tolerant patients, higher buprenorphine infusion rates were administered: 0.1, 0.2 or 0.5 mg/70 kg/h targeting plasma concentrations of 1.0, 2.0 or 5.0 ng/mL, respectively. In both healthy volunteers and opioid-tolerant patients, a 10-fold higher infusion rate was used over the first 15 minutes to speed attainment of steady-state buprenorphine concentrations at the site of action. In order to manage possible gastrointestinal side effects, all participants received 4 mg of ondansetron prior to infusion.

At 120, 180, 240, and 300 minutes after the start of the buprenorphine or placebo infusion, escalating intravenous fentanyl doses (Hameln Pharmaceuticals Ltd., UK) were administered over 90 seconds. The planned fentanyl doses in healthy volunteers were 0.075, 0.15, 0.25 and 0.35 mg/70 kg. In opioid-tolerant patients, the planned fentanyl doses were 0.25, 0.35, 0.50 and 0.70 mg/70 kg.

Arterial blood samples for analysis of buprenorphine and fentanyl plasma concentrations were collected at multiple timepoints over 540 minutes after the start of buprenorphine or placebo infusion. Buprenorphine and fentanyl plasma concentrations were assessed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods validated over a range of 0.02 to 10.0 ng/mL for buprenorphine and 0.1 to 50.0 ng/mL for fentanyl.

Pharmacodynamic and pharmacokinetic outcomes

The primary study endpoint was maximum decrease in minute ventilation, defined as the minimum value of isohypercapnic V_E observed during each fentanyl dosing period compared to pre-fentanyl baseline. The pre-fentanyl baseline value was defined as the average of the last 5 minutes prior to the first fentanyl dose. Secondary endpoints included the number and percentage of participants who experienced apnoea (defined as ⩾20 s loss of respiratory activity) and required verbal stimulation to breath after a fentanyl dose. Any subject who desaturated below 92% without spontaneous recovery within seconds after, was verbally stimulated to breathe, regardless of intervention.

Buprenorphine average plasma concentration (C_{avg}) at steady-state was calculated as the area under the plasma concentration-time curve between 120 and 360 minutes after the start of buprenorphine infusion divided by the time interval. Treatment-emergent adverse events

(TEAE's) were recorded from time of first screening visit through the end of the last visit. Fentanyl dose escalation was halted if a participant did not breathe for a prolonged period or SpO_2 dropped below 85% despite active verbal stimulation by the investigator, or if the investigators deemed necessary (i.e., other TEAE's). Drug plasma concentrations and safety measures (SpO_2, TEAE's) were exploratory endpoints.

Sample size

In the absence of informed priors for the interaction between fentanyl and buprenorphine, no a priori sample size calculation was performed, and statistical testing was descriptive. A post-hoc power analysis, calculated by paired sample t-test for the primary endpoint, showed that a sample size of 8 yields >96% power, when the treatment difference is 50.8%, standard deviation (SD) is 32.7% and alpha is set to 0.05 two-sided.

Randomisation

SEQUENCE GENERATION

Healthy volunteers in Part A were randomly assigned to one of two treatment sequences (buprenorphine-placebo or placebo-buprenorphine). A blocked randomisation schedule was generated by an independent statistician using SAS version 9.4. A block size of 2 was chosen to ensure the best possible balancing if the study would be prematurely halted.

Part B was an open-label, single-sequence crossover study where participants received placebo treatment and then buprenorphine treatment.

ALLOCATION CONCEALMENT AND IMPLEMENTATION

For Part A, participants were single-blinded. The independent statistician who generated the random allocation sequence was not involved in recruiting nor randomising participants. To prevent selection bias, CHDR staff not involved in generating the random allocation sequence assigned the randomisation numbers to participants sequentially, in the order of completed medical screenings. An independent LUMC study pharmacist prepared masked infusion syringes for administration by LUMC staff.

Treatment sequence for Part B was not randomised. Dose group allocation in Part B was performed by the investigators within dose ranges specified per protocol.

Statistical methods

To reduce the impact of technical artifacts that could introduce measurement noise on V_E measures, analysis of V_E endpoints was conducted after post-hoc adjustment of data sets. The adjusted data sets reflect imputations based on clinical notes to account for the impact of concurrent clinical events such as facemask removal, urinating with facemask on and severe itching. Stimulated, nonspontaneous breathing data were set at zero (apnoea) for analyses on ventilation data.

Maximum percent decreases in V_E relative to baseline were compared between treatment groups using a mixed effects model with treatment as a fixed effect. For Part B, all buprenorphine concentration groups were combined to perform the treatment comparison. Maximum percent decreases in V_E were assessed within the first 10 minutes after each fentanyl bolus to accurately characterise the peak pharmacodynamic effect of fentanyl by minimizing the impact of random variation evident over the full 60-minute intervals. Secondary endpoints were compared between treatment groups by exact conditional logistic regression and Fisher's exact test. The exploratory safety endpoint (SpO_2) was analysed in a similar manner to changes in V_E.

The primary and secondary endpoint analyses were performed on participants who received at least 1 dose of fentanyl and had at least 1 post-dose assessment, excluding one participant who received the wrong buprenorphine infusion rate. TEAE's were summarised for participants who received at least one dose of study medication. The buprenorphine and fentanyl plasma concentrations were summarised for all participants who received at least 1 dose of the medication and had an adequate number of pharmacokinetic samples collected.

Statistical analyses were performed using SAS version 9.4.

Results

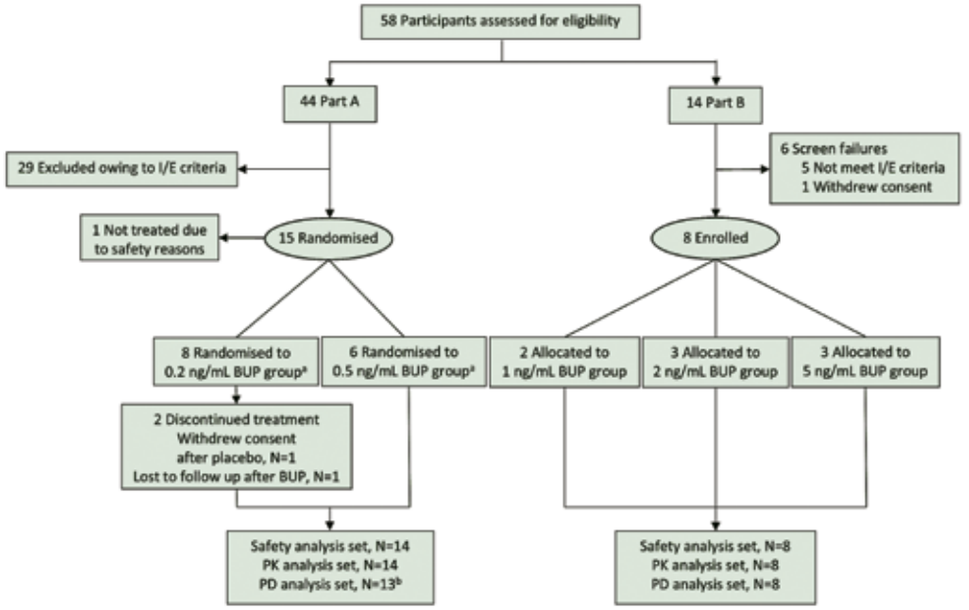
In total, 58 participants were screened for the study, which commenced on 22 March 2018 and completed on 04 January 2019, enrolling a total of 22 participants. Fourteen healthy volunteers and eight opioid-tolerant patients who used high-dose opioids for at least three months (range 0.25–29 years; see **Table 1** for baseline characteristics) were included in the study. The CONSORT diagram summarizes participant disposition (**Figure 2**).

TABLE 1 Participant demographic and clinical characteristics.

	Part A: healthy volunteers		Part B: opioid-tolerant patients			
Buprenorphine concentration	**0.2 ng/mL**	**0.5 ng/mL**	**1 ng/mL**	**2 ng/mL**	**5 ng/mL**	**Grouped**
	(*n* = 8)	**(*n* = 6)**	**(*n* = 2)**	**(*n* = 3)**	**(*n* = 3)**	**(*n* = 8)**
SEX, *N* (%)						
Male	4 (50)	3 (50)	1 (50)	1 (33)	1 (33)	3 (38)
Female	4 (50)	3 (50)	1 (50)	2 (67)	2 (67)	5 (63)
AGE mean (SD) or range, *y*	23.8 ± 4.6	24.5 ± 2.4	44-46	31-43	34-52	42 ± 8
ETHNICITY, *N* (%)						
White	8 (100)	5 (83)	2 (100)	3 (100)	3 (100)	8 (100)
Native Hawaiian		1 (17)				
WEIGHT mean (SD) or range, kg	74.2 ± 6.9	67.9 ± 6.6	70-93	70-87	65-89	78 ± 10
BMI, mean (SD) or range, kg/m^2	23.5 ± 2.2	22.4 ± 1.6	23.6-29.6	22.0-30.8	21.0-31.5	25.9 ± 4.2
Daily MME, mean (SD) or range, mg	NA	NA	90-150	90-480	90-270	203 ± 135
Drug Usage per Participant[a]	NA	NA	- Oxycodone 60 mg/d	- Fentanyl patch 75 mcg/h; oxycodone 90 mg/d; tapentadol 50 mg/d	- Heroin 250 mg/d (smoke); cocaine; marijuana	
			- Fentanyl patch 25 mcg/h; oxycodone 60 mg/d; marijuana	- Buprenorphine 16 mg/d; cocaine; marijuana	- Fentanyl patch 50 mcg/h	
				- Oxycodone 60 mg/d; marijuana	- Fentanyl patch 75 mcg/h; oxycodone 60 mg/d; marijuana	

Abbreviations: BMI = *body mass index;* MME = *Morphine Milligram Equivalents;* NA = *not applicable;* SD = *standard deviation. a. Tailored substitution schedules with oxycodone began a minimum of 48 hours before the first experiment to ensure washout of each patient's usual opioids at baseline.*

FIGURE 2 CONSORT flow diagram.



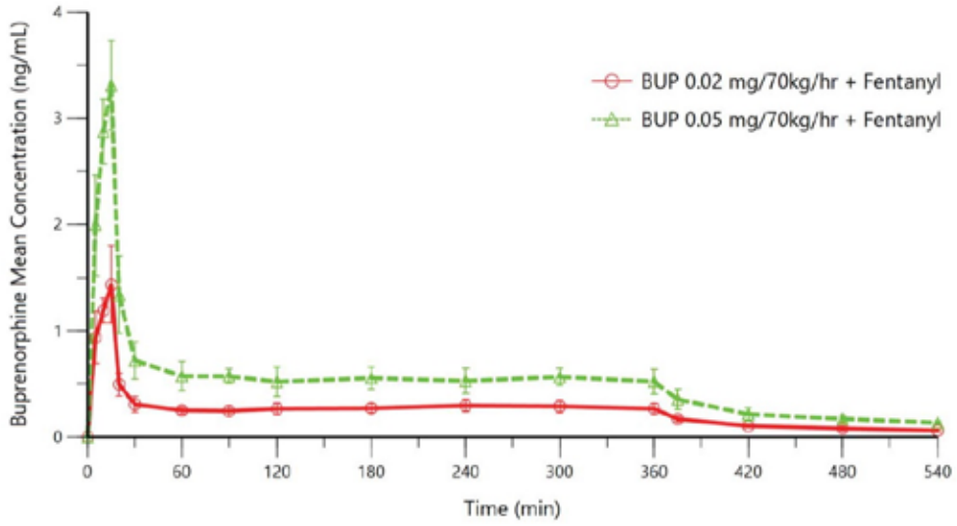
Abbreviations: BUP, *buprenorphine;* I/E = *inclusion/exclusion;* PD, *pharmacodynamic;* PK, *pharmacokinetic. a. Randomised sequences for Part A were Placebo:*BUP *n = 5,* BUP*:Placebo n = 3 for the 0.2 ng/mL group and Placebo:*BUP *n = 2,* BUP*:Placebo n = 4 for the 0.5 ng/mL group. b. One volunteer in the lower dose group received the incorrect buprenorphine dose and was excluded from the* PD *analyses. Data were available for six healthy volunteers in each treatment (placebo and buprenorphine) for the* PD *analyses in the lower dose group due to two volunteers in the lower dose group who completed only one study period.*

In healthy volunteers, steady-state buprenorphine plasma concentrations (mean ± SD) were 0.28 ± 0.05 and 0.54 ± 0.08 ng/mL, respectively (Figure 3), consistent with the target concentrations of 0.2 and 0.5 ng/mL. In opioid-tolerant patients, steady-state buprenorphine plasma concentrations were 1.08 ± 0.33, 2.28 ± 0.40, and 6.12 ± 1.26 ng/mL, respectively (Figure 3), all consistent with the targeted concentrations. Mean fentanyl plasma concentrations are shown for both participant populations in Figure 4.

Table 2 lists fentanyl doses administered to healthy volunteers and opioid-tolerant patients and results for the number of participants who experienced persistent apnoea that required verbal stimulation.

FIGURE 3 Mean buprenorphine plasma concentration-time curves.

Upper panel: Part A, healthy volunteers; Lower panel: Part B, opioid-tolerant patients. In both healthy volunteers and opioid-tolerant patients, a 10-fold higher infusion rate was used over the first 15 minutes to speed attainment of steady-state buprenorphine concentrations at the site of action. Infusions were stopped at 360 min. Steady-state buprenorphine infusion rates are labelled in the graphs.



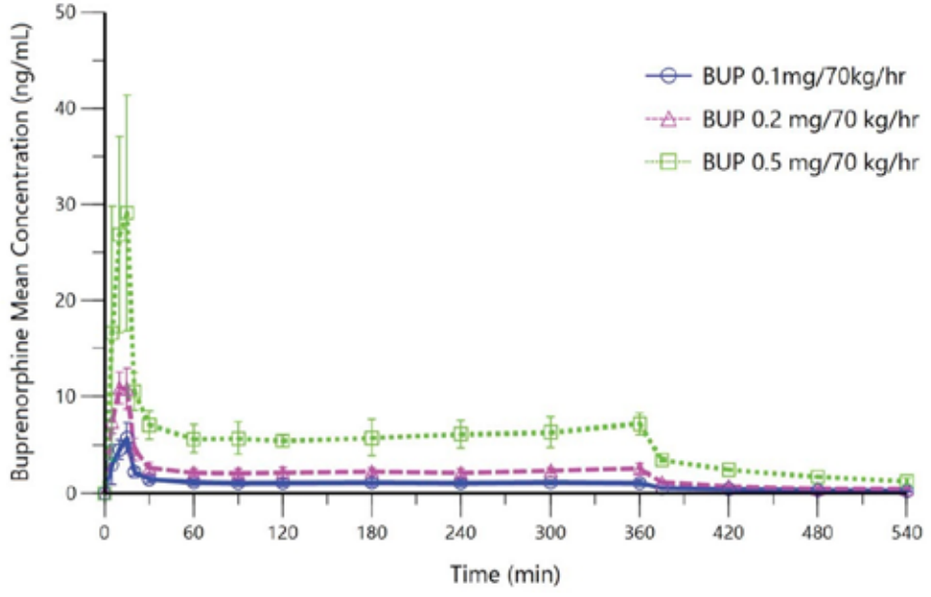
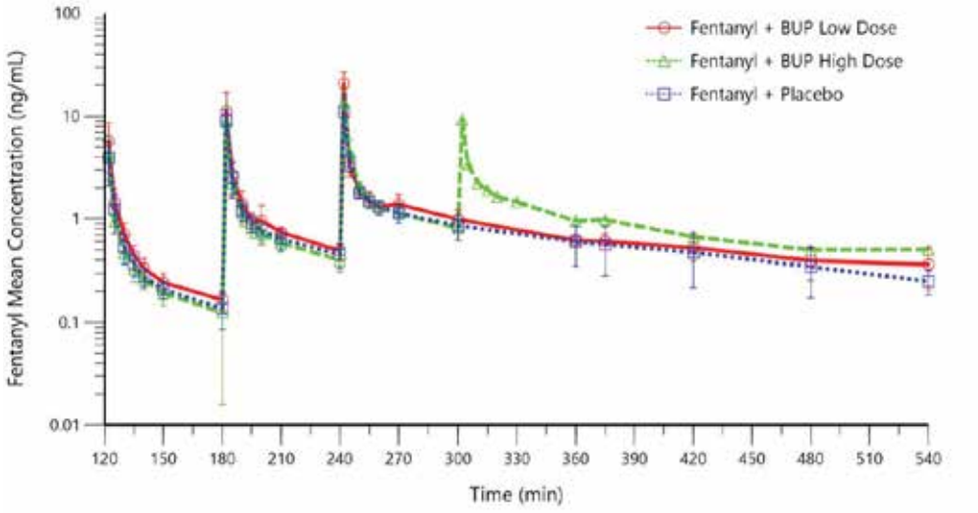


FIGURE 4 Mean fentanyl plasma concentration-time curves.

Upper panel: Part A, healthy volunteers; Lower panel: Part B, opioid-tolerant patients. At 120, 180, 240, and 300 minutes after the start of the buprenorphine or placebo infusion, escalating intravenous fentanyl doses were administered over 90 seconds. Planned fentanyl bolus doses are labelled in the graphs. Higher doses were not administered to participants if they did not tolerate lower fentanyl doses.



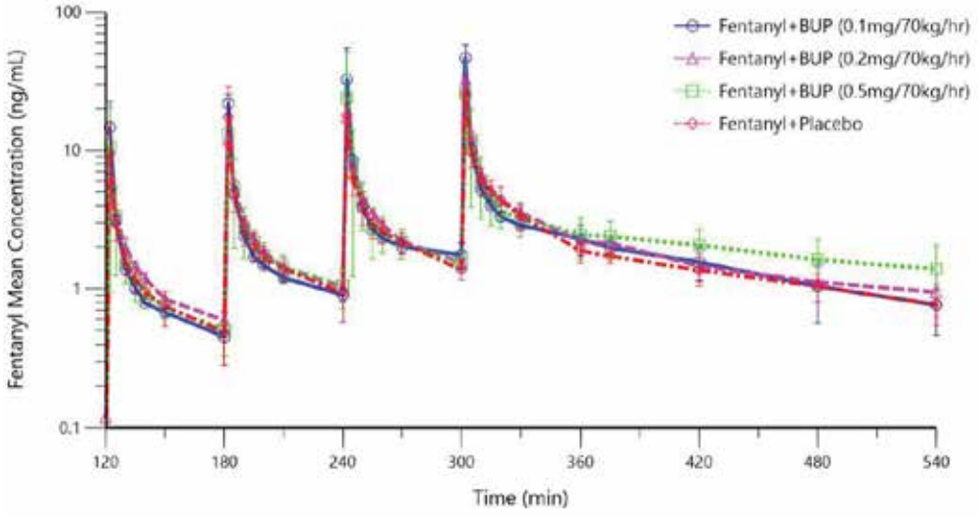


TABLE 2 Number and percentage of participants who experienced apnoea that required stimulation (i.e., persistent apnoea).

	Part A: Healthy Volunteers					Part B: Opioid-tolerant Patients		
Fentanyl Dose	Fentanyl Dose Number	Placebo for 0.2 ng/mL (*n* = 6)	Buprenorphine 0.2 ng/mL (*n* = 6)	Placebo for 0.5 ng/mL (*n* = 6)	Buprenorphine 0.5 ng/mL (*n* = 6)	Fentanyl Dose Number	Placebo (*n* = 8)	Buprenorphine[a] (*n* = 8)
0.075 mg/70 kg	1	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)			
0.15 mg/70 kg	2	1/6 (17)	0/4 (0)[b]	0/6 (0)	0/6 (0)			
0.25 mg/70 kg	3	2/2 (100)[b]	2/4 (50)	3/4 (75)[b]	1/6 (17)	1	0/8 (0)	0/8 (0)
0.35 mg/70 kg	4	0/0	0/0[b]	0/0[b]	0/1 (0)[b]	2	2/8 (25)	0/8 (0)
0.50 mg/70 kg						3	1/6 (17)	0/8 (0)
0.70 mg/70 kg						4	3/4 (75)[b]	0/8 (0)

a. The three buprenorphine dose groups in opioid-tolerant patients (target plasma concentrations of 1, 2 and 5 ng/mL) were grouped for this analysis. b. Some participants did not receive some fentanyl doses due to adverse events, apnea events that did not require stimulation or abnormalities in other ventilatory parameters (i.e., unstable breathing, drop in ventilation or saturation and high end-tidal pCO_2).

During the placebo study periods, five of the six healthy volunteers (83%) who progressed to the third fentanyl dose had persistent apnoea versus only three out of ten (30%) during the buprenorphine study period. Four opioid-tolerant patients progressed to the fourth fentanyl bolus during the placebo period, three of which (75%) experienced persistent apnoea. In contrast, all eight opioid-tolerant patients progressed to the fourth bolus during the buprenorphine study period, and none of them (0%) experienced persistent apnoea. In opioid-tolerant patients, the risk of experiencing apnoea requiring verbal stimulation following fentanyl boluses was significantly lower when receiving buprenorphine than when receiving placebo, with an odds ratio of 0.07 (95% CI, 0.0 to 0.3; $p = 0.001$).

In opioid-tolerant patients, fentanyl reduced V_E up to 49% (21–76%) during buprenorphine infusion (all concentration groups combined) versus up to 100% (68–132%) during placebo infusion ($p = 0.006$). Example tracings for representative opioid-tolerant patients in the 1, 2 and 5 ng/mL concentration groups, show V_E during the placebo and buprenorphine infusion study periods (Figure 5) and graphs depicting individual V_E per concentration level are provided as supplemental material. The tracings indicate that buprenorphine itself decreased V_E compared to placebo; in healthy volunteers, the decrease in ventilation caused by buprenorphine was more pronounced. After fentanyl injections, significant treatment differences for healthy volunteers in the 0.5 ng/mL buprenorphine versus placebo groups were observed, with lower decreases in V_E [least squares mean difference (95% CI), *p*-value] following the first [25.1% (13.4–36.8%), 0.001] and second [31.6%, (19.3–43.8%), < .001] fentanyl bolus compared to pre-fentanyl baseline (Table 3). For the combined group of opioid-tolerant patients, significantly smaller reductions in V_E after fentanyl bolus 1 [29.9% (19.6–40.3%), < .001], 2 [42.8%, (23.8–61.8%), 0.001], 3 [39.4%, (15.7–63.1%), 0.008], and 4 [50.8%, (27.7–73.9%), 0.006] were measured when patients received buprenorphine infusion compared to placebo (Table 3). When the three buprenorphine concentration groups were compared in opioid-tolerant patients, fentanyl effects on V_E appeared greater for the 1 ng/mL group than for the 2 and 5 ng/mL groups.

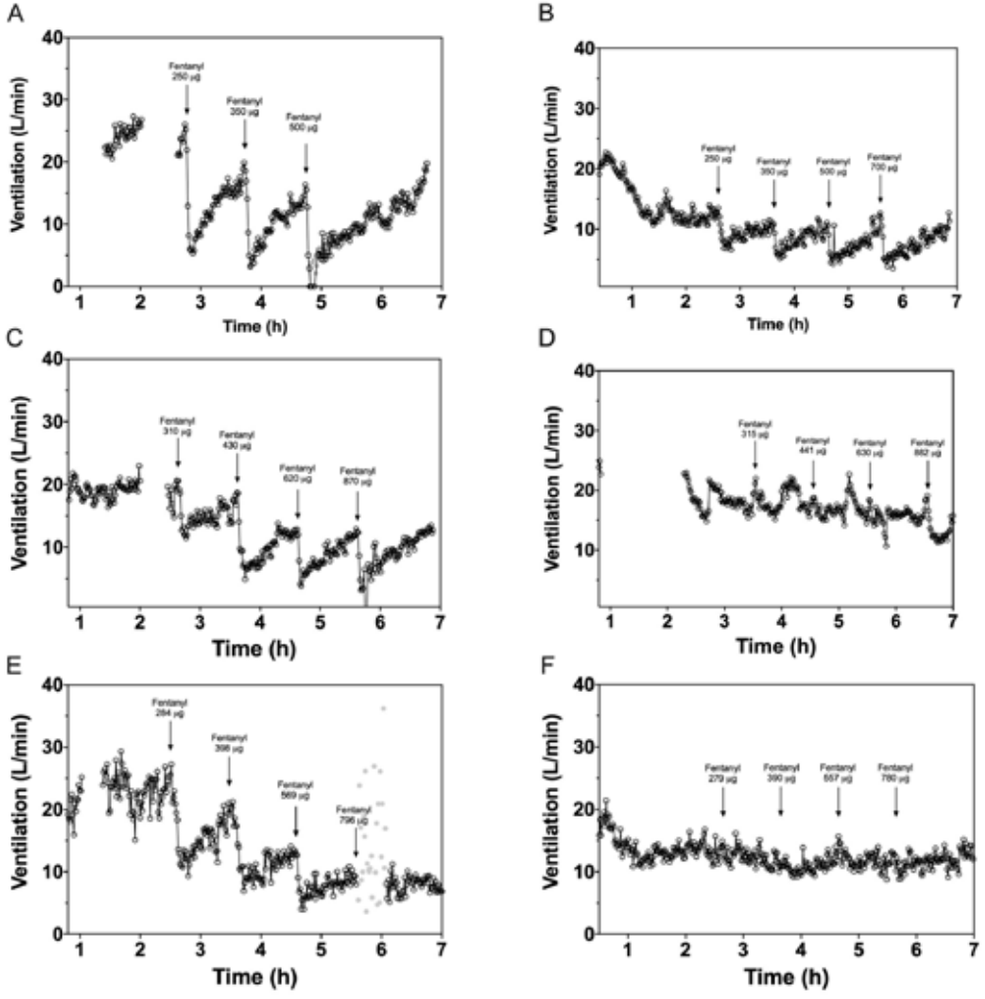
TABLE 3 Maximum decreases in minute ventilation after fentanyl bolus administration (%).

		Healthy volunteers						Opioid-tolerant patients						
		0.2 ng/mL group			0.5 ng/mL group			1 ng/mL group		2 ng/mL group		5 ng/mL group		
		Pl.	BUP	T.D.	Pl.	BUP	T.D.	Pl.	BUP	Pl.	BUP	Pl.	BUP	T.D.[b]
Fentanyl Dose 1[a]	*n*	6	6		6	6		2	2	3	3	3	3	
	LSM (95% CI)	-60.9 (-73.5, -48.2)	-53.1 (-65.8, -40.5)	7.7 (-3.8, 19.3)	-51.3 (-64.3, -38.3)	-26.2 (-40.0, -12.5)	25.1 (13.4, 36.8)	-82.3 (-105.2, -59.3)	-49.2 (-72.1, -26.3)	-44.3 (-63.0, -25.6)	-22.2 (-40.9, -3.5)	-62.5 (-81.2, -43.8)	-26.8 (-45.5, -8.1)	29.9 (19.6, 40.3)
	p-value			0.1590			0.001							<.001
Fentanyl Dose 2[a]	*n*	6	4		6	6		2	2	3	3	3	3	
	LSM (95% CI)	-82.4 (-92.7, -72.0)	-70.3 (-82.8, -57.8)	12.1 (-2.5, 26.7)	-79.0 (-89.4, -68.6)	-47.4 (-57.8, -37.0)	31.6 (19.3, 43.8)	-93.5 (-122.3, -64.6)	-57.3 (-86.1, -28.4)	-68.4 (-91.9, -44.8)	-35.9 (-59.5, -12.4)	-87.5 (-111.0, -63.9)	-30.0 (-53.5, -6.5)	42.8 (23.8, 61.8)
	p-value			0.0916			<.001							0.001
Fentanyl Dose 3[a]	*n*	2	4		4	6		1	2	3	3	2	3	
	LSM (95% CI)	-100.0 (-142.3, -57.7)	-83.2 (-113.2, -53.3)	16.8 (-35.1, 68.7)	-93.6 (-123.6, -63.7)	-71.9 (-96.4, -47.5)	21.7 (-16.9, 60.4)	-100.0 (-145.3, -54.7)	-71.8 (-103.8, -39.8)	-79.3 (-105.5, -53.2)	-46.1 (72.3, -20.0)	-88.1 (-120.1, -56.1)	-30.7 (-56.9, -4.6)	39.4 (15.7, 63.1)
	p-value			0.3788			0.1716							0.008
Fentanyl Dose 4[a]	*n*	0	0		0	1		0	2	3	3	1	3	
	LSM (95% CI)	NA	NA	NA	NA	NA	NA	NA	-68.7 (-140.2, 2.8)	-82.3 (-140.7, -23.9)	-33.7 (-92.1, 24.7)	-116.3 (-189.3, -42.2)	-50.5 (-108.9, 7.8)	50.8 (27.7, 73.9)
	p-value			NA			NA							0.006

Abbreviations: BUP = buprenorphine; CI = confidence interval; LSM = least square mean; NA = not applicable; T.D. = Treatment Difference; Pl. = Placebo.
p<0.05 are presented in bold. Differences are LSM estimated treatment differences between buprenorphine and placebo. a. Maximum changes (%) in minute ventilation during first 10 minutes after each fentanyl administration compared to pre-fentanyl baseline. b. The three buprenorphine concentration level groups in patients were grouped for this analysis.

FIGURE 5 Example graphs showing the effect of fentanyl on minute ventilation in three opioid-tolerant patients during placebo infusion and buprenorphine infusion.

(1) Placebo infusion (A, C and E) and buprenorphine infusion (B, D, F) at target plasma concentrations of 1 ng/mL (top row), 2 ng/mL (middle row) and 5 ng/mL (lower row) (2) Open spaces in the beginning of graphs A, C, D and E relate to concurrent clinical events such as temporary removal of the facemask. (3) Grey dots are stimulated breaths in case of an apnea episode. (4) The time on the x-axis in the graphs is related to the start time of the ventilation experiment, not the timing of the buprenorphine/placebo infusion and fentanyl injections.



All participants in each treatment period reported at least one TEAE and at least one TEAE-related to buprenorphine/placebo treatment. Overall, most events were mild or moderate in severity. In healthy volunteers, the most frequent TEAE's were nausea, apnoea, and somnolence in both periods. The most frequent TEAE's in opioid-tolerant patients were apnoea, dizziness, and somnolence. In the placebo period, 88% of opioid-tolerant patients experienced apnoea compared to 13% during the buprenorphine period. Apnoeas reported as TEAE's did not necessarily require verbal stimulation. All the TEAE's were expected for administration of opioid agonists, including a high incidence of nausea among healthy volunteers who were opioid-naive.

In opioid-tolerant patients, SpO_2 levels were significantly decreased after placebo treatment relative to buprenorphine after the first, third and fourth fentanyl boluses (Table 4). No other consistent differences in safety parameters were observed between treatment groups.

TABLE 4 Maximum change from pre-fentanyl baseline in oxygen saturation (%) in opioid-tolerant patients.

	Placebo	Buprenorphine	Treatment Difference (Buprenorphine-Placebo)
Fentanyl Dose 1			
n	8	8	
LSM	-2.9	-1.5	1.4
95% CI	-3.8, -1.9	-2.4, -0.6	0.1, 2.7
p-value			**0.041**
Fentanyl Dose 2			
n	8	8	
LSM	-5.2	-3.5	1.8
95% CI	-8.2, -2.3	-6.4, -0.5	-2.4, 5.9
p-value			0.353
Fentanyl Dose 3			
n	6	8	
LSM	-8.1	-2.7	5.4
95% CI	-12.1, -4.2	-6.2, -0.7	0.8, 10.0
p-value			**0.030**
Fentanyl Dose 4			
n	4	8	
LSM	-11.2	-2.6	8.6
95% CI	-14.7, -7.6	-5.0, -0.1	4.3, 12.9
p-value			**0.008**

Abbreviations: CI = confidence interval; LSM = least square mean. p<0.05 are presented in bold.

Discussion

The present study is, to the best of our knowledge, the first to provide clinical evidence for the protective effects of buprenorphine in limiting fentanyl-induced respiratory depression. Previous studies in animal models and in healthy volunteers have shown that respiratory depression induced by buprenorphine is characterised by a ceiling effect at higher concentrations.[19–21] It was demonstrated that, unlike some other opioids, respiratory depression associated with buprenorphine is relatively resistant to naloxone reversal, likely because of high receptor affinity and slow dissociation from the receptor.[17,21] The authors hypothesised that because of its special properties, high concentrations of buprenorphine that sustain maximum MOP occupancy could limit the extent of OIRD induced by fentanyl, a potent MOP full agonist.

The results demonstrate that in patients with higher tolerance to the effects of opioids, sustained high plasma concentrations of buprenorphine significantly reduced the magnitude of fentanyl-induced respiratory depression relative to placebo. This effect was observed with escalating fentanyl doses up to 0.70 mg/70 kg (total administered dose 1.8 mg/70kg over 180 minutes). Each fentanyl bolus was infused over 90 seconds, resulting in an immediate ventilatory response. This pharmacodynamic effect was well defined within the first 10 minutes of each fentanyl bolus; the ventilatory response slowly decreased thereafter (i.e., breathing recovered) and became more susceptible to random variation the longer after a bolus was administered. Apnoeic periods directly following a drug injection can be fatal in real-life situations. Therefore, it was regarded justified to only include the ventilatory response during the first 10 minutes after each bolus in the analysis. Buprenorphine administration was itself associated with a decrease in V_E, but at the highest dose there was little or no additional decrease after subsequent fentanyl administration. The numbers in each buprenorphine dose group of opioid-tolerant patients were small, but there was a trend consistent with a buprenorphine concentration-response with highest levels of buprenorphine achieving greater suppression of V_E as evidenced by the tracings in **Figure 5** (suggesting greater MOP occupancy). The impact of the fourth fentanyl bolus on V_E appears to be greater in the highest buprenorphine dose group than in the middle dose group of opioid-tolerant patients. This is due to a few isolated low values directly following fentanyl administration in two thirds of patients in the high-dose group. These data points were not excluded from the analysis but might be considered outliers. Because dose level groups were small, the statistical analysis was performed by grouping data across the buprenorphine dose levels.

Apnoea events were less frequent and less severe following fentanyl administration during buprenorphine infusion than during placebo infusion. Opioid-tolerant patients treated with the highest dose of buprenorphine had no meaningful apnoea events or changes in SpO_2 after fentanyl boluses. At lower buprenorphine doses, fentanyl had an appreciable effect on ventilation. These results are consistent with the expected greater MOP occupancy at higher buprenorphine plasma levels.[24] Inhibition of fentanyl-induced respiratory depression by buprenorphine in healthy volunteers was observed only to a limited extent in this study. Although fentanyl did cause respiratory depression during buprenorphine infusion in healthy volunteers, especially at high-dose buprenorphine infusion, the decrease in V_E was significantly lower compared to the fentanyl effect during placebo infusion. Comparisons were difficult for the third and fourth fentanyl boluses, as only six of 12 healthy volunteers progressed to the third bolus during the placebo period compared to ten during the buprenorphine treatment. The only healthy volunteer who tolerated all four fentanyl boluses received buprenorphine at the highest dose. Collectively, the results suggest that buprenorphine at high concentrations reduces respiratory depression induced by fentanyl administration and suggest that sustained high concentrations of buprenorphine, such as those achieved with some extended-release injections used to treat OUD,[25] may protect against inadvertent fentanyl overdose.

A possible limitation of this study is the relatively small number of participants with limited racial diversity. Moreover, the opioid-tolerant patient group is somewhat heterogeneous, including six patients chronically using opioids for pain, and two chronic drug abusers, and might not fully represent the real-world population of patients with OUD. However, ventilatory responses to buprenorphine and fentanyl were consistent between all opioid-tolerant patients with relatively low inter-subject variability. In addition, the observed effects of buprenorphine on fentanyl-induced respiratory depression were substantial and significant, so the authors regard these results as clinically relevant despite the small sample size, and valid from the perspective of a single-centre trial.

In conclusion, data from this study provide clinical evidence that buprenorphine reduces the harmful effects of fentanyl on ventilation and protects against fentanyl-induced respiratory depression in a concentration-dependent manner. Future research, including studies with larger sample sizes and combining other populations in clinical practice, designed to confirm the potential protective effect of buprenorphine against this fatal consequence of opioid misuse, is warranted.

SUPPORTING INFORMATION

S1 Clinical study protocol:
https://doi.org/10.1371/journal.pone.0256752.s004
S2 Individual V_E per concentration level:
https://doi.org/10.1371/journal.pone.0256752.s003

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CHAPTER 3

MODELLING BUPRENORPHINE REDUCTION OF FENTANYL-INDUCED RESPIRATORY DEPRESSION

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Abstract

BACKGROUND Potent synthetic opioids, such as fentanyl, are increasingly abused, resulting in unprecedented numbers of fatalities from respiratory depression. Treatment with the high-affinity mu-opioid receptor partial agonist buprenorphine may prevent fatalities by reducing binding of potent opioids to the opioid receptor, limiting respiratory depression.

METHODS To characterize buprenorphine-fentanyl interaction at the level of the mu-opioid receptor in 2 populations (opioid-naive individuals and individuals who chronically use high-dose opioids), the effects of escalating IV fentanyl doses with range 0.075–0.35 mg /70 kg (opioid naive) and 0.25–0.70 mg/70 kg (chronic opioid use) on iso-hypercapnic ventilation at 2–3 background doses of buprenorphine (target plasma concentrations range: 0.2–5 ng/mL) were quantified using receptor association/dissociation models combined with biophase distribution models.

RESULTS Buprenorphine produced mild respiratory depression, while high doses of fentanyl caused pronounced respiratory depression and apnoea in both populations. When combined with fentanyl, buprenorphine produced a receptor binding–dependent reduction of fentanyl-induced respiratory depression in both populations. In individuals with chronic opioid use, at buprenorphine plasma concentrations of 2 ng/mL or higher, a protective effect against high-dose fentanyl was observed.

CONCLUSION Overall, the results indicate that when buprenorphine mu-opioid receptor occupancy is sufficiently high, fentanyl is unable to activate the mu-opioid receptor and consequently will not cause further respiratory depression in addition to the mild respiratory effects of buprenorphine.

Introduction

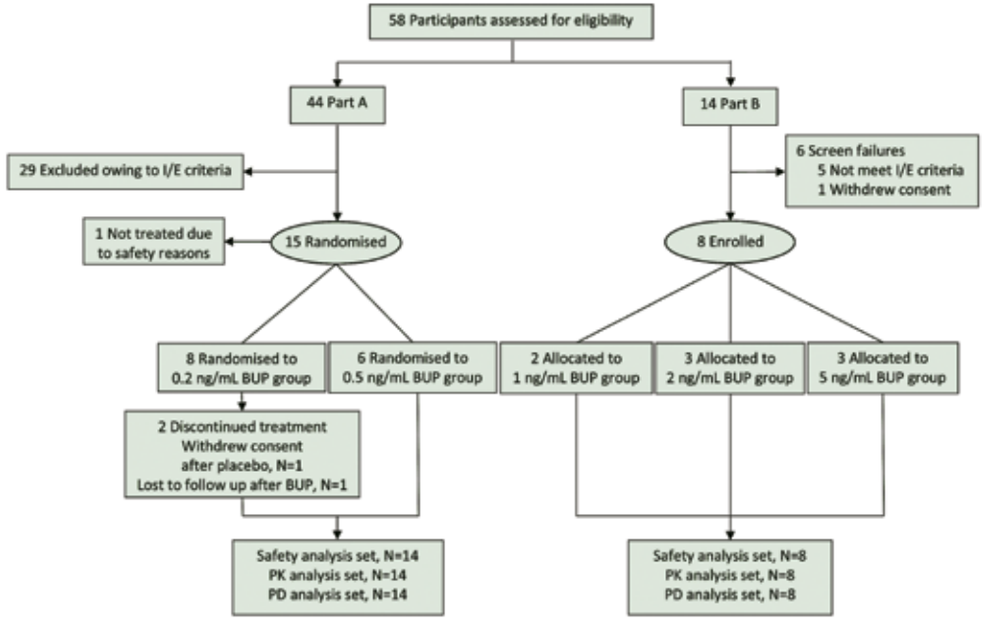
Over the past 2 decades, abuse of illicit or prescribed opioids has caused a soaring number of fatalities from opioid-induced respiratory depression.[1,2] According to the CDC, around 76,000 people in the United States died from an opioid overdose in the 12 months ending in June 2021.[3] The majority of these deaths are the result of synthetic opioids such as fentanyl being ingested as a substitute for heroin or with psychostimulants (such as cocaine and methamphetamine) that had been adulterated with the opioid.[2] This major health and socioeconomic burden to society requires immediate and ongoing attention. One option to reduce the risk of a fatal respiratory depression from an opioid overdose in patients with an opioid use disorder is treatment with buprenorphine.[4,5] Buprenorphine is a semisynthetic opioid derived from the morphine precursor thebaine and is a mu-opioid receptor (MOP) partial agonist.[6-8] We previously showed that the high affinity of buprenorphine at the MOP, which is directly related to its slow receptor kinetics, makes reversal of buprenorphine effects difficult with the opioid antagonist naloxone, even at high naloxone doses.[7] Subsequent pharmacokinetic/pharmacodynamic modelling studies suggest that buprenorphine at sufficiently high plasma concentrations prevents binding of potent opioids to the MOP, causing less respiratory depression and other opioid-related unwanted effects, including opioid craving.[8,9] However, this requires experimental proof. In the current pharmacokinetic/pharmacodynamic modelling study, we examined the interaction of buprenorphine and the potent opioid fentanyl on ventilation in opioid-naive volunteers and individuals with chronic opioid use. The main goal of this study was to characterise buprenorphine-fentanyl interactions at the MOP and to determine buprenorphine plasma levels needed to protect against the respiratory depression induced by fentanyl in individuals with chronic opioid use.

Results

The study was performed in 2 parts: part A was conducted in 14 opioid-naive volunteers (7 men/7 women, on average 24 years old) and part B was conducted in 8 individuals with chronic opioid use (3 men/5 women, on average 42 years old) (**Figure 1**). Both parts included 2 study periods (period 1 and period 2), during which participants received continuous IV infusion of buprenorphine, or a placebo co-administered

with up to 4 escalating fentanyl IV doses (Table 1). Opioid-naive volunteers had the option to participate in a third study period (period 3) where they received an IV infusion of buprenorphine alone. Two of the opioid-naive volunteers only participated to period 1 (1 volunteer withdrew consent after completion of the first experimental session; the other did not return for unknown reasons); available data of both individuals were included in the analyses (i.e., 1 placebo/fentanyl data set and 1 buprenorphine/fentanyl data set). Five volunteers participated in period 3. All individuals with chronic opioid use completed periods 1 and 2. They had used on average 220 ± 134 mg morphine equivalents per day for an average of 7 years (range: 3 months to 29 years) and were transitioned to 114 mg oxycodone per day (range: 50–315 mg) for the study, with the last dose of oxycodone given at least 15 hours prior to each period. Their opioid use was related to chronic pain (n = 6) or opioid use disorder (n = 2).

FIGURE 1 CONSORT flow diagram.



Abbreviations: BUP, *buprenorphine;* I/E = *inclusion/exclusion;* PD, *pharmacodynamic;* PK, *pharmacokinetic.*

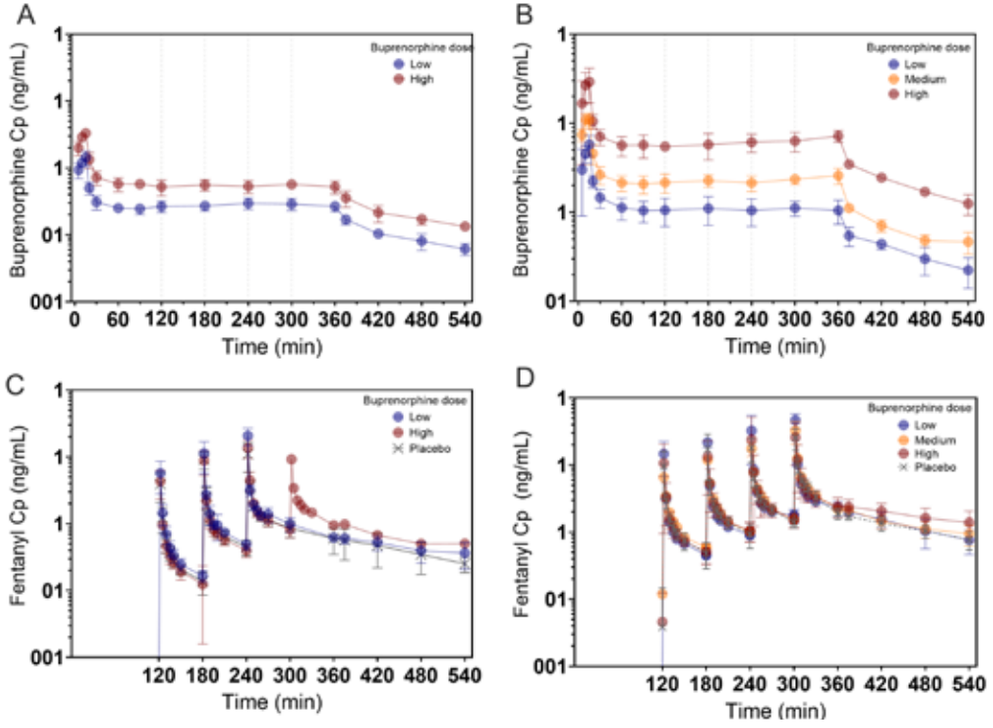
TABLE 1 Buprenorphine and fentanyl dosing and blood sampling scheme.

Opioid-naive volunteers		
Low-dose buprenorphine (target plasma conc. 0.2 ng/mL)	t = 0 min	0.05 mg/70 kg in 15 min
	t = 15 min	0.02 mg/70 kg per h
High-dose buprenorphine (target plasma conc. 0.5 ng/mL)	t = 0 min	0.125 mg/70 kg in 15 min
	t = 15 min	0.05 mg/70 kg per h
Fentanyl (in both groups)	t = 120 min	0.075 mg/70 kg in 90 s
	t = 180 min	0.15 mg/70 kg in 90 s
	t = 240 min	0.25 mg/70 kg in 90 s
	t = 300 min	0.35 mg/70 kg in 90 s
Individuals with chronic opioid use		
Low-dose buprenorphine (target plasma conc. 1 ng/mL)	t = 0 min	0.25 mg/70 kg in 15 min
	t = 15 min	0.1 mg/70 kg per h
Medium-dose buprenorphine (target plasma conc. 2 ng/mL)	t = 0 min	0.5 mg/70 kg in 15 min
	t = 15 min	0.2 mg/70 kg per h
High-dose buprenorphine (target plasma conc. 5 ng/mL)	t = 0 min	1.25 mg/70 kg in 15 min
	t = 15 min	0.5 mg/70 kg per h
Fentanyl (in all 3 groups)	t = 120 min	0.25 mg/70 kg in 90 s
	t = 180 min	0.35 mg/70 kg in 90 s
	t = 240 min	0.50 mg/70 kg in 90 s
	t = 300 min	0.70 mg/70 kg in 90 s
Blood sampling		
Buprenorphine samples at:	0 (pre-dose), 5, 10, 15, 20, 30, 60, 90, 120, 180, 240, 300, 360, 375, 420, 480, and 540	
Fentanyl samples at:	120 (pre-bolus), 122, 125, 130, 135, 140, 150, 180 (pre-bolus), 182, 185, 190, 195, 200, 210, 240 (pre-bolus), 242, 245, 250, 255, 260, 270, 300 (pre-bolus), 302, 305, 310, 315, 320, 330, 360, 375, 420, 480, and 540	

All study participants (opioid-naive volunteers and those with chronic opioid use) completed their experimental sessions without any unexpected adverse events (see ref. 10 for details on safety findings). Eight opioid-naive volunteers received low-dose buprenorphine (target plasma concentration: 0.2 ng/mL), 6 others high-dose buprenorphine (target concentration: 0.5 ng/mL), with matching measured plasma concentrations (mean ± SD) of 0.28 ± 0.05 and 0.54 ± 0.08 ng/mL, respectively (Figure 2). Individuals with chronic opioid use received low-dose (n = 2, target concentration: 1 ng/mL), medium-dose (n = 3, target concentration: 2 ng/mL), or high-dose (n = 3, target concentration: 5 ng/mL) buprenorphine, with matching measured plasma concentrations of 1.08 ± 0.33, 2.28 ± 0.40, and 6.12 ± 1.26 ng/mL, respectively (Figure 2). Because

of the occurrences of apnoeic events, the number of fentanyl doses was restricted to either 2 or 3 (irrespective of treatment) in 13/14 opioid-naive volunteers with just 1 individual receiving all 4 fentanyl doses at high-dose buprenorphine (Figure 2). In individuals with chronic opioid use, all 4 doses were given during the buprenorphine session but 2–4 doses during the placebo session.

FIGURE 2 Mean ± SD pharmacokinetic profiles.
Buprenorphine plasma concentrations (Cp) in opioid-naive volunteers receiving low- or high-dose buprenorphine targeting a plasma concentration of 0.2 or 0.5 ng/mL, respectively (A) and individuals with chronic opioid use receiving low-, medium-, or high-dose buprenorphine targeting a plasma concentration of 1, 2, or 5 ng/mL, respectively (B). Fentanyl plasma concentrations (Cp) in opioid-naive volunteers (C) and individuals with chronic opioid use (D) receiving multiple doses of fentanyl across treatment groups (opioid-naive: 0.075, 0.15, 0.25, and 0.35 mg/70 kg; chronic opioid use: 0.25, 0.35, 0.50, and 0.70 mg/70 kg).



Population pharmacokinetic analyses

Three-compartment models best described buprenorphine and fentanyl plasma concentration data. Concentrations were log-transformed for the analysis and an additive error model was retained in each case (which can be interpreted as a proportional error model on untransformed data). The parameter estimates of the final pharmacokinetic models are given in Table 2. No significant differences between the 2 populations were identified during the covariate analysis. Goodness-of-fit plots are given in Supplemental Figure 1; and prediction- and variability-corrected visual predictive checks (PVCVPC's), comparing observations with model predictions, are given in Supplemental Figures 2 and 3. Examples of data fits are given in Figure 3 and Figure 4. Overall, the inspection of goodness-of-fit plots and PVCVPC's indicated that the models well described the concentration data for both buprenorphine and fentanyl.

TABLE 2 Pharmacokinetic parameter estimates from the combined data set of opioid-naive volunteers and those with chronic opioid use.

Parameter	Estimate ± SE (%RSE)	ω^2 ± SE (%CV)	v^2 ± SE (%CV)
Buprenorphine			
V_1 (L/70 kg)	5.6 ± 1.6 (29)	-	0.50 ± 0.25 (80)
V_2 (L/70 kg)	6.4 ± 0.82 (13)	-	-
V_3 (L/70 kg)	130 ± 6.4 (4.9)	0.029 ± 0.018 (17)	-
CL (L/min/70 kg)	1.3 ± 0.055 (4.1)	0.028 ± 0.010 (17)	0.0053 ± 0.0030 (7.3)
Q_2 (L/min/70 kg)	1.8 ± 0.43 (24)	-	-
Q_3 (L/min/70 kg)	1.4 ± 0.093 (6.8)	0.041 ± 0.037 (20)	0.036 ± 0.023 (19)
σ^2	0.019 ± 0.0023 (12)		
Fentanyl			
V_1 (L/70 kg)	10.5 ± 1.5 (14)	0.43 ± 0.11 (73)	0.047 ± 0.020 (22)
V_2 (L/70 kg)	14.4 ± 2.7 (18)	0.52 ± 0.12 (82)	-
V_3 (L/70 kg)	166 ± 9.6 (5.8)	0.058 ± 0.018 (25)	-
CL (L/min/70 kg)	1.3 ± 0.080 (6.3)	0.087 ± 0.019 (30)	0.0088 ± 0.0032 (9.4)
Q_2 (L/min/70 kg)	2.0 ± 0.29 (14)	0.39 ± 0.088 (69)	0.022 ± 0.018 (15)
Q_3 (L/min/70 kg)	2.3 ± 0.17 (7.6)	0.098 ± 0.038 (32)	0.018 ± 0.0067 (14)
σ^2	0.021 ± 0.0028 (13)		

CV is coefficient of variation for interindividual variability (calculated as $\sqrt{exp(\omega^2)-1}$ multiplied by 100) or inter-occasion variability (same formula with v^2); RSE, relative standard error; ω^2, variance for interindividual variability; v^2, variance for inter-occasion variability; V_{1-3}, volumes of compartments 1–3; CL, clearance; $Q_{2,3}$, intercompartmental clearances; σ^2, additive residual error variance. '–' denotes the parameter was not estimable and was fixed to zero.

FIGURE 3 Example of data results and analyses of an opioid-naive individual.
Data fits and predicted receptor occupancy driving the effect of fentanyl (ascending doses of 0.075, 0.15, and 0.25 mg/70 kg) on minute ventilation at the background of placebo infusion (B, D, and F) and high-dose buprenorphine infusion targeting a plasma concentration of 0.5 ng/mL (A, C, E, and G) in an opioid-naive volunteer. (A) Measured buprenorphine plasma concentration (Cp) (gray circles) and data fits (continuous line). (B and C) Measured fentanyl plasma concentration (Cp) (black circles) and data fits (continuous lines). (D and E) Predicted receptor occupancy for fentanyl (broken line, F) and buprenorphine (continuous line, B). (F and G) Measured ventilation (blue circles) and data fit of the model with a Kalman filter (green line) and data fit of the model without a Kalman filter (red line). Acquisition of ventilation data was sometimes interrupted for various reasons (see text); in this case because the individual had to urinate (F and G).

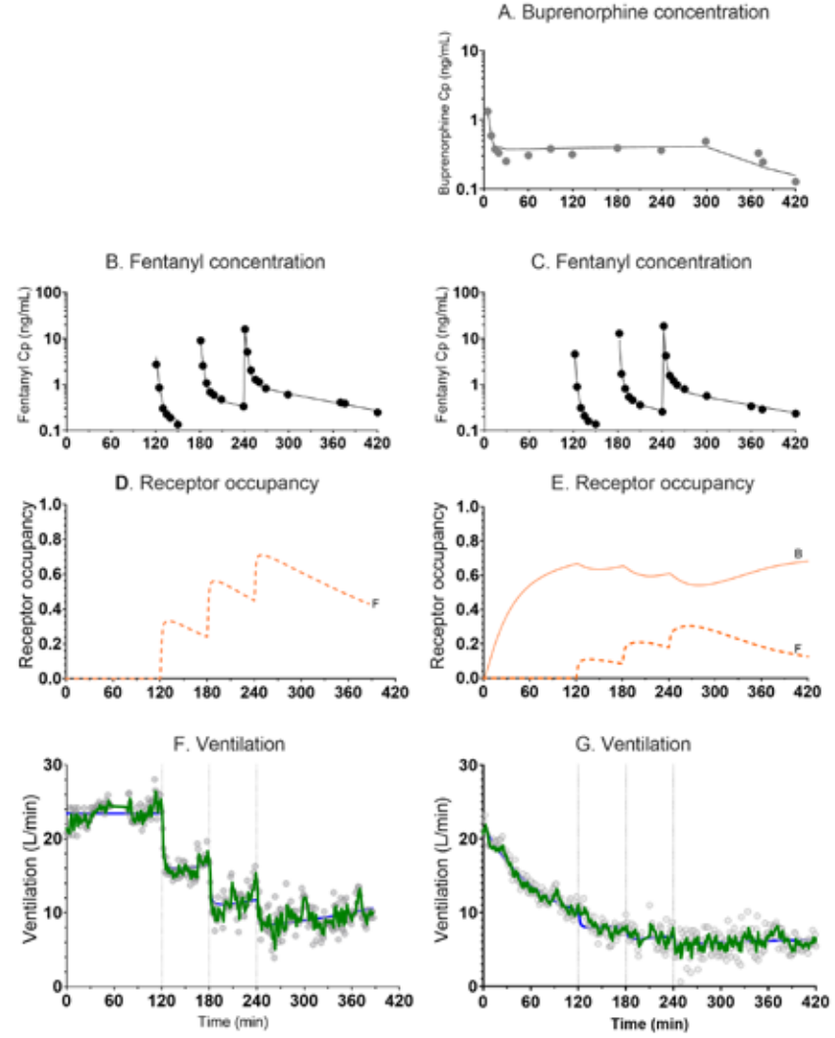
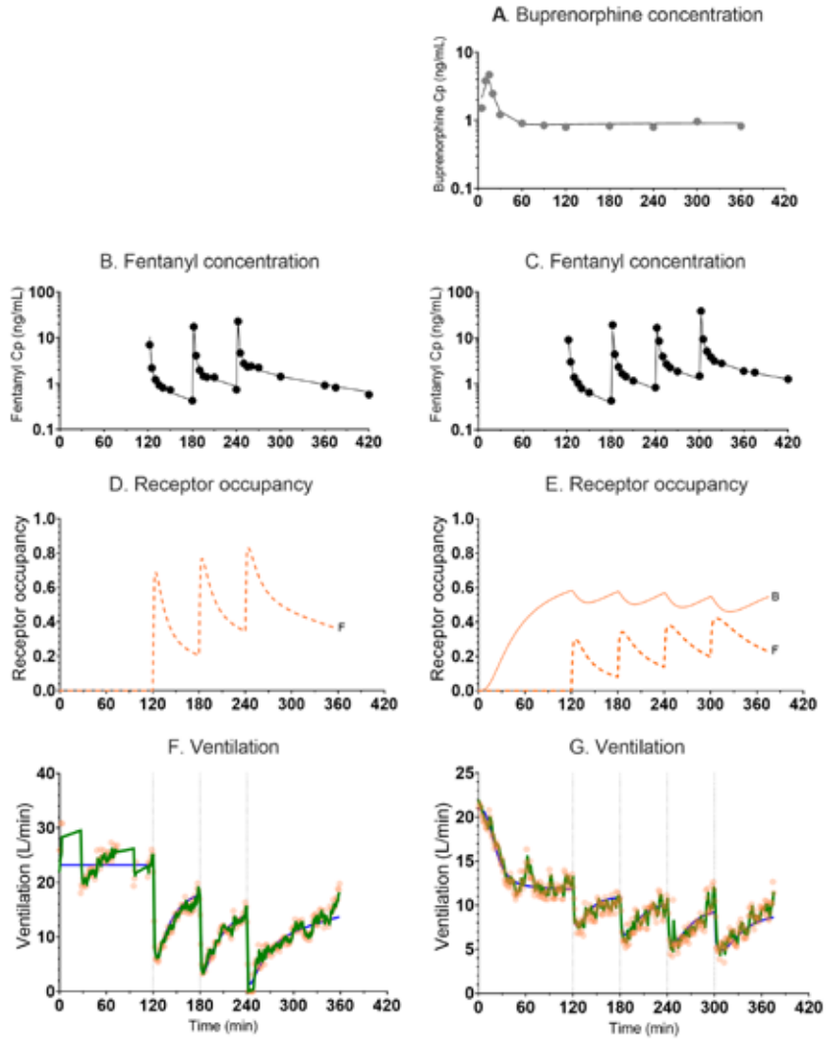


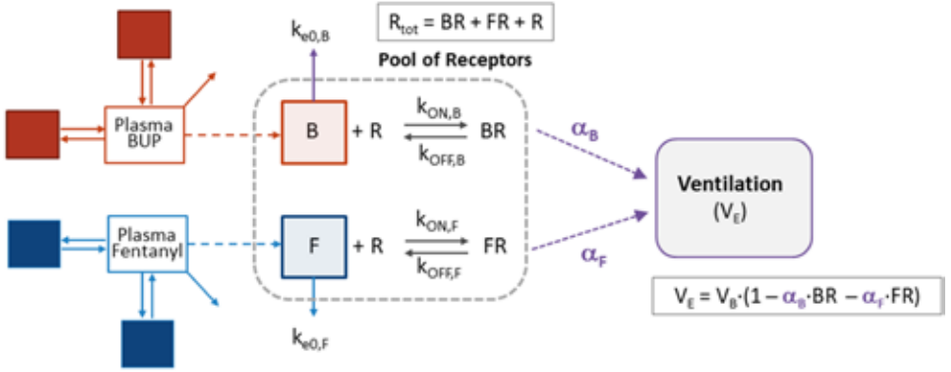
FIGURE 4 Example of data results and analyses of an individual with chronic opioid use.
Data fits and predicted receptor occupancy driving the effect of fentanyl (ascending doses of 0.25, 0.35, 0.50, and 0.70 mg/70 kg) on minute ventilation at the background of placebo infusion (B, D, and F) and low-dose buprenorphine infusion targeting a plasma concentration of 1 ng/mL (A, C, E, and G) in an individual with chronic opioid use. (A) Measured buprenorphine plasma concentration (Cp) (gray circles) and data fits (continuous line). (B and C) Measured fentanyl plasma concentration (Cp) (black circles) and data fits (continuous lines). (D and E) Predicted receptor occupancy for fentanyl (broken line, F) and buprenorphine (continuous line, B). (F and G) Measured ventilation (orange circles) and data fit of the model with a Kalman filter (green line) and data fit of the model without a Kalman filter (red line). Acquisition of ventilation data was sometimes interrupted for various reasons; in this case because the individual had to urinate (F).



Population pharmacokinetic/pharmacodynamic analyses

Minute ventilation data measured in opioid-naive volunteers and those with chronic opioid use were modelled simultaneously using a population pharmacokinetic/pharmacodynamic modelling approach. The integrated pharmacokinetic/pharmacodynamic model, incorporating receptor association/dissociation models with biophase distribution models, is represented in **Figure 5**. Because fentanyl associates and dissociates rapidly from MOPS, C_{50F} (= $k_{OFF,F}/k_{ON,F}$) was estimated in place of fentanyl association and dissociation rate constants $k_{ON,F}$ and $k_{OFF,F}$.

FIGURE 5 Integrated pharmacokinetic/pharmacodynamic model.
α_B, α_F: intrinsic activity of buprenorphine and fentanyl, respectively (which also accounts for receptor reserve); B, F: effect site concentrations for buprenorphine and fentanyl, respectively; BR, FR: concentrations of receptors bound to buprenorphine and fentanyl, respectively; BUP: buprenorphine; $k_{e0,B}$, $k_{e0,F}$: equilibration rate constants for buprenorphine and fentanyl, respectively; $k_{ON,B}$, $k_{OFF,B}$: buprenorphine association and disassociation rate constants; $k_{ON,F}$, $k_{OFF,F}$: fentanyl association and disassociation rate constants; R: concentration of unbound receptors; V_B: baseline ventilation; V_E: minute ventilation. Because fentanyl associates and dissociates rapidly from the receptors, $C_{50,F}$ (=$k_{OFF,F}/k_{ON,F}$) was estimated in place of $k_{OFF,F}$ and $k_{ON,F}$.



A Kalman filter was implemented to account for the correlations in residual noise (i.e., measurement noise). Typically, pharmacokinetic/pharmacodynamic models assume that the residual error is uncorrelated. However, with ventilation measurements every minute, correlations between residuals are likely to occur and can affect the estimation of model parameters if not appropriately accounted for. The parameter estimates of the final models with and without implementation of a Kalman filter are given in **Table 3** and **Supplemental Table 1**, respectively. Upon model comparison, the model without the Kalman filter displayed an overprediction of the variability and a misprediction of the probability of apnoea (see **Figure 6** and **Figure 7** compared with **Supplemental Figures 4** and **5**). Additionally, the model without the Kalman filter produced correlated noise when examining the autocorrelation and cross-correlation functions. This is exemplified in **Supplemental Figure 6**, which shows the autocorrelation function of the residuals for an analysis without (blue line) and with the Kalman filter (black line). The function shows 'white' noise for residuals when a parallel noise component was added to the structural model, while they were correlated in the model without such a component. Since the model with the Kalman filter addressed these issues satisfactorily, it was considered the final model.

The analyses revealed several significant differences between opioid-naive volunteers and those with chronic opioid use in terms of buprenorphine association rate constant $k_{ON,B}$ (opioid naive: 0.26 ± 0.02 $mL \cdot ng^{-1} \cdot min^{-1}$ versus chronic opioid use: 0.085 ± 0.009 $mL \cdot ng^{-1} \cdot min^{-1}$), buprenorphine-intrinsic activity α_B (opioid naive: 0.81 ± 0.03 versus chronic opioid use: 0.48 ± 0.03), and fentanyl potency $C_{50,F}$ (opioid naive: 0.68 ± 0.13 ng/mL versus chronic opioid use: 2.23 ± 0.53 ng/mL) (see **Table 3**). These results suggest differences in receptor kinetics between the 2 populations, with a lower buprenorphine and fentanyl sensitivity in individuals with chronic opioid use compared with opioid-naive volunteers. In both populations, αB was less than 1 while α_F was fixed to 1 with no associated variability, suggesting that apnoea occurs at infinite fentanyl concentrations. However, the use of the Kalman filter allowed adequate prediction of apnoea (**Figures 6** and **7**). In opioid-naive volunteers, differences in the magnitude of residual noise were observed between study occasions, with less residual noise when buprenorphine was given compared with a placebo (**Table 3**). Examples of data fits are given in **Figures 3** and **4** for 1 opioid-naive volunteer and 1 individual with chronic opioid use, respectively. They show that the model with the Kalman filter (green lines in **Figure 3F** and **G**, and **Figure 4F** and **G**) well described the minute ventilation data. Also, the output of the model without the Kalman filter is included (red lines in **Figure 3F** and **G**, and **Figure 4F** and **G**). Goodness-of-fit plots are given in **Supplemental Figure 1, G–I**. The pvcVPCs, comparing observations with model predictions, are given in **Figure 6** (opioid naive) and **Figure 7** (chronic opioid use) for the model with the Kalman filter and in **Supplemental Figures 4** and **5** for the model without the Kalman filter. Taken together, the model with the Kalman filter adequately described the ventilation data and gave reliable predictions of the probability of apnoea.

FIGURE 6 Prediction- and variability-corrected visual predictive checks of the pharmacodynamic model with a Kalman filter in opioid-naive individuals for the various drug administrations and probabilities of apnoea for the same conditions.

(A and B) Fentanyl given at the background of placebo infusion. (C and D) Fentanyl given at the background of buprenorphine infusion. (E and F) Just buprenorphine. The dots in A, C, and E are the 1-minute ventilation averages; the broken lines are the observed percentiles (dark orange: median, dark blue: 2.5th and 97.5th percentiles); the bins are the 95% confidence intervals of simulated percentiles (orange bins: median, blue bins: 2.5th and 97.5th percentiles). The right panels (B, D, and F) give the probability of apnoea. The red symbols are the probabilities of the observed apnoeic episodes; the orange bins are the simulated 95% confidence intervals of the probability of apnoea.

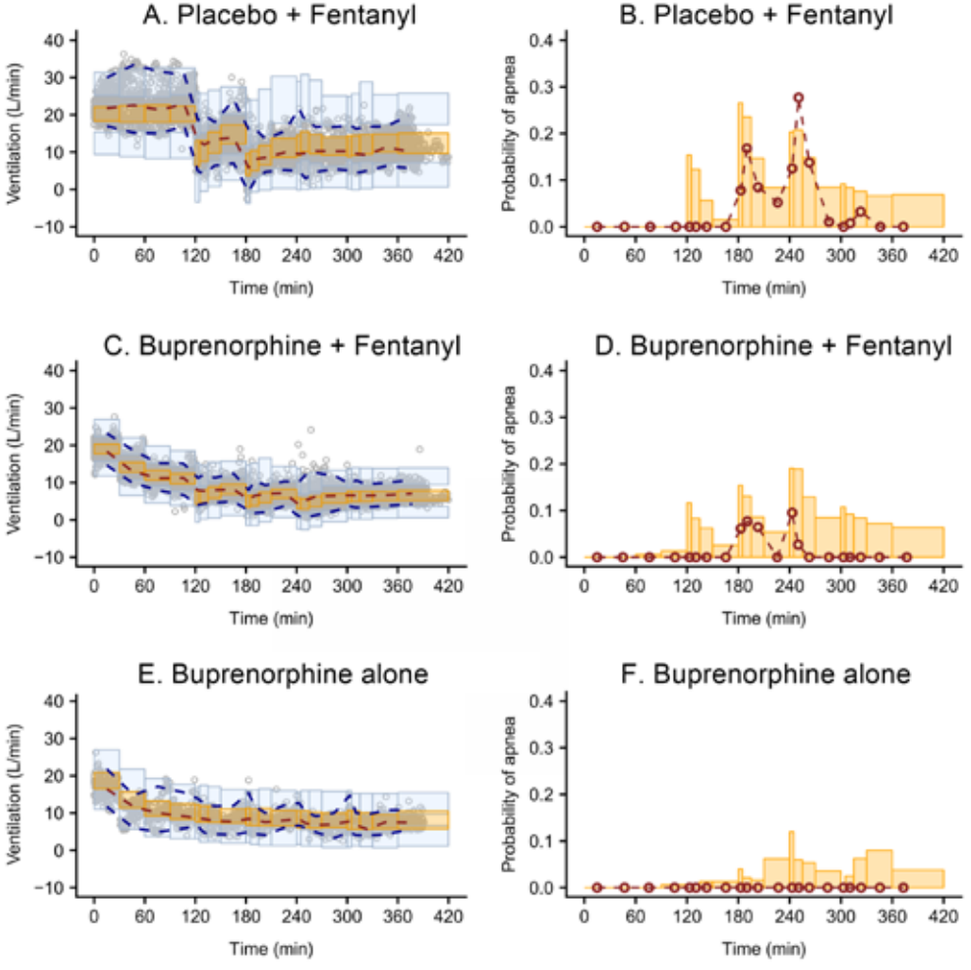


FIGURE 7 Prediction- and variability-corrected visual predictive checks of the pharmacodynamic model with a Kalman filter in opioid-naive individuals for the various drug administrations and probabilities of apnoea for the same conditions.

(A and B) Fentanyl given at the background of placebo infusion. (C and D) Fentanyl given at the background of buprenorphine infusion. (E and F) Just buprenorphine. The dots in A, C, and E are the 1-minute ventilation averages; the broken lines are the observed percentiles (dark orange: median, dark blue: 2.5th and 97.5th percentiles); the bins are the 95% confidence intervals of simulated percentiles (orange bins: median, blue bins: 2.5th and 97.5th percentiles). The right panels (B, D, and F) give the probability of apnoea. The red symbols are the probabilities of the observed apnoeic episodes; the orange bins are the simulated 95% confidence intervals of the probability of apnoea.

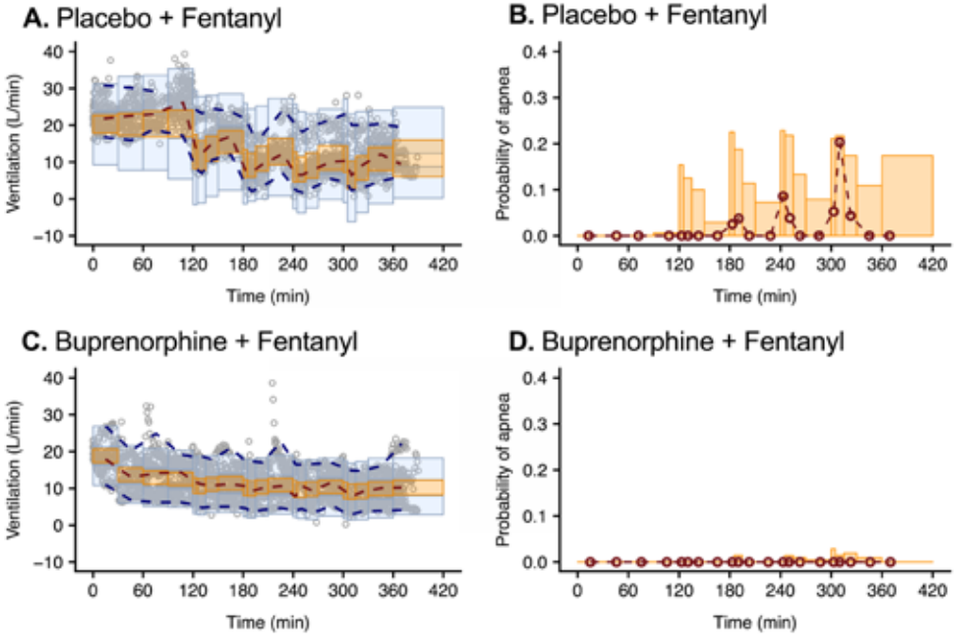


TABLE 3 Pharmacodynamic parameter estimates for the model with a Kalman filter

Parameter	Estimate ± SE (%RSE)	ω^2 ± SE (%CV)	v^2 ± SE (%CV)
Baseline ventilation V_B (L/min)	20.2 ± 0.35 (1.7)	FIXED to 0.001 (3.2)	0.0064 ± 0.0016 (8.0)
$k_{ON,B}$ (mL·ng^{-1}·min^{-1})			
Opioid-naive	0.26 ± 0.023 (9.0)	FIXED to 0.001 (3.2)	-
Chronic opioid use	0.25 ± 0.029 (12)		-
$k_{OFF,B}$ (min^{-1})	0.019 ± 0.014 (7.5)	FIXED to 0.001 (3.2)	-
$k_{E0,B}$ (min^{-1})	0.0028 ± 0.00020 (7.1)	FIXED to 0.001 (3.2)	-
α_B			
Opioid-naive	0.81 ± 0.030 (3.7)		
Chronic opioid use	0.48 ± 0.027 (5.6)		
$C_{50,F}$ (ng/mL)			
Opioid-naive	0.68 ± 0.13 (18)	0.37 ± 0.25 (67)	-
Chronic opioid use	2.23 ± 0.53 (24)		
α_F	FIXED to 1	FIXED to 0.001 (3.2)	-
$k_{E0,F}$ (min^{-1})	0.10 ± 0.024 (24)	1.16 ± 0.30 (148)	-
σ		FIXED to 0.001 (3.2)	0.17 ± 0.054 (44)
Occ1	1.37 ± 0.12 (9.1)		
Occ2-5/Occ1	0.69 ± 0.063 (9.1)		
σ_v		FIXED to 0.001 (3.2)	FIXED to 0.001 (3.2)
Occ1	0.95 ± 0.026 (2.8)		
Occ2-5/Occ1	0.58 ± 0.024 (3.7)		
τ (min)	28.7 ± 2.7 (9.3)	FIXED to 0.001 (3.2)	-

SE, standard error of the estimate; RSE, relative standard error; ω^2, variance for interindividual variability; CV, coefficient of variation for interindividual variability (calculated as $\sqrt{exp(\omega^2) - 1}$ multiplied by 100) or interoccasion variability (same formula with v^2); v^2, variance for interoccasion variability; $k_{ON,B}$ and $k_{OFF,B}$, association and dissociation rate constant for buprenorphine; $C_{50,F}$ (=$k_{OFF,F}/k_{ON,F}$), fentanyl effect-site concentration causing a 50% decrease in ventilation; a_B and a_F, intrinsic activity parameters for buprenorphine and fentanyl, respectively, that also account for receptor reserve; $k_{Eo,B}$ and $k_{Eo,F}$, effect-site equilibration rate constant for buprenorphine and fentanyl, respectively; σ, standard deviation of residual error; Mechanism of action of opiorphin, standard deviation of process noise; τ, time constant determining correlation in time of process noise; Occ, occasion. Opioid-naive: Occ1, placebo + fentanyl; Occ2, buprenorphine + fentanyl; Occ3, buprenorphine only. Chronic opioid use: Occ4, placebo + fentanyl; Occ5, buprenorphine + fentanyl. '–' denotes the parameter was not estimable and was fixed to zero.

Simulations

Simulations were performed to better understand the interaction between fentanyl and buprenorphine on ventilation in individuals with chronic opioid use. In **Figure 8**, we show the effect of 4 subsequent IV fentanyl doses, 0.25, 0.35, 0.50, and 0.70 mg/70 kg, at 3 target buprenorphine plasma concentrations, 0 (placebo), 1, and 5 ng/mL. **Figure 8, D–F**, show that buprenorphine reduced the number of fentanyl-bound receptors in a concentration-dependent manner, resulting in a smaller fentanyl effect on ventilation (**Figure 8, G–I**) (see **Supplemental Figure 7** for complete results, including 2 ng/mL buprenorphine). **Figure 9A** shows the probability of fentanyl-induced apnoea as a function of buprenorphine plasma concentration at increasing doses of fentanyl (0.1 to 5 mg/70 kg); the probability was calculated as the percentage of simulated individuals experiencing at least 1 episode of apnoea. Overall, simulation results aligned with experimental results in the study. During the placebo session, 2 of 8 (25%) individuals with chronic opioid use had apnoea with a cumulative fentanyl dose of 0.6 mg/70 kg, and 7 of 8 (88%) had apnoea after a cumulative fentanyl dose of 1.8 mg/70 kg. Under placebo conditions, the predicted percentage of study participants with apnoea was 16% for 0.5 mg/70 kg fentanyl and 59% for 2 mg/70 kg fentanyl. During the buprenorphine session, fentanyl effects on ventilation were reduced, and only 1 of 8 (13%) individuals with chronic opioid use experienced apnoea after a cumulative dose of 1.8 mg/70 kg. In the simulations, the percentage of individuals with apnoea after 2 mg/70 kg fentanyl ranged between 2.1% (5 ng/mL buprenorphine) and 6.4% (1 ng/mL buprenorphine). **Figure 9B** shows the effect of increasing doses of fentanyl (0.05 to 5 mg/70 kg) on the change in minute ventilation relative to pre-fentanyl baseline as a function of buprenorphine plasma concentration. Both simulations showed that relatively high plasma concentrations of buprenorphine (2 ng/mL or higher) reduced the probability of apnoea corresponding with a smaller ventilatory depression. The best clinical outcomes were observed at a buprenorphine concentration of 5 ng/mL for the highest dose of fentanyl investigated.

FIGURE 8 Results of simulation study: probabilities of apnoea and decrease in ventilation.
Simulations in a representative ('typical') individual with chronic opioid use showing the effect of 4 subsequent fentanyl IV doses (0.25, 0.35, 0.50, and 0.70 mg/70 kg) on top of a buprenorphine plasma concentration of 0 (placebo), 1, and 5 ng/mL. (A–C) Fentanyl and buprenorphine plasma concentrations (Cp). (D–F) Fentanyl and buprenorphine receptor occupancy. (G–I) Ventilation.

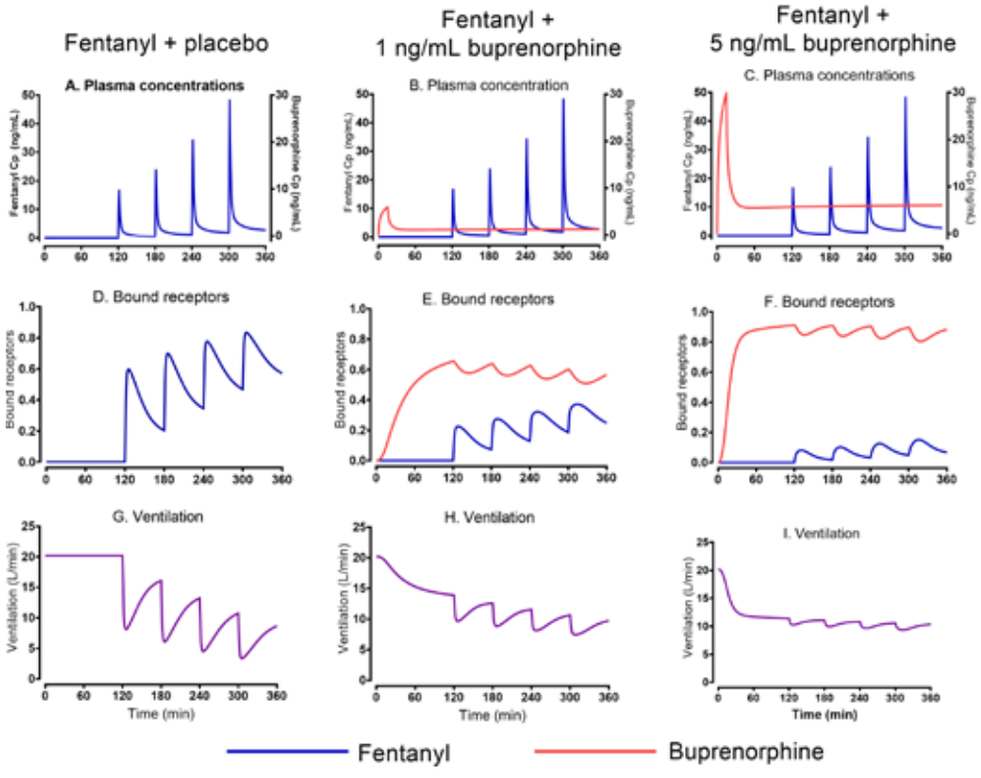
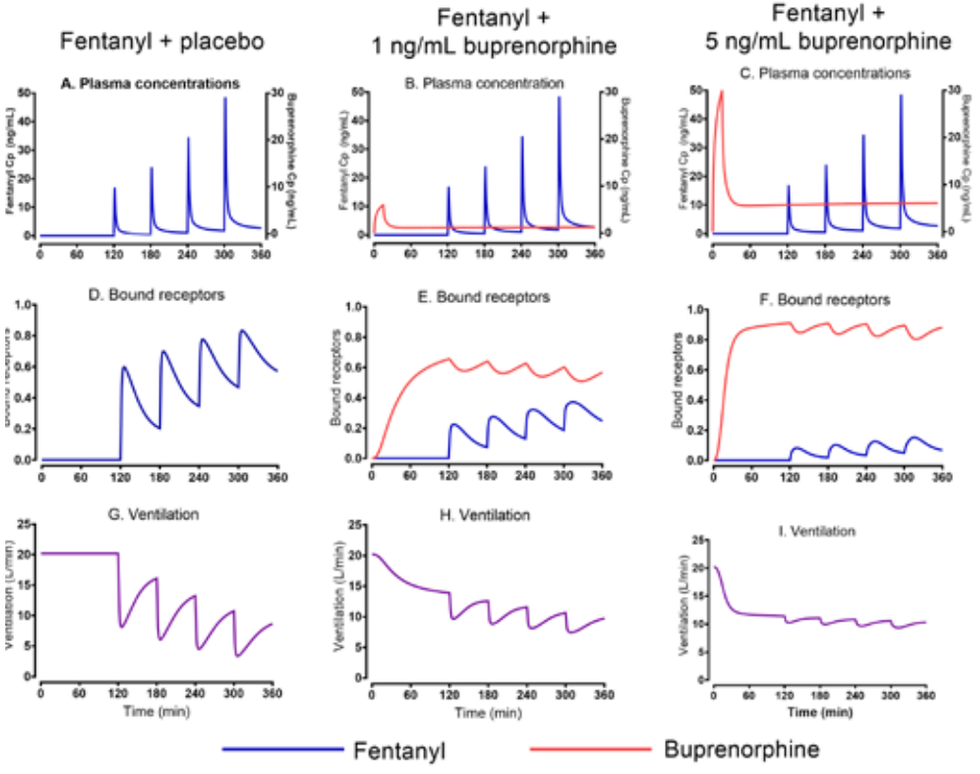


FIGURE 9 Results of simulation study: plasma concentrations, receptor binding, and ventilation.
Simulations showing the probability of apnoea (A) and median peak decrease in ventilation (B) at various fentanyl bolus doses ranging from 0.05 to 5 mg/70 kg (dose in mg/70 kg given within the panels before their respective effect lines) against the steady-state buprenorphine plasma concentration in individuals with chronic opioid use. The peak drop in ventilation was calculated from pre-fentanyl value and expressed a percentage of ventilation baseline (V_B).



Discussion

We tested whether the MOP partial agonist buprenorphine was able to effectively prevent respiratory depression induced by the MOP full agonist fentanyl in opioid-naive volunteers and in individuals with chronic opioid use. The relationship between opioid (fentanyl and buprenorphine) plasma concentrations and respiratory effects were analysed using a population pharmacokinetic/pharmacodynamic modelling approach. The following are the main results from the analyses: (A) The population pharmacokinetic/pharmacodynamic model, developed from the combined analysis of data from opioid-naive volunteers and those with chronic opioid use, was able to adequately describe the complex interaction of buprenorphine and fentanyl on ventilation using receptor association/dissociation models. (B) The parameter α, which incorporates intrinsic ligand activity and receptor reserve, was 1 for fentanyl in both populations but differed between the 2 populations for buprenorphine, with values of 0.8 in opioid-naive volunteers and just 0.5 in individuals with chronic opioid use; the lower α for buprenorphine is consistent with the previously reported buprenorphine ceiling effect on respiratory depression.[8] (C) Fentanyl sensitivity differed 3.3-fold between the 2 populations, with reduced fentanyl sensitivity in individuals with chronic opioid use ($C_{50,F}$ = 2.23 ng/mL) compared with opioid-naive individuals ($C_{50,F}$ = 0.68 ng/mL), indicative of substantial tolerance to the respiratory effects of fentanyl in individuals with chronic opioid use. (D) Despite the decreased fentanyl sensitivity in individuals with chronic opioid use, apnoea did occur in this population with a probability approaching 1 at high-dose fentanyl administration (**Figure 9**); consequently, a protective pharmacological approach remains a necessity in this particular population. (E) Buprenorphine displayed slow receptor kinetics with a dissociation rate constant k_{OFF} estimated at 0.019 min^{-1}, which corresponds to a half-life of 37 minutes. (F) Like fentanyl, buprenorphine sensitivity was reduced in individuals with chronic opioid use (apparent potency K = k_{OFF}/k_{ON} = 0.22 ng/mL) compared with opioid-naive individuals (K = 0.072 ng/mL). (G) The plasma/effect-site equilibration half-life, $t_{½\,keo}$, was 6.9 minutes for fentanyl and 251 minutes for buprenorphine; no differences between the 2 populations were noted. (H) Finally, buprenorphine produced a concentration-dependent (i.e., receptor binding dependent) reduction of the ability of fentanyl to induce respiratory depression; this was further confirmed in simulation studies showing that buprenorphine plasma concentrations of 2 ng/mL and higher exerted effective protective effects even with high-dose fentanyl administrations (e.g., 2–5 mg/70 kg).

Hence, our data demonstrated that buprenorphine formulations that deliver sustained plasma concentrations of 2 ng/mL or higher would have protective effects against respiratory depression and apnoea induced by full-opioid agonists, such as fentanyl. Although medium-dose buprenorphine (target plasma concentration: 2 ng/mL) was effective toward low doses of fentanyl, high-dose buprenorphine (target plasma concentration: 5 ng/mL) appeared to provide a larger protective effect toward high doses of fentanyl, such as the 5 mg/70 kg dose as presented in the simulations.

Concentration-dependent effects resulting from the competitive interaction of buprenorphine and (ab)used opioid at the MOP are also noted for other clinical effects of buprenorphine during treatment of opioid use disorder. In a study evaluating the ability of a buprenorphine extended-release formulation (BUP-XR) to block the subjective effects of the full MOP agonist hydromorphone, buprenorphine plasma concentrations of 2 ng/mL or higher produced a more consistent response toward opioid blockade.[11] Additionally, a subgroup analysis of a phase III randomized clinical trial of BUP-XR indicated that patients who used opioids by the IV route (thereby exposed to higher concentrations of opioids) had higher abstinence rates following plasma exposure to 5–6 ng/mL buprenorphine (maintenance dose of 300 mg) compared with 2–3 ng/mL buprenorphine (maintenance dose of 100 mg).[12]

The mechanisms of the reduction in opioid sensitivity in individuals with chronic opioid use relative to opioid-naive individuals, as described by differences in fentanyl and buprenorphine potency parameters C_{50} and K (k_{OFF}/k_{ON}), respectively, have been discussed previously.[13] In brief, apart from possible pharmacokinetic effects, such as upregulation of efflux transporters in brain endothelial cells, the observation of a reduced opioid potency in individuals with chronic opioid use is related to one of several cellular and molecular changes that occur during chronic opioid exposure, including receptor desensitization, endocytosis, degradation, and downregulation. Possible mediators in these processes are PKC, β-arrestin, adenylate cyclase, NMDA receptors, and glial cells.[13] All of these factors are involved in the development of tolerance to opioid analgesia; only PKC has been shown to be involved in the tolerance to opioid-induced respiratory depression.[14]

The effects of fentanyl and buprenorphine on the ventilatory control system have been previously described in opioid-naive volunteers,

albeit never in combination and not in individuals with chronic opioid use.[7–9,15] The parameter estimates of the current study obtained in opioid-naive individuals are in close agreement with these prior findings. We previously observed that the ability of the competitive MOP antagonist naloxone to reverse buprenorphine-induced respiratory depression is reduced compared with reversal of fentanyl- and morphine-induced respiratory depression.[7,8,16] We related this to the slow buprenorphine receptor kinetics and related high receptor affinity, and these same factors contribute to our current findings that buprenorphine had protective effects against fentanyl-induced respiratory depression. When buprenorphine receptor occupancy is sufficiently high, fentanyl with its rapid receptor kinetics is unable to activate the MOP and consequently will not cause additional respiratory depression on top of the respiratory effects of buprenorphine. Buprenorphine respiratory effects are imposed by its intrinsic activity, which was relatively small in individuals with chronic opioid use. Although the study was conducted in a controlled setting in a relatively small number of individuals with chronic opioid use and may not be broadly applicable to the range of patients treated for opioid use disorder, this is an important finding and supports the protective effect of buprenorphine in limiting life-threatening respiratory depression in individuals with chronic opioid use, at a background of just asymptomatic respiratory depression caused by buprenorphine alone. These data warrant further investigation in a large outcome study where a BUP-XR formulation maintaining plasma concentrations at or above 2 ng/mL could be used.

To accurately describe the variability in the data and improve the accuracy of model parameter estimates, we incorporated a Kalman filter. Compared with the pharmacokinetic/pharmacodynamic model without a Kalman filter, adding a noise filter reduced the presence of autocorrelations and cross-correlations within the model residuals and resulted in improved model predictions. We discussed previously that this indicates improvement in model performance with more reliable estimates of variability and structural model parameters.[17] If we compare the model parameter estimates of the final models with and without a Kalman filter, the differences are relatively small. Most importantly, the speed of onset/offset of the fentanyl response increased from 18.7 to 6.9 minutes ($t_{½\,keo}$), whereas the buprenorphine dissociation rate constant k_{OFF} remained similar ($t_{½\,kOFF}$ = 43 versus 37 minutes). The fentanyl $t_{½\,keo}$ of 6.9 minutes is a more realistic estimate of fentanyl dynamics as it corresponds with its value for changes in the power spectrum of the electroencephalogram ($t_{½\,keo}$ = 6.4 minutes).[18] Earlier analyses of pharmacokinetic and pharmacokinetic/pharmacodynamic data sets similarly favoured stochastic models with a Kalman filter to remove correlated residual noise.[17,19,20]

Conclusions

Buprenorphine has been shown to reduce all-cause mortality and opioid-related mortality following treatment after a nonfatal opioid overdose.[4,21] Although it is well established that maintenance treatment with buprenorphine reduces illicit opioid use, the current study describes a second mechanism through which buprenorphine may reduce opioid overdose deaths. Our data showed that buprenorphine had a protective effect against fentanyl-induced respiratory depression at plasma concentrations of 2 ng/mL and higher, with a reduced probability of apnoea even at high fentanyl doses. This indicates that when buprenorphine receptor occupancy is sufficiently high, fentanyl is unable to activate the MOP and consequently will not cause additional respiratory depression on top of the mild respiratory effects of buprenorphine. Although this small experimental medicine study was not performed in real-life conditions and warrants the conduct of a large outcome study, the ability of buprenorphine to reduce the risk of serious respiratory events was clearly demonstrated.

Methods

The study was conducted from March 2018 through January 2019 in Leiden, Netherlands. Dosing and respiratory testing were performed at the Department of Anaesthesiology of the Leiden University Medical Centre; all other procedures were performed at the Centre for Human Drug Research, both located in Leiden, Netherlands. The study design and primary clinical outcomes were previously published;[10] the modelling of fentanyl versus placebo responses were previously published.[13] Here, we report on the population pharmacokinetic/pharmacodynamic modelling of the complete data set characterizing the buprenorphine-fentanyl interaction in opioid-naive volunteers and those with chronic opioid use.

Study design

The study had 2 parts: part A was conducted in opioid-naive volunteers and part B was conducted in individuals with chronic opioid use treated for chronic pain or opioid use disorder (see CONSORT flow diagram in **Figure 1**).

PART A

Part A had a single-blind, 2-sequence crossover design and was performed in 14 nonsmoking (including e-cigarettes) healthy study participants of either sex, aged 18–45 years, with a BMI of 18–30 kg/m^2 and without any history of any medical or psychiatric disease, including substance use disorder. Study participants were randomized to receive a continuous IV infusion of buprenorphine or a placebo on 2 occasions (period 1 and period 2). The randomization schedule was generated by an independent statistician using PC SAS version 9.4. Up to 4 identical IV escalating fentanyl challenges (in mg/70 kg) were administered in both periods on top of a buprenorphine or placebo infusion (**Table 1**). In some study participants, a third period (period 3) was added, in which they only received a buprenorphine infusion in an open-label manner. Participation in period 3 was optional. The time between study periods was 10–17 days for adequate washout.

PART B

Part B had an open-label, single-sequence crossover design and was conducted in 8 opioid-tolerant individuals, aged 18–55 years, with a BMI of 18–32 kg/m^2 and using at least 90 mg oral morphine equivalents daily. All participants were only enrolled if they were in stable physical and mental condition as defined by the investigators and based on their medical, neurological, and psychiatric history; blood and urine chemistry; and electrocardiogram. They were admitted to the clinic 2–5 days prior to period 1 and, if not already using oxycodone, transitioned to oral oxycodone. To ensure washout of each participant's usual opioids, tailored substitution schedules with oxycodone began a minimum of 48 hours before the first experiment, and the last dose of oxycodone was administered at least 15 hours before any study drug administration. During period 1, all participants received 4 escalating fentanyl IV doses on top of a placebo infusion. During period 2, the participants received a buprenorphine infusion combined with the identical fentanyl doses (in mg/kg) as given during period 1. This fixed dosing sequence was chosen to optimize the fentanyl dose escalation before the fentanyl-buprenorphine combination was administered. Because of the short half-life of fentanyl, period 2 occurred at least 40 hours after period 1. During this washout period, participants again received oxycodone for opioid substitution.

On the experiment days, all study participants were transferred to an anaesthesia suite where they received an IV line in one arm for drug administration and an arterial line in the contralateral radial artery for blood sampling. Arterial oxygen saturation was measured by finger probe (Masimo Corporation) and heart rate using 3 chest electrodes (Datex Cardiocap).

Respiratory testing

Ventilation was measured on a breath-to-breath basis at iso-hypercapnia and iso-normoxia, using the end-tidal forcing technique.[22,23] The end-tidal oxygen concentration was kept constant at 13.5 vol.%, and the end-tidal CO_2 concentration was kept constant at the level that caused a minute ventilation of at least 20 L/min at baseline (i.e., prior to any drug administration). See previous studies.[22,23] for a detailed description of the technique. In brief, a face mask was placed over the nose and mouth, which was connected to a pneumotachograph/pressure transducer system (Hans Rudolph Inc.) for measurement of ventilation and 3 mass flow controllers (Bronkhorst High Tech) maintained delivery of O_2, CO_2, and N_2. The mass flow controllers were driven by a computer running custom-made software RESREG/ACQ (Leiden University Medical Centre, Netherlands) allowing strict control of inspired gas concentrations. Gas concentrations were measured at the mouth by a capnograph (Datex Capnomac). Respiration was measured from placement of the mask until the end of the buprenorphine or placebo infusion. All breath-to-breath data were averaged over 1 minute for pharmacokinetic/pharmacodynamic data analyses.

Drug dosing in part A

After ventilation had stabilized, a 6-hour continuous infusion of buprenorphine (Indivior UK Ltd.) or placebo (normal saline) was started. Two buprenorphine dose cohorts, low and high, were evaluated, targeting 7 individuals per cohort and aiming to achieve buprenorphine plasma concentrations of 0.2 and 0.5 ng/mL. Escalating IV bolus doses of fentanyl (Hameln Pharmaceuticals Ltd.) were administered in the range of 0.075–0.35 mg/70 kg at t = 2, 3, 4, and 5 hours after the start of the buprenorphine or placebo infusion. See **Table 1** for the buprenorphine and fentanyl dosing schemes. Fentanyl dose escalations were limited if a procedure-related adverse event occurred, defined by the loss of respiratory activity for 60 seconds or longer despite active stimulation of the participant, end-tidal CO_2 concentration greater than 67.5 mmHg, oxygen saturation less than 85% for at least 2 minutes, or any

other situation or condition that could interfere with the health of the participant as judged by the investigators. If an apnoeic event occurred and the individual was verbally stimulated to breathe, the individual did not proceed to the next fentanyl dose level.

Drug dosing in part B

After ventilation had stabilized, a 6-hour continuous infusion of buprenorphine or placebo was started. Three buprenorphine dose cohorts, low, medium, and high, were evaluated with 2–3 individuals per group aiming to achieve buprenorphine plasma concentrations of 1, 2, and 5 ng/mL, respectively, corresponding to 50%, 70%, and 80% MOP occupancy.[24] Escalating IV bolus doses of fentanyl were administered in the range of 0.25–0.70 mg/70 kg at t = 2, 3, 4, and 5 hours after the start of the buprenorphine or placebo infusion. See **Table 1** for the buprenorphine and fentanyl dosing schemes. As for opioid-naive volunteers, fentanyl dose-escalation was limited when a procedure-related adverse event occurred.

Adjudication of respiratory data

The minute ventilation data collected in part A and part B were adjudicated to account for the impact of concurrent events, such as stimulation to breathe, face mask removal, urinating while the face mask was on, and severe itching. The adjudication of the data, driven by clinical observations and inspection of the raw data, was performed as follows: (A) minute ventilation data measured under respiratory stimulation during a period of respiratory arrest were set to zero (apnoea), indicating that there was no spontaneous breathing; (B) minute ventilation data measured within ± 5 minutes of the mask removal were set to missing due to artefacts in ventilation associated with the removal/placement of the mask; (C) minute ventilation data measured while the study participant was urinating while in the face mask were set to missing values over the corresponding time interval ± 5 minutes; (D) minute ventilation data measured while the study participant experienced severe itching leading to the elevation of ventilation were set to missing values over the corresponding time interval ± 5 minutes; and (E) for apnoea events lasting less than 60 seconds, minute ventilation was corrected for zero values during apnoea (weighted average). Data adjudication was performed in R software version 3.5.1.

Blood samples and fentanyl and buprenorphine assays

In parts A and B, 8 mL arterial blood samples were drawn at predefined times as specified in **Table 1** for measurement of fentanyl and buprenorphine plasma concentrations. When no fentanyl bolus was given, sampling continued at hourly intervals and again according to schedule from 360 minutes on. Plasma was separated within 30 minutes of blood collection and stored at –20°C until analysis. Plasma concentrations of buprenorphine and fentanyl were determined using 2 validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays. Briefly, human K2EDTA plasma containing the analytes and the deuterated internal standards (buprenorphine-d4 or fentanyl-d5) were extracted with methyl tert-butyl ether/hexane for buprenorphine or with methyl tert-butyl ether after the addition of sodium carbonate for fentanyl (liquid-liquid extraction). After extraction, the organic phase was dried down under nitrogen in a water bath at 40°C, reconstituted, and transferred to plastic injection vials for buprenorphine. For fentanyl, a small portion of the organic phase obtained after extraction was transferred to an autosampler vial that contained formic acid in water. The peak area of the *m/z* 468.5→414.4 buprenorphine product ion was measured against the peak area of the *m/z* 472.5→414.4 buprenorphine-d4 internal standard production. The peak area of the *m/z* 337→188 fentanyl product ion was measured against the peak area of the *m/z* 342→188 fentanyl-d5 internal standard product ion. Quantitation was performed using weighted ($1/x^2$) linear least squares regression analyses generated from calibration standards. Both assays were fully validated for linearity, selectivity, recovery, matrix effect, accuracy, precision, and stability before application to the sample analysis. The calibration range was 0.020–10.0 ng/mL for buprenorphine and 0.100–50.0 ng/mL for fentanyl. The overall accuracy and precision for quality control samples during the sample analyses were all within 5.3%. All plasma samples were analysed within the established stability window.

Statistics

PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES

The analyses were conducted in 2 stages (sequential pharmacokinetic/pharmacodynamic modelling). In a first stage, population pharmacokinetic models were fitted to buprenorphine and fentanyl plasma

concentration data. In a second stage, empirical Bayes estimates of individual pharmacokinetic parameters obtained from population pharmacokinetic modelling served as input for the pharmacokinetic/pharmacodynamic model describing the respiratory interaction of buprenorphine and fentanyl. Data analyses were performed using NONMEM, version 7.4.4 (ICON Development Solutions), a nonlinear mixed effects modelling software package. Perl-speaks-NONMEM (PsN; https://uupharmacometrics.github.io/PsN/) version 4.9.0 was used to operate NONMEM.

PHARMACOKINETIC MODEL DEVELOPMENT

Population pharmacokinetic models of buprenorphine and fentanyl were developed separately; 2- and 3-compartment models were evaluated for both drugs. Interindividual variability was estimated assuming log-normal distributions for individual pharmacokinetic parameters. When pharmacokinetic data were obtained at multiple occasions, additional random effects were included to estimate between-occasion variability. Clearances and volumes were allometrically scaled by body weight (standardized to a body weight of 70 kg), using the well-established power model and exponents of 0.75 for clearances and 1 for volumes of distribution.[25] Model selection was based on standard diagnostic plots, changes in minimum objective function value, and the robustness/precision of parameter estimates. The likelihood ratio test was applied to nested models with a nominal α-level of 0.05.

Given the small sample size (22 individuals in total) and because the purpose of analysis was to provide individual predictions for pharmacokinetic/pharmacodynamic modelling, only 1 covariate was explored, i.e., the opioid use state, to evaluate differences between studied populations. Differences between populations were tested on each pharmacokinetic parameter using an automated procedure by PsN's stepwise covariate model building utility (forward selection: $p < 0.05$; backward selection: $p < 0.001$).

PHARMACOKINETIC/PHARMACODYNAMIC MODEL DEVELOPMENT

Negative ventilation data were allowed by the model to describe long periods of apnoea and were censored at zero using M3 methodology.[26] Model estimation was performed in NONMEM using the stochastic approximation expectation-maximization (SAEM) algorithm. The importance sampling (IMP) algorithm was used to calculate the −2 log likelihood value at the final model parameter estimates and to obtain the asymptotic standard errors of estimates.

As the purpose of the present analysis was to assess the interaction of buprenorphine and fentanyl on ventilation via activation of the MOP system, receptor association/dissociation models were used and combined with biophase distribution models to account for buprenorphine and fentanyl hysteresis. The equations describing receptor association/dissociation for each molecule are as follows:[8,16]

$$d[BR]/dt = k_{ON,B} \times [B] \times [R] - k_{OFF,B} \times [BR]$$

$$d[FR]/dt = k_{ON,F} \times [F] \times [R] - k_{OFF,F} \times [FR]$$

where B, F, and R denote buprenorphine, fentanyl, and receptors, respectively; [B] and [F] denote effect-site concentrations for buprenorphine and fentanyl (i.e., concentrations in their respective effect compartment). [BR] and [FR] denote the concentrations of receptors bound to buprenorphine and fentanyl, respectively. [R] denotes the concentration of unbound receptors, and k_{ON} and k_{OFF} are association and disassociation rate constants, respectively. Because the dissociation rate constant for fentanyl ($k_{OFF,F}$) was large in previous analyses,[8] we assume that $k_{ON,F} \times [F] \times [R] - k_{OFF,F} \times [FR] = 0$, leading to $[FR] = [F] \times [R]/C_{50,F}$ with $C_{50,F} = k_{OFF,F}/k_{ON,F}$ or the fentanyl concentration at the postulated effect-site causing 50% of maximal depression of ventilation.

Assuming that the total number of receptors [R_{TOT}] is equal to the sum of drug-bound receptors and unbound receptors: $[R_{TOT}] = [R] + [BR] + [FR]$, and after normalizing [BR] and [FR] by setting $[R_{TOT}] = 1$, we obtain: $[FR] = (1 - [BR]) \times ([F]/C_{50,F}) / (1 + [F]/C_{50,F})$.

The relationship between the bound receptor concentrations and ventilation was described using a linear transduction function as follows:[8]

$$V_E = V_B \times (1 - \alpha_B \times [BR] - \alpha_F \times [FR])$$

where V_E is minute ventilation, V_B is the iso-hypercapnic baseline ventilation (i.e., prior to any drug given), and α_B and α_F are parameters for buprenorphine and fentanyl, respectively, that combine receptor reserve and intrinsic ligand activity.

Interindividual variability was estimated assuming log-normal distributions for individual pharmacokinetic/pharmacodynamic parameters. An additive model structure was tested for the residual error, with and without inclusion of interindividual variability. Overestimation of interindividual variability can occur when intraindividual stochastic noise processes are not appropriately accounted for.[27] With the present

data sets, there were 350–450 minute-ventilation measurements per individual, and those data points were most likely correlated. Hence, the standard modelling assumption that observations would be independent and normally distributed conditionally to individual-specific random effects may not be valid. Therefore, a simple Kalman filter was added to model process noise.[19,28,29] The Kalman filter has 3 components: σ = the standard deviation of the residual (intraindividual) noise, σ_γ = the standard deviation of the parallel noise (which is also intraindividual), and τ the time constant determining correlation of process noise in time. Interindividual variability and inter-occasion variability were tested on some of those parameters (σ and σ_γ).

The effect of opioid-use state was first examined based on the empirical Bayes estimates of individual random effects (η) obtained from the base model and evaluated graphically and statistically by Kruskal-Wallis rank-sum test. Significant ($p < 0.05$) relationships were further tested in NONMEM using a forward/backward selection procedure. Significance levels of 0.05 and 0.001 were used for forward and backward selection, respectively. Additional criteria for covariate selection included the pharmacological relevance of the effect and the convergence of the estimation and covariance routines.

MODEL EVALUATION

pvcVPCs were generated to ensure that the models were able to reproduce the data used for model building. Additionally, standard goodness-of-fit plots were produced, including individual and population diagnostic plots. When the Kalman filter was implemented, autocorrelation (i.e., correlation between residuals shifted by Δt) functions and cross-correlation functions (correlation between residuals and pharmacodynamic model input shifted by Δt) were plotted and inspected for model inadequacies according to Ljung (28). When the residuals are white (uncorrelated), the autocorrelation function is zero if $\Delta t > 0$; when $\Delta t = 0$, a residual has a correlation of 1 with itself. If the model fully explains the data, the cross-correlation is zero (i.e., the residuals are completely random).

SUPPORTING INFORMATION

Supplemental Tables and Figures:
https://insight.jci.org/articles/view/156973/sd/1

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CHAPTER 4

FIRST-IN-HUMAN TRIAL TO ASSESS THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF STR-324, A DUAL ENKEPHALINASE INHIBITOR FOR PAIN MANAGEMENT

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Abstract

AIM Dual enkephalinase inhibitors (DENKIS) are involved in the regulation of nociception via opioid receptors. The novel compound STR-324 belongs to the DENKI pharmacological class. This first-in-human study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of STR-324 in healthy male participants.

METHODS This was a randomised, double-blind, placebo-controlled ascending dosing study in two parts: in part 1, 30 participants received 0.004-11.475 mg h^{-1} of STR-324 or placebo (ratio 4:1) by 4 h intravenous infusion in a two-group, partial crossover design with four treatment periods separated by 1 month wash-out, and in part 2, 48 participants divided into three groups received either the active drug (1.25-11.25 mg h^{-1}) or placebo (ratio 3:1) by 48 h intravenous infusion. Safety and tolerability parameters, pharmacokinetics and pharmacodynamic effects on neurocognitive and neurophysiological tasks and on a nociceptive test battery were evaluated.

RESULTS No clinically relevant changes in safety parameters were observed. All treatment-emergent adverse events were mild and transient. The pharmacokinetics of STR-324 could not be determined due to most concentrations being below quantifiable limits. STR-324 metabolite concentrations were measurable, showing dose proportionality of C_{max} and AUC_{inf} with an estimated $t_{½}$ of 0.2-0.5 h. Significant changes in pharmacodynamic parameters were observed, but these were not consistent or dose-dependent.

CONCLUSION STR-324 displayed favourable safety and tolerability profiles at all doses up to 11.475 mg h^{-1}. Although pharmacokinetic characterisation of STR-324 was limited, dose proportionality could be assumed based on major metabolite data assayed as proxy. No clear effects on nociceptive thresholds or other pharmacodynamic measures were observed.

Introduction

Currently, the standard of care for patients who suffer from pain includes a wide range of analgesic compounds, depending on the perceived severity of the pain. In 1987, the World Health Organization (WHO) published a three-step analgesic ladder as the guideline for developing treatment plans for pain, originally intended for use in cancer pain management.[1] At the third step, for the highest severity of pain, opioids are indicated. Although the efficacy of opioids on severe acute pain has been established, emerging data showed that use of opioids for chronic noncancer pain is controversial due to the lack of evidence of the long-term efficacy in that patient population. In addition, opioids are associated with many adverse effects, including respiratory depression induced by high doses administered in an uncontrolled setting, eventually leading to hypoxaemia and death.[2] Moreover, opioids are reported to have a relatively high potential for abuse compared to other analgesic drug classes. Opioid use disorder is a major source of morbidity and mortality worldwide.[3] This class of drugs has increasingly been prescribed in recent decades, and a concurrent rise in the number of fatalities attributable to opioid misuse and overdose has been reported.[4] Therefore, there is an unmet need for highly effective analgesics with fewer associated risks, especially for the treatment of chronic pain. New drugs with less potential for abuse are preferred over classic opioid therapies, if available.

A class of opioidergic drugs that has recently gained attention is that of dual enkephalinase inhibitors (DENKIS). In healthy humans, the neural process of nociception is regulated by endomorphines including enkephalins, which play a major role in the modulation of pain. However, this natural modulation is of short duration. Enkephalins are synthesised intracellularly and stored in large synaptic vesicles, only to be released locally in response to nociceptive information. Enkephalins are present at the different levels of signal transmission from the peripheral sensory neurons through the spinal dorsal horn up to the brain. Outside the cells, enkephalins briefly interact with opioid receptors, reducing nociceptive transmission, before their action is disturbed by the metallopeptidases aminopeptidase-N (APN) and neprilysin (NEP). These two peptidases generate inactive metabolites and thus contribute

to the modulation of nociceptive signalling. No enkephalinase inhibitors are yet available for the treatment of pain. Inhibiting both APN and NEP, and thereby increasing the bioavailability of enkephalins is a new therapeutic paradigm in the management of pain. DENKIS have the capacity of inhibiting both peptidases APN and NEP. Enkephalinase inhibitors have the advantage of being active only when enkephalins are produced in response to a pain sensation.[5]

STR-324 is the pyroglutamised form of opiorphin and both penta-peptides are naturally present in humans as endogenous compounds with an opiorphin:STR-324 ratio of 70:30.[6] Like its parent molecule, STR-324 is a DENKI that targets APN and NEP.[7, 8] A schematic representation of the putative mode of action of STR-324 is presented in **Figure 1**. In preclinical studies (unpublished) a favourable safety and analgesic activity profile of STR-324 supported the administration in humans for further research.[9, 10] Preclinically, measurements of STR-324 appeared challenging but its main metabolite allowed adequate characterisation. Isolated as a compound, STR-324 has not been previously administered to humans. STR-324 is currently being developed as a therapeutic candidate for pain management. In contrast to other compounds in its drug class, STR-324 is naturally present in the human body and therefore, theoretically, has a reduced risk for toxicities of any type. The observation of an improved benefit-risk ratio compared to commonly prescribed opioids, such as morphine and fentanyl, would justify a place for STR-324 in the armamentarium for the management of pain.

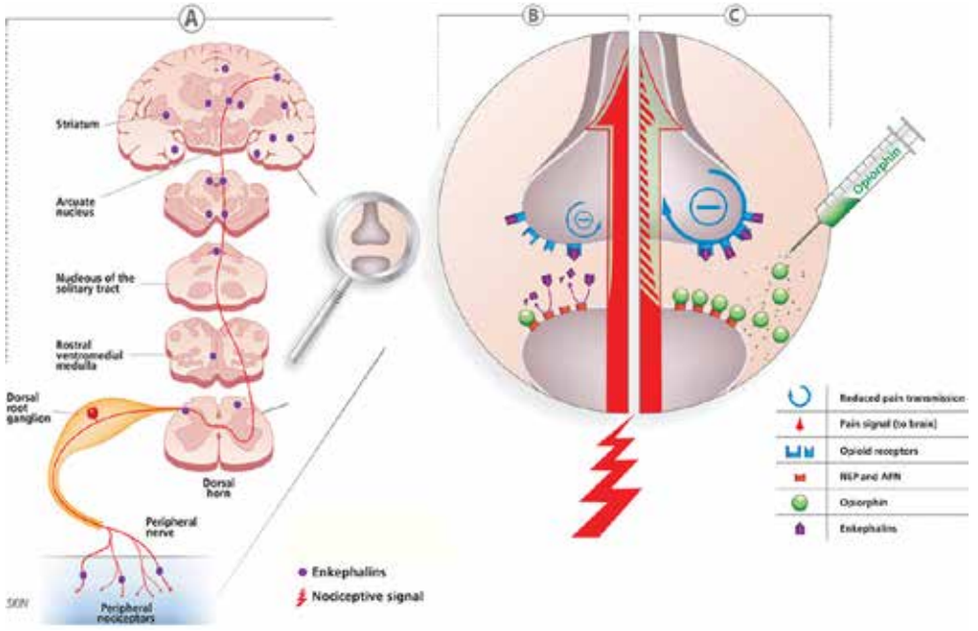
The aim of this study was to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of STR-324 intravenous infusions in healthy males.

Methods

This first-in-human, randomised, double-blind, placebo-controlled, ascending dosing study was conducted at the Centre for Human Drug Research (Leiden, the Netherlands), in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH GCP) and ethical principles as referenced in EU Directive 2001/20/EC. The protocol was approved by the Medical Review and Ethics Committee of the BEBO foundation (Assen, the Netherlands).

This trial was prospectively registered in EudraCT (number 2014-002402-21) and at toetsingonline.nl (CHDR-1725, ABR-number 63085).

FIGURE 1 Mechanism of action of opiorphin/STR-324.[9]



APN, *aminopeptidase-N;* NEP, *neprilysin*

Study participants

Healthy male volunteers aged 18-45 years, with a body mass index of 18-30 kg/m2, could be enrolled after successfully completing a medical screening. No female participants were included in this trial due to the reported hormonal influence on pain thresholds in women. Written informed consent was obtained from all participants before any study-specific procedures were performed. Key exclusion criteria were any clinically significant medical conditions, in particular any conditions that could affect sensitivity to cold and pain, any active or chronic disease or condition that could interfere with the conduct of the study, previous history of seizures or epilepsy, pain tolerance >80% on nociceptive test battery as determined at screening, history of any substance use disorder, smoking >5 cigarettes (or equivalent products) per day, alcohol consumption ⩾21 units/week and use of any medication, especially analgesics, within 14 days before dosing. Participants were required to practice effective contraception throughout the study.

Study design

This ascending dosing study consisted of two parts. Part 1 had a partial crossover, four out of five design with 30 participants divided into two equal groups following an interleaving dosing schedule with 4-week wash-out periods. Participants received escalating STR-324 doses and randomly placed placebo in four visits. Of the 15 participants per group, 12 received a placebo once and three received active drug during all visits. Part 2 had a parallel study design and included 48 participants divided into three equal groups. In this part, participants randomly received placebo or one of the three STR-324 dose levels over a 48 h infusion, randomised in a 3:1 ratio (active versus placebo). Dose levels in part 2 were selected based on tolerability in part 1 and expected analgesic activity but did not exceed the protocol-defined maximum dose of 11.475 mg h^{-1} for 24 h (based on preclinical no observed adverse effect level [NOAEL]). Administration of each new dose level was performed using a sentinel approach. An overview of the treatment groups and dose levels is presented in **Tables 1** and **2**. All doses were prepared by the pharmacy of Leiden University Medical Centre (Leiden, the Netherlands).

Safety and tolerability assessments

Safety and tolerability were assessed based on treatment-emergent (serious) adverse events (TE[S]AE's), clinical laboratory tests, vital signs, electrocardiogram (ECG) and continuous Holter monitoring and continuous oxygen saturation. TE(S)AE's were recorded from the time the participant signed consent until the follow-up visit and were classified according to MedDRA v20.1. Measurements of safety parameters were repeatedly performed throughout the study period.

Because increased levels of angiotensin II (Ang-II) have been reported in rats after a bolus injection of opiorphin,[11] Ang-II plasma levels were measured pre-dose and directly after infusion stop as additional safety measure; whole blood was collected in K2EDTA BD Vacutainer tubes and analysed by Ardena Bioanalytical Laboratory, the Netherlands, using a qualified ELISA method (EIA-ANGII, RayBiotech, Norcross, USA). The lower limit of quantification (LLOQ) was 5 pg/mL.

Pharmacokinetic assessments

To characterise the pharmacokinetics (PK) of STR-324 and its major metabolite, called STR-324M, blood samples were taken at various time points and urine was collected according to specified intervals (Appendix 1). Based on preclinical data, measurement of plasma concentrations of STR-324 was expected to be challenging. However, metabolisation occurred systemically in blood and STR-324M allowed for adequate characterisation. Therefore, it was decided to use the plasma concentrations of the inactive metabolite STR-324M as a proxy of the exposure of the parent product in humans. Blood was collected in K2EDTA BD Vacutainer tubes, immediately mixed with ethanol in a prefilled polypropylene tube and stored at ⩽–70 °C. For each urine PK collection interval, each fraction of collected urine was homogenised and a sample was taken to be handled similar to blood samples.

All PK samples were analysed by Ardena Bioanalytical Laboratory, the Netherlands, using a specific good laboratory practice (GLP) validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method compliant with the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) guidelines. Briefly, products were extracted by acidifying the received clinical samples, followed by evaporation and reconstitution. Chromatographic separation was performed on a Waters BEH300 C18 column (Waters Chromatography BV, Milford Massachusetts, USA) using gradient elution and quantification using an API 6500 + LC-MS/MS system with a turbo ion spray probe (AB Sciex, Framingham Massachusetts, USA and Shimadzu, Kyoto, Japan). Data acquisition was performed using Analyst software (version 1.6.3) from AB Sciex. Following peak area integration, regression was also performed using Analyst. Concentrations were calculated using nine-point curves with weighted linear regression. The LLOQ for the PK assay was 0.1 ng/mL for blood, 2 ng/mL for urine.

Pharmacodynamic assessments

To determine the effect of STR-324 on pain thresholds, measurements using a validated battery of nociceptive tests were performed at set times throughout the day. The nociceptive test battery was previously validated and used to show the analgesic profile of a wide variety of compounds.[12–15] At screening, a training session was performed to reduce possible learning effects, and to exclude participants conform the eligibility criteria. Assessments were performed with the participant sitting comfortably in a chair, leg raised, in a quiet room that was fitted with ambient lighting. Each participant was assigned to a separate room to minimise any distraction. During part 1 of the study, the

test battery was performed twice predose as baseline assessment and six times over 5 h postdose on each study drug administration day. In part 2 the test battery was performed twice predose and at 1, 8, 24, 48 and 56 h postdose. Nociceptive tests used in this study are listed in Supporting Information Table S2.

In part 2 a series of neurocognitive and neurophysiological measurements was also performed, using a validated test battery measuring a wide range of central nervous system (CNS) functions as described previously.[16] Additionally, pupillometry was performed using a digital camera with flash, and pupil/iris ratio was calculated as a measure of pupil size (Qpupil, Leiden University Medical Centre, Leiden, the Netherlands). A complete overview of the measurements performed is provided in the Supporting Information.

In part 2, the 49-item Addiction Research Center Inventory (ARCI)[17] and bowel function index (BFI)[18] were taken to explore the opioidergic drug effects of STR-324.

Exploratory biomarkers

Given STR-324 inhibits the action of APN and NEP, a reduction in the plasma levels of these two molecules may be measurable. In literature, Big Endothelin 1 (Big ET-1) has been reported to be an indirect biomarker for the effects of two neprilysin inhibitors[19]and was included as proxy biomarker for NEP in this study.

APN and Big ET-1 concentrations in peripheral blood were included as exploratory endpoints in part 2 only. The blood samples to measure APN were collected in sodium heparin tubes, and for Big ET-1 K2EDTA BD Vacutainer tubes were used. Both biomarkers were analysed by the Ardena Bioanalytical Laboratory using qualified ELISA methods. The LLOQS were 0.400 nmol/well for APN and 0.100 fmol/well for Big ET-1.

Statistical analysis

The primary endpoint (safety of STR-324) was descriptively reported, summarizing TEAE's by system organ class and preferred term. For the PK parameters, a noncompartmental analysis was performed. PK parameters assessed for each individual profile are shown in Tables 3 and 4. Additionally, urinary excretion (%) was calculated. The methods of analysis of pharmacokinetic and pharmacodynamic endpoints are provided in the supporting information (Appendix 3). Noncompartmental analysis and programming of PK tables and figures was conducted with R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019). All safety and statistical programming was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Since statistical testing was exploratory, statistical hypothesis testing and power calculations were not performed.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to Pharmacology, and are permanently archived in the Concise Guide to Pharmacology 2019/20.[20-22]

TABLE 1 Treatment groups and baseline characteristics (mean and SD)

	Treatment STR-324 (or matching placebo)				
	Part 1 (4h infusion)		Part 2 (48h infusion)		
Treatment Group	1	2	3	4	5
Participants, N	15	21	16	16	16
Dose level (mg h^{-1})	0.004 - 5.748	0.021 - 11.475	1.25	3.75	11.25
AGE, Y					
Mean (SD)	27.4 (6.4)	26 (6.9)	23.6 (3.1)	22.9 (2.1)	22.9 (3.0)
Range	20-40	19-45	19-32	20-27	18-29
GENDER, %					
Male	100	100	100	100	100
ETHNICITY, N (%)					
Caucasian	12 (80)	18 (85.7)	9 (75)	11 (91.7)	12 (100)
Mixed	1 (6.7)	-	1 (8.3)	1 (8.3)	-
Black/African	1 (6.7)	3 (14.3)	1 (8.3)	-	-
Other	1 (6.7)	-	1 (8.3)	-	-
HEIGHT, CM					
Mean (SD)	182.2 (7.2)	184.3 (6.6)	184.2 (5.2)	182.1 (6.3)	183.7 (6.5)
Range	169.4-193.1	172.9-199.4	176.1-197.4	169.7-194.6	171.8-202.4
WEIGHT, KG					
Mean (SD)	76.8 (11.1)	77.9 (10.3)	76.1 (9.1)	71.7 (9.8)	73.8 (8.8)
Range	56.1-93.8	59.5-96.4	58.7-95.8	58.4-93.0	60.7-91.3
BMI, KG/M^2					
Mean (SD)	23.1 (2.3)	22.9 (2.2)	22.5 (2.6)	21.6 (2.4)	21.8 (1.7)
Range	19.5-26.5	19.4-26.5	18.1-28.0	17.6-26.3	19.5-25.7

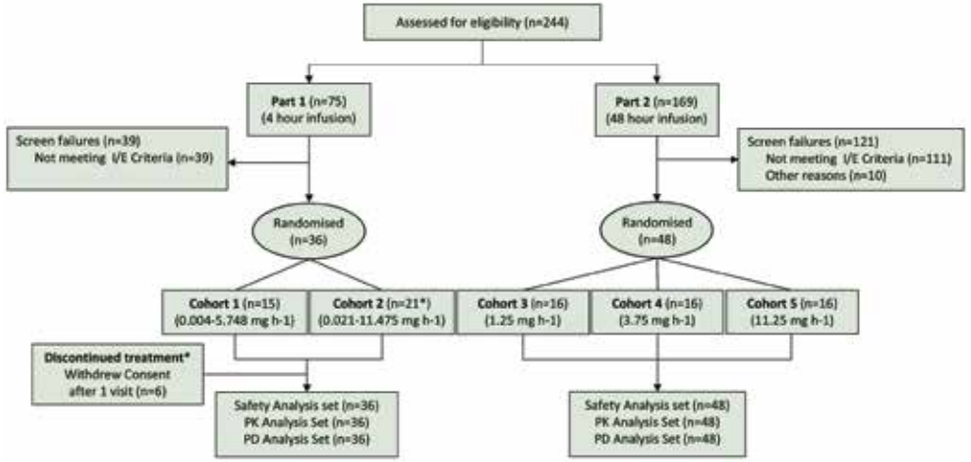
Abbreviations: BMI, body mass index; N, sample size; SD, standard deviation; y, years.

Results

Between 20 February 2018 and 07 November 2018, 36 participants were enrolled in part 1 of the study and 48 participants in part 2. Due to the premature discontinuation of six participants in part 1, replacement participants were enrolled to complete data for all study visits (Figure 2). Demographics and other baseline characteristics are presented in Table 1.

FIGURE 2 Study flow diagram.

**Four initially randomised participants withdrew consent after one visit due to personal reasons. Replacement participants completed the remaining visits, of whom one withdrew consent after one treatment period and a second replacement participant was used. In addition, this replacement participant withdrew consent after one treatment period and a third replacement participant was used.*



Safety and tolerability

An overview of TEAE's that were assessed as at least possibly related to treatment can be found in Table 2. Two serious adverse events (SAE's) occurred postdose but were not considered related to STR-324 administration: acute appendicitis in part 1 and testicular torsion in part 2. No participants withdrew from the study due to adverse effects. Headache, fatigue and somnolence were frequently reported in all STR-324 dose groups, but no dose-dependent increase in adverse events and no evident differences compared to placebo were observed. In part 2 there were five mild adverse events recorded related to the infusion site versus zero in the placebo group. However, no dose-dependent increase in events was observed. Additionally, the events did not reoccur after infusion cannula replacement. Most adverse events were mild in severity. Seven moderate adverse events were reported, in part 1 only. All moderate and severe adverse events were classified as being unlikely or not related to the study drug and were reported in both STR-324 and placebo groups. No clinically relevant changes in blood chemistry, haematology, urinalysis, Ang-II plasma levels, vital signs or ECG tests were identified.

TABLE 2 Summary of treatment-emergent adverse events possibly or probably related to treatment in both study parts (by MedDRA preferred term).

	Part 1 (4h infusion)																	
Dose Level (mg h^{-1})	**0.004**		**0.021**		**0.106**		**0.319**		**0.956**		**2.869**		**5.748**		**11.475**		**Placebo**	
Participants, N	**12**		**12**		**12**		**12**		**12**		**12**		**12**		**12**		**24**	
Treatment relationship	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**
TEAE, N																		
Fatigue	-	-	-	1	-	1	-	-	-	1	-	1	-	-	-	1	-	-
Dizziness	-	1	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	3
Headache	-	1	-	1	-	2	-	-	-	1	-	1	-	2	-	-	-	2
Somnolence	-	-	-	3	-	-	-	-	-	-	-	1	-	1	-	3	-	4
Nausea	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-
Non-cardiac chest pain	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-

	Part 2 (48h infusion)							
Dose Level (mg h^{-1})	**1.25**		**3.75**		**11.25**		**Placebo**	
Participants, N	**12**		**12**		**12**		**12**	
Treatment relationship	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**	**PROB**	**POS**
TEAE, N								
Fatigue	-	-	-	1	-		-	-
Dizziness	-	-	-	2	-	1	-	1
Headache	-	-	-	3	-	1	-	1
Somnolence	-	-	-	-	-	-	-	2
Nausea	-	1	-	-	-	-	-	-
Diarrhoea	-	1	-	-	-	-	-	-
Palpitations	-		-	-	-	-	-	1
Chest pain	-	1	-	-	-	-	-	-
Feeling abnormal	-	1	-	-	-	-	-	-
Infusion site pruritus	-	1	-	-	-	-	-	-
Neck pain	-	1	-	-	-	-	-	-

MedDRA, Medical Dictionary for Regulatory Activities; N, sample size; POS, Possibly; PROB, Probably; TEAE, treatments-emergent adverse event.

Pharmacokinetics

Following intravenous administration of STR-324, plasma concentration-time profiles were characterised. STR-324 plasma concentrations were below the limit of quantification (BLQ) for most dose levels during the 4 and 48 h infusion periods. Due to the paucity of results, it was not considered appropriate to undertake a full toxicokinetic interpretation of these data except for the highest dose level in part 1. A summary of C_{max}, t_{max} and AUC_{last} for STR-324 is provided in **Table 3**.

TABLE 3 Summary of STR-324 pharmacokinetics.

	Dose level (mg h^{-1})	N	Mean	SD	CV	GeoMean	GeoCV	Median	Min	Max
Part 1 (4h infusion)	**2.869**									
	C_{max}, ng/ml	12	11.94	41.27	345.74	1.32	377198.74	0	0	143
	t_{max}, h	3	3.4	1.21	35.66	3.23	43.29	4.08	2	4.12
	AUC_{last}, ng*h/ml	12	12.75	44.09	345.95	0.89	3006866.09	0	0	152.76
	5.748									
	C_{max}, ng/ml	12	0.07	0.08	109.74	0.14	25.62	0.05	0	0.19
	t_{max}, h	6	3.69	0.83	22.43	3.58	29.14	4	2	4.08
	AUC_{last}, ng*h/ml	12	0.09	0.12	131.88	0.14	110.79	0.01	0	0.28
	11.475									
	C_{max}, ng/ml	12	0.2	0.04	18.9	0.2	19.5	0.2	0.15	0.26
	t_{max}, h	12	3.08	1.23	39.87	2.81	48.93	4	1.5	4.08
	AUC_{last}, ng*h/ml	12	0.59	0.22	36.67	0.55	43.39	0.62	0.28	0.93
Part 2 (48h infusion)	**3.75**									
	C_{max}, ng/ml	12	0.06	0.07	125.78	0.14	16.57	0	0	0.16
	t_{max}, h	5	21.01	18.14	86.36	11.73	300.34	24	1	48.02
	AUC_{last}, ng*h/ml	12	1.37	2.2	160.13	2.6	94.35	0	0	6.13
	11.25									
	C_{max}, ng/ml	12	0.21	0.08	38.38	0.23	21.45	0.22	0	0.32
	t_{max}, h	11	35.33	18.99	53.76	26.8	118.4	48.02	4.22	48.2
	AUC_{last}, ng*h/ml	12	8.62	3.51	40.77	9.17	23.34	8.53	0	14.58

$AUC_{o\text{-}last}$, Area under the concentration-time curve from time o to last quantifiable concentration; C_{max}, maximum concentration; CV, coefficient of variation; GeoCV, geometric mean coefficient of variation; GeoMean, geometric mean; h, hour; N, sample size; SD, Standard Deviation. t_{max}, time to reach C_{max}.

In contrast, plasma concentrations for the metabolite STR-324M were measurable for all dose levels with the exception for dose level 1 in part 1 where concentrations were BLQ. PK profiles of STR-324M for all dose levels are presented in **Figure 3**. Plasma concentrations of the metabolite STR-324M increased immediately after the infusion started and decreased rapidly after the end with a bi-exponential decline. A summary of the PK parameters is shown in **Table 4**. Terminal half-life and half-life related parameters could not be determined for the first four dose levels of part 1, as only the first part of the bi-exponential decay was above the LLOQ. Infusion rate normalised C_{max} and dose normalised AUC_{last} for STR-324M were equal across dose levels (indicative for a dose proportional PK). All observations for STR-324 in urine were BLQ except at the highest dose in either study part. Excretion of the metabolite into urine ranged from 12.5% to 17.7% in part 1 (dose levels 0.004 to 11.475 mg h–1, respectively), which is in line with the dose-linear exposure and stable elimination constant. Similarly, excretion of the metabolite into urine ranged from 16.2% to 16.5% in the 1.25 to 11.25 mg h^{-1} dose range in part 2.

Pharmacodynamics

No consistent, dose-dependent pharmacodynamic effects related to STR-324 could be observed in this study. Significant differences from placebo in multiple parameters were observed at varying dose levels for the cold pressor, electrical burst, pressure pain and conditioned pain modulation response in both study parts. Results of the nociceptive tests are shown in the Supporting Information.

Of the other pharmacodynamic results measured, the following effects are noteworthy: a significant decrease in pupil/iris ratio (miosis) was observed during the 48 h infusions at all three doses in part 2 for both the right and left eyes (overall treatment effect $P < .05$) when compared to placebo. The largest decrease was measured at the lowest dose in both the right (–0.04272 [95% confidence interval [CI] –0.06099, –0.02444, $P < .05$]) and left eyes (–0.04278 [95% CI –0.06544, –0.02012, $P < .05$]). Example changes from baseline graphs are shown in **Figure 4**. Compared to placebo, a significant increase in APN activity of 57.7% (95% CI 1.5-113.8, $P < .05$) was observed at the highest dose of STR-324 in part 2. The increase was observed during the first 24 hours of dosing only.

FIGURE 3 Means with standard deviations of concentration-time profiles of STR-324M in plasma, per treatment (part 1 left, part 2 right).

The last point in the graph reflects the last measurable concentration after discontinuation of the infusion.

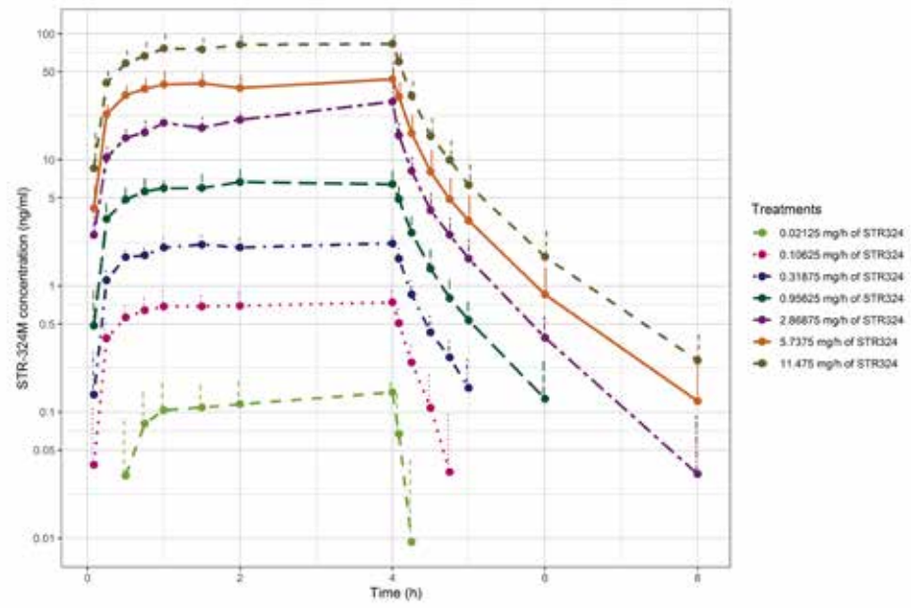


TABLE 4 Summary of STR-324M pharmacokinetics.

	Dose level (mg h^{-1})	N	Mean	SD	CV	GeoMean	GeoCV	Median	Min	Max
Part 1 (4h infusion)	**0.956**									
	C_{max}, ng/ml	11	7.01	1.58	22.57	6.85	22.29	6.93	5.25	9.54
	t_{max}, h	11	2.32	1.15	49.44	2.08	52.93	2	1	4
	AUC_{last}, ng*h/ml	11	25.64	6.24	24.33	24.97	24.35	24.74	18.15	35.61
	AUC_{inf}, ng*h/ml	11	25.81	6.28	24.33	25.14	24.31	24.87	18.25	35.85
	$t_{½}$, h	11	0.49	0.13	26.76	0.48	27.42	0.5	0.31	0.77
	CL, L/h	11	99.92	23.23	23.25	97.4	24.31	98.46	68.29	134.12
	2.869									
	C_{max}, ng/ml	12	30.35	29.31	96.57	25.12	55.43	22.9	16.8	123
	t_{max}, h	12	3.1	1.18	38	2.84	49.74	4	1	4.12
	AUC_{last}, ng*h/ml	12	89.42	32.45	36.29	85.65	29.09	85.17	54.46	187.25
	AUC_{inf}, ng*h/ml	12	89.6	32.48	36.25	85.83	29.08	85.31	54.55	187.49
	$t_{½}$, h	12	0.5	0.1	20.54	0.49	19.63	0.47	0.39	0.68
	CL, L/h	12	88.43	21.67	24.51	85.58	29.08	86.1	39.17	134.65
	5.748									
	C_{max}, ng/ml	12	45.01	9.69	21.54	44.02	22.6	46.6	31.6	58.3
	t_{max}, h	12	2.88	1.4	48.72	2.49	66.34	4	1	4.02
	AUC_{last}, ng*h/ml	12	163.34	33.82	20.7	160.11	21.23	166.25	116.3	221.89
	AUC_{inf}, ng*h/ml	12	163.55	33.88	20.72	160.31	21.24	166.53	116.37	222.16
	$t_{½}$, h	12	0.56	0.13	22.93	0.55	24.66	0.62	0.37	0.7
	CL, L/h	12	93.51	19.75	21.12	91.63	21.24	88.23	66.12	126.23
	11.475									
	C_{max}, ng/ml	12	87.47	15.21	17.39	86.33	16.78	82.75	70	117
	t_{max}, h	12	3.04	1.21	39.93	2.76	52.5	4	1	4
	AUC_{last}, ng*h/ml	12	323.39	56.71	17.54	318.95	17.44	306.29	233.19	423.13
	AUC_{inf}, ng*h/ml	12	323.68	56.77	17.54	319.23	17.44	306.43	233.68	423.68
	$t_{½}$	12	0.64	0.08	12.76	0.64	12.32	0.63	0.56	0.81
	CL, L/h	12	93.23	16.07	17.23	91.95	17.58	95.89	69.34	125.73

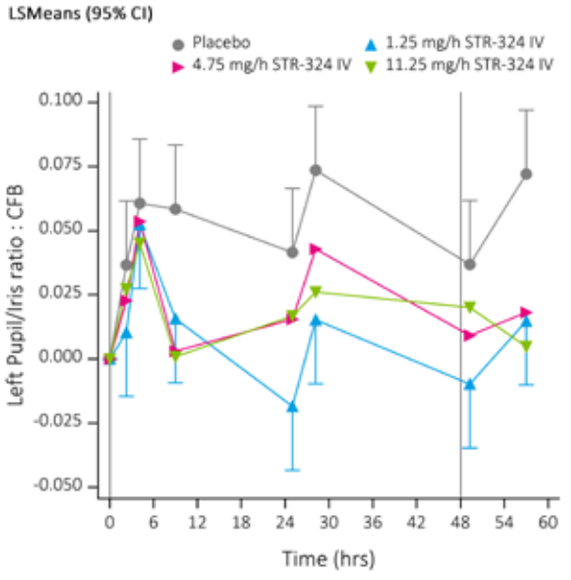
CONTINUATION TABLE 4

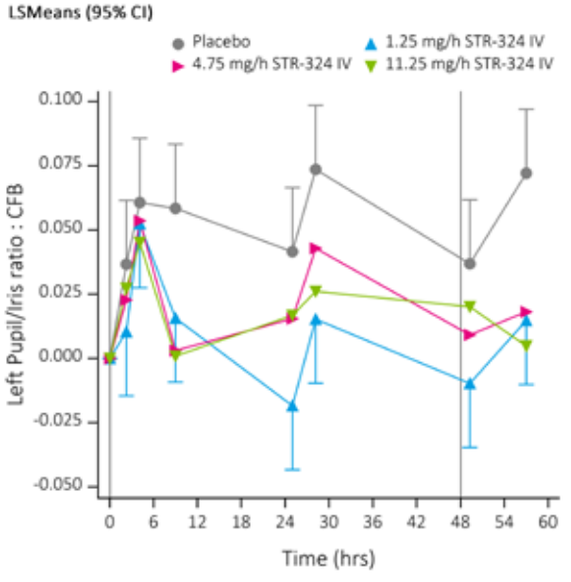
	Dose level (mg h^{-1})	N	Mean	SD	CV	GeoMean	GeoCV	Median	Min	Max
Part 2 (48h infusion)	**1.25**									
	C_{max}, ng/ml	12	10.23	1.78	17.41	10.1	15.84	9.66	8.27	14.9
	t_{max}, h	12	18.35	15.57	84.9	11.16	169.33	16	2	48.08
	AUC_{last}, ng*h/ml	12	435.76	89.31	20.5	428.62	18.46	414.79	357.04	665.63
	AUC_{inf}, ng*h/ml	12	435.93	89.34	20.49	428.79	18.46	415	357.17	665.83
	$t_{½}$, h	12	0.49	0.09	18.2	0.48	19.91	0.49	0.3	0.6
	CL, L/h	12	90.85	14.94	16.44	89.57	18.46	92.62	57.68	107.53
	3.75									
	C_{max}, ng/ml	12	35.56	7.61	21.39	34.79	22.37	34.95	23.1	45.8
	t_{max}, h	12	16.67	16.05	96.28	8.56	242.03	8.01	1	48.02
	AUC_{last}, ng*h/ml	12	1443.61	352.5	24.42	1400.16	27.26	1383.86	739.52	2031.29
	AUC_{inf}, ng*h/ml	12	1443.85	352.56	24.42	1400.4	27.26	1384	739.63	2031.64
	$t_{½}$, h	12	0.68	0.18	26.19	0.66	28.63	0.72	0.44	0.93
	CL, L/h	12	85.24	25.96	30.45	82.27	27.26	83.45	56.71	155.77
	11.25									
	C_{max}, ng/ml	12	86.96	13.68	15.73	86.02	15.27	81.55	71.4	115
	t_{max}, h	12	8.77	9.65	109.98	4.93	177.96	8	1	32
	AUC_{last}, ng*h/ml	12	3609.86	661.29	18.32	3556.46	18.04	3535.46	2861.62	4828.51
	AUC_{inf}, ng*h/ml	12	3610.45	661.47	18.32	3557.03	18.05	3536.08	2862.14	4829.5
	$t_{½}$, h	12	0.76	0.08	10.13	0.76	9.54	0.75	0.67	0.96
	CL, L/h	12	98.58	17.12	17.37	97.17	18.05	98.09	71.57	120.76

$AUC_{0\text{-}last}$, Area under the concentration-time curve from time 0 to last quantifiable concentration; $AUC_{0\text{–}inf}$, AUC from time 0 to infinity; CL, clearance; C_{max}, maximum concentration; CV, coefficient of variation; GeoCV, geometric mean coefficient of variation; GeoMean, geometric mean; h, hour; N, sample size; SD, Standard Deviation t_{max} time to reach C_{max}; $t_{½}$, terminal half-life.

FIGURE 4 Least square means (LSMeans) change from baseline (CFB) in pupil/iris ratio of the right eye in part 2.

The 95% confidence interval (CI) is shown for the outer lines in each graph. Vertical grey lines indicate the start and stop of 48 h infusion.





Discussion

In this study, we combined a typical first-in-human study design in healthy volunteers with extensive nociceptive and CNS testing, with the aim to not only determine the safety, tolerability and pharmacokinetic profile of STR-324 but to also obtain an impression of its analgesic and opioid-like (adverse) effects potential.

The presented safety data indicates that STR-324 has a favourable safety and tolerability profile in healthy males at all doses up to 11.475 mg h^{-1} for 48 h of infusion. Although some adverse effects were recorded as being possibly related to the study drug, the absence of dose-dependency and of evident differences with participants who received placebo reject any relatedness to STR-324. Although several participants prematurely discontinued participation, none withdrew consent as a result of events related to the study drug. As expected from preclinical data, measuring plasma concentrations of STR-324 was challenging and limited its pharmacokinetic characterisation. STR-324M, its main metabolite, therefore served as a proxy of the plasma concentrations and exposure of STR-324. No significant, dose-dependent analgesic or other pharmacodynamic effects related to STR-324 could be observed in this study except for the paradoxical increase in APN at the higher dose in part 2.

Based on the sparse concentrations measured in this study, we can confirm that STR-324 is a short-lived compound that is quickly metabolised and distributes outside the blood compartment almost immediately after administration. This is in line with preclinical findings, where the parent compound provided analgesia only during a short period of time when given by bolus opiorphin[23] and proved highly unstable after administration by intravenous infusion. This instability hampered the pharmacokinetic characterization of STR-324 in animals, in contrast to its metabolite. In this study, PK profiles of STR-324 and its main, inactive metabolite were characterised in plasma and urine. We anticipated the challenge of measuring plasma concentrations of STR-324 by using the metabolite as a proxy to get an impression of the PK profile of the parent compound and to support dose escalations. The metabolite could be consistently measured throughout the infusion periods and dose proportionality was observed. Similarity between profiles was confirmed by visual inspection of available individual plots of the parent. Therefore, the notion that the PK of STR-324 could be monitored appropriately and also showed dose proportionality (albeit only briefly available in plasma) was assumed to be justified.

Several DENKI's are currently being developed for the treatment of pain. This is the first published report of a study that aimed to demonstrate the analgesic effects of a DENKI with use of evoked pain models in humans. In response to a painful stimulus, enkephalins are released into the synaptic cleft and interact with opioid receptors for a brief period before being rapidly metabolised, which results in a short half-life of approximately 12 minutes.[5,24,25] The effect of DENKI's can be expected to occur shortly after a painful stimulus is applied, as it will only be active as long as enkephalins are released. As no dose-dependent effects were observed in any of the evoked pain tests performed, the observed effects are unlikely to be due to the pharmacological effects of the compound and are most likely due to chance. No alpha correction for multiple testing was performed in this study. In preclinical studies, STR-324 showed strong analgesic activity in murine models simulating postoperative pain and neuropathic pain, but also without dose dependency. The highest dose level in this study was approximately 20-fold higher than the human equivalent of the lowest pharmacological active dose in murine models, suggesting that the lack of observed effect is not due to insufficient exposure. It is important to note that the parent compound could also not be measured in animal models, and the comparison is therefore based on allometric scaling and not on actual pharmacokinetic data. Because of the short action of enkephalins, a longer time of exposure to the study drug at the dose levels explored in this study is not expected to yield different results.

The fact that no consistent analgesic effect was observed in this proof-of-concept study might be due to limited involvement of enkephalinases in the acute nociceptive pain models that were used. Possibly, an effect of a DENKI only becomes clinically measurable after acute or prolonged exposure to a nociceptive stimulus or can be best demonstrated in evoked pain models simulating neuropathic pain. It could also be hypothesised that pain intensity may modulate the quantity of released enkephalins, thus modulating the extent of efficacy of DENKI's; human-evoked pain models possibly do not allow sufficient pain intensity to be reached to model real-world painful stimuli that activate a measurable enkephalin response.

Decrease of the pupil/iris ratio (miosis) is a well-known opioidergic effect. In this study a statistically significant treatment effect was observed compared to placebo, although the effect was greatest at the lowest dose. The treatment effect is the result of an increase in the pupil/iris ratio (mydriasis) in the placebo group, while the ratio remained

quite stable in all three (active) treatment groups during infusion. The observed decrease in pupil/iris ratio in this study (<10% ratio relative reduction) was minor compared to the miosis induced by opioids such as buprenorphine that show a decrease in ratio of up to 50%.[26] Although it is possible that the relative static pupils were a pharmacodynamic effect of the compound, the observed treatment effect was most likely a result of unexpected placebo group behaviour, not a true opioidergic effect. An increase in pupil size (mydriasis) could hypothetically be caused by other factors, such as nociceptive tasks or circadian rhythm. However, pupillometry measurements were not (immediately) preceded by nociceptive tests.

Several parameters of the CNS test battery showed a statistically significant effect. Although pharmacological effects cannot be excluded, this is unlikely due to inconsistency of the effects regarding time of measurement and dose level.

We hypothesised that, given the expected mode of action and kinetics of the enkephalin degradation, administration of STR-324 would lead to a decrease in activity of APN and BigET-1 during and shortly after infusion. However, no changes in BigET-1 were observed and an unexpected increase in activity of APN at the highest tested dose was measured. It is possible that STR-324-induced modification of the levels of APN and NEP may be only measured locally instead of systemically, and should therefore be measured in a different compartment than blood. Although the pharmacokinetic profile in this study supports the hypothesis that STR-324 remains in the plasma in its active form at least temporarily, preclinical data demonstrated that STR-324 and opiorphin distribute to other compartments within seconds after administration, which may also explain the absence of measurable clinical changes related to other biological interactions that APN and NEP have. Alternatively, variability might have been too large with respect to a limited effect size. It is important to emphasise the exploratory nature of these biomarkers and the (pre)clinically unconfirmed relation with the mode of action of STR-324. In addition, the analytical methods were qualified but not validated thus there is no absolute certainty regarding the accuracy of the obtained results for these exploratory biomarkers.

Because intrasubject variability of evoked pain models is lower than intersubject variability, a partial crossover design was used where each participant could serve as his own control while keeping the ascending dose fashion of a typical first-in-human study. This approach benefits the efficient assessment of the effects of a novel compound despite limitations on study size. Although the number of included participants is common for phase I first-in-human studies and despite the innovative study design, smaller analgesic effects possibly remain undetected due to group sizes. Given that only male participants were included in this trial, this limits generalisability.

Finally, the evoked pain models that were included can profile a drug to a certain extent, but not all types of pain were evaluated. A phase IIa proof-of-concept study on postoperative pain to confirm the analgesic potential of STR-324 is ongoing.

In conclusion, this study suggests that STR-324 was observed to be generally well tolerated at all dose levels and infusion regimens tested in this healthy male population. Pharmacokinetic profiling of STR-324 was limited. However, dose proportionality can be assumed based on metabolite data. No clear dose-dependent effects were observed on the nociceptive thresholds or on tests related to CNS or bowel functioning.

SUPPORTING INFORMATION

APPENDIX 1

Supplementary Table 1: Pharmacokinetic sampling time points:
https://bpspubs.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fbcp.14931&file=bcp14931-sup-0001-Table+S1.docx

APPENDIX 2

Supplementary Table 2: PainCart evoked pain model results (estimates of the difference compared to placebo including 95% confidence interval:
https://bpspubs.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fbcp.14931&file=bcp14931-sup-0002-Table+S2.docx

APPENDIX 3

Methods of Analysis of Pharmacokinetic and Pharmacodynamic Measurements:
https://bpspubs.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fbcp.14931&file=bcp14931-sup-0003-Data+S1.docx

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CHAPTER 5

NEUROCOGNITIVE EFFECT OF BIASED μ-OPIOID RECEPTOR AGONIST OLICERIDINE, A UTILITY FUNCTION ANALYSIS AND COMPARISON WITH MORPHINE

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Abstract

BACKGROUND Oliceridine (Olinvyk) is a μ-opioid receptor agonist that in contrast to conventional opioids preferentially engages the G-protein–coupled signalling pathway. This study was designed to determine the utility function of oliceridine versus morphine based on neurocognitive tests and cold pressor test.

METHODS The study had a randomized, double-blind, placebo-controlled, partial block three-way crossover design. Experiments were performed in 20 male and female volunteers. The subjects received intravenous oliceridine (1 or 3 mg; cohorts of 10 subjects/dose), morphine (5 or 10 mg; cohorts of 10 subjects/dose), or placebo on three separate occasions. Before and after dosing, neurocognitive tests, cold pressor test, and plasma drug concentrations were obtained at regular intervals. Population pharmacokinetic–pharmacodynamic analyses served as the basis for construction of a utility function, which is an objective function of probability of benefit minus probability of harm. Antinociception served as the measure of benefit, and slowing of saccadic peak velocity and increased body sway as the measures of neurocognitive harm.

RESULTS The oliceridine and morphine C_{50} values, i.e., the effect-site concentrations causing 50% effect, were as follows: antinociception, 13±2 and 23±7 ng/ml; saccadic peak velocity, 90±14 and 54±15 ng/ml; and body sway, 10±2 and 5.6±0.8 ng/ml, respectively. The ratio oliceridine/morphine of the therapeutic indices, C_{50}(benefit) /C_{50}(harm), were 0.34 (95% CI, 0.17 to 0.7; $p < 0.01$) for saccadic peak velocity and 0.33 (0.16 to 0.50; $p < 0.01$) for body sway. The oliceridine utility was positive across the effect-site concentration 5 to 77 ng/ml, indicative of a greater probability of benefit than harm. The morphine utility was not significantly different from 0 from 0 to 100 ng/ml. Over the concentration range 15 to 50 ng/ml, the oliceridine utility was superior to that of morphine ($p < 0.01$). Similar observations were made for body sway.

CONCLUSIONS These data indicate that over the clinical concentration range, oliceridine is an analgesic with a favourable safety profile over morphine when considering analgesia and neurocognitive function.

Introduction

Although μ-opioid receptor agonists appear to have the same molecular site of action, i.e., the μ-opioid receptor,[1,2] evidence is accumulating that large differences exist among μ-opioids in their efficacy and adverse effects profile.[3,4] These variations can be attributed to multiple factors, including pharmacokinetics, receptor kinetics, intraneuronal translation pathways, and pharmacodynamics.[3,4] Recently, a new class of opioid was discovered, biased toward G-protein intraneural activation,[5,6] of which the opioid oliceridine (Olinvyk) was approved in the United States for use in adults in the management of acute pain severe enough to require an intravenous opioid analgesic and for whom alternative treatments are inadequate. We earlier showed in young and older volunteer populations that oliceridine has advantages over morphine with respect to respiratory depression.[7,8] For example, in young volunteers, we demonstrated that the oliceridine utility function is superior to that of morphine.[7] The utility function, U_1, is defined by the probability of benefit (i.e., antinociception) minus the probability of harm (e.g., respiratory depression) or $U_1 = P(B) - P(H)$.[7,9] While oliceridine utility was positive over the clinical concentration range, morphine utility was negative and significantly different from oliceridine.[7] These data indicate that oliceridine has a greater likelihood for pain relief than respiratory depression, while the reverse is true for morphine. This marked difference may be due to a specific postreceptor engagement by oliceridine, which shows preferential (i.e., biased) postreceptor activation of G-protein signalling and reduced β-arrestin recruitment and receptor internalization, unlike morphine.[5,6]

In addition to respiratory depression, opioids induce psychomotor effects such as sedation and motor effects affecting balance, which may also be undesirable. These neurocognitive effects have been associated with the inability to mobilize or a high likelihood of falling, memory loss, and confusion, and in the elderly, delirium and possibly progression of already existing cognitive impairment.[10–15]

This study aimed to compare the neurocognitive impact of oliceridine versus morphine using a validated neurocognitive test battery in a population of healthy male and female volunteers.[16] Utility functions were constructed based on pharmacokinetic and pharmacodynamic modelling and derived from two measures of neurocognitive function: saccadic peak velocity and body sway, and antinociception as measured

by the cold pressor test. We chose saccadic peak velocity as a surrogate biomarker of sedation and body sway as a surrogate for balance or motor stability.[16–18] The hypothesis is that oliceridine has superior utility compared to morphine in these pharmacodynamic domains. A description of the complete neurocognitive data set will be reported elsewhere.

Materials and Methods

Ethics and Registration

The ethics committee BEBO (Stichting Beoordeling Ethiek Biomedisch Onderzoek, Assen, The Netherlands) and the national competent authority, the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands), approved the protocol. All study procedures were conducted in accordance with the principles of the declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent before any assessments. The study was conducted from February 4, 2022, to June 10, 2022, and was registered at the World Health Organization International Clinical Trials Registry Platform (https://trialsearch.who.int/) on June 2, 2021, under identifier ISRCTN13308001 with Geert Jan Groeneveld as principal investigator.

Subjects

Twenty subjects, both male and female, were enrolled in the study after an initial screening visit. The main inclusion criteria were age (18 to 55 yr, inclusive), body mass index (18 to 32 kg/m^2, inclusive), absence of a current or history of any medical or psychiatric disease, and use of any illicit or prescribed drugs. Subjects who were identified as poor metabolizers of CYP450 2D6 substrates through genotyping at screening were excluded (as such phenotypes are associated with poor oliceridine metabolism and consequently high oliceridine concentrations),[8] as were subjects with a known medical condition affecting sensitivity to cold and those who indicated the pain test to be intolerable or achieved pain tolerance at more than 80% of maximum input intensity for the cold pressor pain at screening. The complete list of inclusion and exclusion criteria is given in Supplemental Digital Content 1.

Study Design and Treatment

The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, The Netherlands, and had a randomized, double-blind, placebo-controlled, dose-ranging, partial block three-way crossover design. All participants were tested on three separate days and randomly assigned to receive one of five treatments on each study day, excluding any previously received treatment. A total of 20 unique sequences (of 60 possible unique sequences) were used with treatments: placebo (5% dextrose), 1 mg oliceridine, 3 mg oliceridine, 5 mg morphine, or 10 mg morphine. All treatments were dissolved in 5% dextrose. The administration of each treatment occurred more than 60 s through an intravenous access line in a volume of 3 ml. Oliceridine was obtained from Trevena Inc. (USA); morphine and 5% dextrose were obtained from the local accredited pharmacy (Leiden University Medical Centre). Consequently, each unique treatment was given to 12 subjects, and each drug combination occurred six times, but in different sequences. There were at minimum 7-day washout periods in between the dosing days.

The oliceridine and morphine doses were chosen as they were considered approximately equianalgesic assuming a 1:5 potency ratio oliceridine:morphine of Olinvyk[19] and were anticipated to cause measurable neurocognitive effects with manageable other adverse effects.[7,8] Clinically, oliceridine doses of 1 to 2 mg are given intravenously with additional bolus doses of 1 to 3 mg every 1 to 3 h as needed.[7] According to the label, higher single oliceridine doses are not recommended. The morphine doses were based on clinical use, with 10 mg morphine considered the highest dose manageable in an outpatient study with healthy volunteers.

Randomization and Blinding

After successful screening, each subject was assigned a unique subject number. The treatment assignments were randomized using a computer-generated randomization list created in SAS 9.4. The randomization code was shared exclusively with the pharmacy of the Leiden University Medical Centre and remained confidential until the study was completed and the data lock was lifted. Emergency envelopes containing unblinding information were prepared as a precautionary measure to ensure subject safety, but they were not utilized during the study. On dosing days, the pharmacy provided the investigators the appropriate study medication in masked syringes.

Measurements

Venous blood samples of 4 ml each were collected from a large vein in the arm opposite to the arm of drug infusion. The samples were

obtained at specific timepoints, including predose and postdose at 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360, and 720 min. A window of ±2 min was applied for samples obtained in the first 60 min, and ±5 min for subsequent samples. The collected blood samples were shipped to Labcorp Bioanalytical Services (USA), where the quantitation of oliceridine, morphine, and morphine-6-glucuronide plasma concentrations was performed using validated high-performance liquid chromatography with tandem mass spectrometry bioanalytical assays.[7,8]

Additionally, all subjects underwent repeated neurocognitive testing using the validated NeuroCart test battery.[16] This battery consists of a range of tests that assess central nervous system functioning, which are administered sequentially in a fixed sequence; we have demonstrated previously that the tests do not influence each other and have negligible learning effects. The assessment of antinociception was also conducted using the cold pressor test. The primary endpoint of the study was *a priori* designated as the drug effect on one specific neurocognitive test: saccadic eye movement peak velocity. Secondary endpoints included anterior–posterior body sway and the pain detection tolerance threshold. The current analysis performed at Leiden University Medical Center (by E.O. and A.D.) aimed to construct utility functions using these three endpoints.

During the screening visit, all subjects were familiarized with the saccadic peak velocity test.[16–18] On study days, the test was performed twice predose and postdose (with dosing at t = 0 min) at 30, 60, 120, 180, 240, 300, and 360 min. The eye movement recordings took place in a quiet room with dimmed lighting. Disposable silver–silver chloride electrodes were placed on the subject's forehead and beside the lateral canthi of both eyes for registration of the electro-oculographic signals. The skin resistance was reduced to less than 5 kΩ before measurements to ensure adequate readings. Head movements were restricted using a fixed head support. The subject was requested to track a moving dot displayed on a computer screen. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15º in both directions. Fifteen saccades were recorded, with random interstimulus intervals varying from 3 to 6 s. The average values of saccadic peak velocity from all correctly executed saccades were used as input to the pharmacodynamic model.

Body sway was assessed using a body sway meter while subjects had their eyes closed and were wearing comfortable low-heeled shoes.[16–18] The body sway meter, a pot string meter from Celesco (Intertechnology Inc., Canada) permits measurement of body movements in a single plane and provides a measure of postural stability via a string attached to the subject's waist. Before starting a measurement, subjects were asked to stand still with their feet approximately 10 cm apart and their hands in a relaxed position alongside their body. All body movements over a 2-min period were integrated and expressed as mm of sway. Measurements were obtained before dosing and at the specific time points after dosing (t = 0 min): 50, 80, 140, 200, 260, 320, and 380 min.

Nociceptive tolerance thresholds were measured using the cold pressor pain test.[7,20,21] During screening, a training session was conducted to exclude subjects who found the pain test intolerable or reached tolerance at more than 80% of the maximum input intensity, with a cut-off time of 120 s. During the test, the subjects placed their nondominant hand into a warm-water bath at 35 ± 0.5°C for 120 s. After 105 s, a blood pressure cuff on the upper arm was inflated to a pressure of 20 mmHg below their resting diastolic blood pressure, limiting compensatory blood flow without causing pain. At 120 s, the subject transferred their hand from the warm-water bath to a cold-water bath of similar size, with circulating water, maintained at a temperature of 1.0 ± 0.5°C. Subjects were instructed to indicate when they reached their pain tolerance, by moving the slider of an electronic visual analogue scale to the rightmost position. Alternatively, if the limit of 120 s was reached, before reaching pain tolerance, the study ended. Either way, when the subjects removed their hand from the water, the blood pressure cuff deflated. Measurements were obtained before dosing and at specific time points after dosing (t = 0 min): 20, 90, 150, 210, 270, 330, and 390 min.

Pharmacokinetic–Pharmacodynamic Data Analysis and Construction of Utility Functions

We used NONMEM 7.5.1 (ICON plc., USA) to describe the population pharmacokinetics and pharmacodynamics of oliceridine and morphine[7]; for specifics, see Supplemental Digital Content. In brief, the analysis had two stages. In the first stage, the pharmacokinetic data were analysed using a two compartment PK model, in agreement with earlier modelling studies using venous samples.[7] This resulted in individual empirical Bayesian pharmacokinetic model parameter estimates. These were used in the second stage: the pharmacodynamic analysis. This resulted in baseline parameters and potency parameters, C_{50} values,

with their interindividual and inter-occasion variances. The observed hysteresis between plasma concentration and effect site was characterized as a first-order process with half-life $t_{½}k_{e0}$. The data from the two drugs were analysed separately, but the analgesia and neurocognitive data were combined. Parameter estimates are reported as median ± standard error of the estimate.

Neurocognitive Effects

Saccadic peak velocity and body sway were analysed using sigmoid E_{MAX} models. For saccadic peak velocity, a possible maximum effect was assumed to be zero; for body sway, the possible maximal effect was assumed to be infinite. Saccadic peak velocity C_{50} indicates a 50% decrease from baseline, while body sway C_{50} indicates a 50% increase in sway from baseline. Shape parameter γ was fixed to 1 in the analyses.

Cold Pressor Test

Although the analysis of the cold pressor test was published before,[7] we provide a description for the sake of completeness. In the analysis of the hand latency withdrawal data, a log-logistic distribution was assumed, considering the 120-s cutoff times. The predicted latency time was estimated as the median of the log-logistic distribution using the following equation:

$$\text{Predicted latency}(t) = \text{Baseline} \times (1 + 0.5 \times C_E(t)/C_{50})$$

where Baseline is the baseline latency (i.e., before opioid administration), $C_E(t)$ is the drug concentration in the effect site at time t, and C_{50} is the effect-site drug concentration causing 50% increase in withdrawal latency time. In cases in which the hand withdrawal latency reached the cutoff value, the probability of the censored observation was:

$\log P(\text{withdrawal time} > \text{cutoff}) = \text{survival}$ and $\text{survival} = -\log[1 + (\text{observation}/\text{prediction})^Z]$, where Z is a shape factor; otherwise:

$$\log P(\text{withdrawal time} = \text{observation}) = \log(Z) - \log(\text{prediction}) + (Z \times \log(\text{observation}/\text{prediction}) + 2 \times \text{survival})$$

Goodness of Fit

To assess the adequacy of the data fits, plots of the individual predicted *versus* measured data, population predicted *versus* measured data, and residuals *versus* time were created and inspected. To further evaluate the final pharmacokinetic or pharmacodynamic models, visual predictive checks were conducted by estimation of the normalized prediction discrepancies. We visually confirmed that the normalized prediction discrepancies *versus* time showed no discernible trends, heteroscedasticity, or both.

Utility Functions

We defined the utility function as the probability of the desired effect (analgesia) minus the probability of an adverse effect (in this study, the specified neurocognitive effects).[7,9] The threshold for the desired effect was *a priori* set at a 50% or more increase in hand-withdrawal latency relative to the predrug baseline values (benefit or analgesia greater than or equal to 0.50); the threshold for neurocognitive effects was *a priori* set at a change of at least 25% of the response (saccadic peak velocity or body sway) relative to predrug baseline levels (harm greater than or equal to 0.25). We constructed two utility functions:

$U_1 = P(\text{benefit} \geq 0.50) - P(\text{harm} \geq 0.25)$, and

$U_2 = (P \text{ benefit AND NOT harm})$,

where P is the probability

Utility functions were constructed as a function of the opioid effect-site concentrations and as a function of time after a 1-min infusion of either 3 mg oliceridine or 10 mg morphine.[9] The utilities were calculated from the population pharmacokinetic and pharmacodynamic parameter estimates and their interindividual variability parameters, ω (**Tables 1** and **2**).

Utility probabilities were calculated by counting the number of times either outcome (benefit or harm) occurred with 10,000 simulations and then dividing the counts by 10,000. At each simulation step, individual values for the model parameters were generated by random number generators based on the typical values and interindividual and inter-occasion variances. These procedures were run for the two drugs and comparison endpoints separately. Utility functions are presented ± 95% CI.

Results

Participants

A total of 73 participants were screened, 23 of whom were randomized (10 females, 13 males). Nineteen (8 females, 11 males) completed all three study visits as planned per protocol. Two of the initially enrolled

participants did not complete all planned visits (one was discontinued after the first visit due to symptomatic COVID-19 disease, and the other withdrew consent after the second visit) and were replaced. One replacement participant withdrew consent after one visit and a second replacement was scheduled. The other replacement withdrew consent after two visits and was not replaced. No participants withdrew consent due to adverse events; no serious adverse events were reported during the study. Characteristics of the randomized population are given in Table 3. The full analysis of all data derived from the NeuroCart test battery will be reported elsewhere.

TABLE 1 Pharmacokinetic Parameter Estimates.

Parameter	Estimate ± SEE (%RSE)	ω ± SEE (%RSE) [%CV]	Lower, Upper Values
Oliceridine			
V_1, l	65.9 ± 2.9 (4)	0.15 ± 0.03 (20) [40]	60.0, 71.6
k_{10}, h^{-1}	0.68 ± 0.03 (4)	*	0.63, 9.74
k_{12}, h^{-1}	0.29 ± 0.05 (20)	0.38 ± 0.10 (30) [68]	0.20, 0.42
k_{21}, h^{-1}	0.78 ± 0.07 (9)		0.65, 0.93
σ	0.16 ± 0.01 (5)		0.15, 0.18
V_2, l†	23.9 ± 2.8 (10)		18.5, 29.8
CL_1, l/h†	44.9 ± 2.3 (5)		40.3, 49.3
CL_2, l/h†	18.8 ± 3.2 (20)		13.3, 26.3
Morphine			
V_1, l	38.0 ± 3.4 (9)	‡	31.2, 45.5
k_{10}, h^{-1}	3.2 ± 0.2 (9)		2.7, 3.7
k_{12}, h^{-1}	9.1 ± 0.7 (7)		7.8, 10.4
k_{21}, h^{-1}	1.8 ± 0.1 (4)		1.7, 2.0
σ	0.17 ± 0.01 (5)		0.16, 0.19
V_2, l†	186 ± 10 (5)		169, 205
CL_1, l/h†	119 ± 5 (5)		110, 131
CL_2, l/h†	342 ± 19 (5)		308, 381

**The interoccasion variability for oliceridine k_{10} is 0.14 ± 0.03 with RSE of 20%, CV of 39%, lower value of 0.09, and upper value of 0.22. †Derived from the one sampling importance resampling step, performed after the importance sampling step using NONMEM and the 'table_resample' utility. ‡The interoccasion variability for morphine V1 is 0.12 ± 0.03 with RSE of 30%, CV of 36%, lower value of 0.07, and upper value of 0.19. CL_1, clearance from compartment 1; CL_2, intercompartmental clearance between compartments 1 and 2; CV, coefficient of variation for interindividual or interoccasion variability, which is calculated as $\sqrt{[\exp(\omega^2) - 1] \times 100}$; k_{10}, elimination rate constant; k_{12}, rate constant in between compartments 1 and 2; k_{21}, rate constant in between compartments 2 and 1; RSE, relative standard error; SEE, standard error of the estimate; V_1, volume of compartment 1; V_2, volume of compartment 2; σ is additive residual error variance; ω, interindividual variability.*

TABLE 2 Pharmacodynamic Parameter Estimates.

Parameter	Estimate ± SEE (%RSE)	ω ± SEE (%RSE) [%CV]	Lower, Upper Values
Oliceridine			
Saccadic peak velocity			
Baseline, °/s	453 ± 8.5 (2)	0.08 ± 0.01 (20) [30]	435, 466
C_{50} (ng/ml)	90 ± 14 (20)	0.46 ± 0.12 (20) [76]*	66, 116
$t_{½}k_{e0}$ (h)	0.7 ± 0.1 (20)	0.56 ± 0.19 (30) [86]	0.4, 0.9
σ	21 ± 1 (6)		19, 23
Body sway			
Baseline (mm)	174 ± 19 (10)	0.46 ± 0.12 (20) [76]	137, 221
C_{50} (ng/ml)	10 ± 2 (20)		7, 16
$t_{½}k_{e0}$ (h)	1.1 ± 0.2 (20)		0.7, 1.4
σ	64 ± 4 (6)		58, 73
Cold pressor test			
Baseline (s)	29 ± 4 (10)	0.60 ± 0.09 (20) [90]	21, 37
C_{50} (ng/ml)	13 ± 2 (10)	0.46 ± 0.12 (20) [76]*	10, 17
$t_{½}k_{e0}$ (h)	0.07 ± 0.03 (40)		0.03, 0.13
Z	9.15 ± 0.62 (7)		8.1, 10.3
Morphine			
Saccadic peak velocity			
Baseline (°/s)	459 ± 11 (2)	0.10 ± 0.10 (20) [32]	439, 480
C_{50} (ng/ml)	54 ± 15 (30)	0.41 ± 0.13 (30) [71]†	30, 85
$t_{½}k_{e0}$ (h)	3.4 ± 1.0 (30)		2.0, 5.5
σ	21.9 ± 1.3 (6)		20, 25
Body sway			
Baseline (mm)	229 ± 28 (10)	0.44 ± 0.09 (20) [75]	176, 287
C_{50} (ng/ml)	5.6 ± 0.8 (10)	0.41 ± 0.13 (30) [71]†	4, 8
$t_{½}k_{e0}$ (h)	2.7 ± 2.5 (90)	1.81 ± 0.332 (20) [230]	0.3, 9.8
σ	132 ± 11 (8)		115, 154
Cold pressor test			
Baseline (s)	32 ± 4 (10)	0.52 ± 0.08 (20) [82]	24, 41
C_{50} (ng/ml)	23 ± 7 (30)	0.41 ± 0.13 (30) [71]†‡	13, 40
$t_{½}k_{e0}$ (h)	1.0 ± 0.2 (20)		0.6, 1.6
Z	8.9 ± 0.70 (8)		7.6, 10.3

*When parameters were omitted, they were not estimable. *The interoccasion variability for oliceridine $^{t}{}_{½}k_{e0}$ (body sway) is 0.52 ± 0.08 with RSE of 20% and CV of 82%. For oliceridine, one ω was included in the statistical model for C_{50} of all three endpoints. †Similarly, for morphine, one ω was included in the statistical model for C_{50} of all three endpoints. ‡One additional ω was added for morphine C_{50} (cold pressor test), which was 0.91 ± 0.31 with RSE of 30% and CV of 120%. CV, coefficient of variation for interindividual or interoccasion variability, calculated as $\sqrt{[\exp(\omega^2) - 1] \times 100}$; C_{50}, potency parameter (i.e., the effect-site drug concentration causing a 50% effect); RSE, relative standard error; SEE, standard error of the estimate; $t_{½}k_{e0}$, the blood-effect-site equilibration half-life; Z, steepness coefficient of the log-logistic distribution; σ, residual noise component; ω is interindividual variability.*

TABLE 3 Subject Demographics.

Characteristic	Randomized Population (N = 23)
Sex	
Female	10
Male	13
Race	
Native American	1
African American	1
Other	2
White	19
Age, yr*	32 ± 12 (19 to 53)
Weight, kg*	73 ± 13 (55 to 105)
Body mass index, kg/m²*	23 ± 3 (20 to 28)

**Mean ± SD (range).*

Pharmacokinetic Analyses

The last samples (t = 720 min) of all subjects were excluded from the analyses as they had unexpectedly high values relative to the other samples. The individual pharmacokinetic data and mean plasma concentrations for low- and high-dose oliceridine and morphine are given in supplemental figure 1, A to D. The pharmacokinetic models were parameterized with volumes (V_1) and rate constants, as these were the best in terms of objective function and standard errors of the variability parameters. This may be due to fewer covariances between the parameters compared to the parameterization with volumes and clearances. Additionally, we derived V_2 and clearances from the one sampling importance resampling step as explained in the Supplemental Digital Content.[22] All pharmacokinetics parameter estimates are given in **Table 1**. The population model output is plotted in supplemental figure 1A (oliceridine) and supplemental figure 1C (morphine) for low- and high-dose drug administration. Inspection of the individual data fits and goodness-of-fit plots (supplemental figure 1, E to L) indicate that the pharmacokinetic models adequately describe the data.

Pharmacodynamic Analyses

Data from one subject (ID 16) were excluded from the pharmacodynamic analyses, as this subject was unresponsive with respect to drug effect in the cold pressor test and had rather unexpected small baseline cold pressor test latency values. **Supplemental Figures 2** to **4** present the pharmacodynamic responses and data analyses. Both opioids exhibit a dose-response relationship for all three endpoints. The adequacy of the data fits was determined by examining individuals fits (data not shown) and the goodness-of-fit plots (supplemental figures 2 to 4).

The estimated parameter values are given in **Table 2**. For the potency parameter C_{50}, a single between-subject variability parameter, ω, was included in the statistical model for all three endpoints, while an additional ω was added for the cold pressor morphine C_{50}. Compared to morphine, oliceridine C_{50} values were greater for both neurocognitive endpoints but not for the cold pressor test, indicative of a lower potency for developing adverse effects but greater potency for analgesia. In addition, the hysteresis parameter $t_{½}k_{e0}$ differed between the two drugs with a more rapid onset/offset for oliceridine compared to morphine, irrespective of the measured endpoint. In the cold pressor test, oliceridine displayed an almost instantaneous response ($t_{½}k_{e0}$, 0.07 ± 0.03 h), suggestive of a rapid equilibration between plasma concentration and effect site. Morphine $t_{½}k_{e0}$ ranges (1 to 3 h) indicate the rather slow passage of this opioid across the blood-brain barrier. When comparing the C_{50} values across endpoints, oliceridine showed similar potency values for body sway and the cold pressor test (i.e., equipotency), while peak saccadic velocity was approximately one tenth as potent compared to the other two endpoints. For morphine, the highest potency was observed for body sway, followed by cold pressor test (factor 4) and finally peak saccadic velocity (factor 10).

Therapeutic Indices

Adverse effects relative to cold pressor test were analysed by comparing therapeutic indices as follows: [C_{50}(cold pressor test)/C_{50}(saccadic peak velocity) oliceridine]/[C_{50}(cold pressor test)/C_{50}(saccadic peak velocity) morphine] = 0.34 with 95% CI, 0.17 to 0.71. For body sway, this ratio was 0.33 (0.16 to 0.50). Both indicate the favourable behaviour of oliceridine compared to morphine as based on these therapeutic indices.

Utility Functions

The utility functions are given in **Figure 1** for U_1 = P(B) − P(H) with their 95% CI. All four curves indicate that the oliceridine utilities were superior to those of morphine. For saccadic peak velocity (**Figure 1A**), the largest probability value for U_1 as a function of effect-site concentration was 0.63 (95% CI, 0.44 to 0.83), at an oliceridine concentration of 21 ng/

ml, which we consider a large effect. The equivalent value for morphine was 0.09 (–0.11 to 0.26) at a morphine concentration of 8 ng/ml. While oliceridine had a positive probability U_1 over the concentration range of 5 to 77 ng/ml (otherwise not significantly different from 0), morphine probability U_1 was not significantly different from 0 over the plasma concentration range 0 to 100 ng/ml. Based on the 95% CI ranges,[23] the two curves differed significantly from 15 to 50 ng/ml. Similar observations were made for body sway (**Figure 1B**), with significant differences in probability between opioids over the concentration range of 12 to 87 ng/ml. In addition, for U_2 = P(B *and not* H), indicative of the probability of occurrence of antinociception without any neurocognitive harm, oliceridine was superior to morphine (**fig. 2**). For saccadic peak velocity, the peak probability U_2 was 0.63 (0.45 to 0.83) at an oliceridine concentration of 20 ng/ml and 0.20 (0.05 to 0.49) at a morphine concentration of 15 ng/ml with significant differences in probabilities between the two opioids from 18 to 30 ng/ml. For body sway, equivalent values were 0.44 (0.25 to 0.74) at an oliceridine concentration of 17 ng/ml, 0.09 (0.01 to 0.21) at a morphine concentration of 14 ng/ml, and significantly different over the concentration range of 16 to 52 ng/ml.

For the utility functions as a function of time after 3 mg intravenous oliceridine or 10 mg intravenous morphine administration, we refer to **Figure 1, C** and **D**, for U_1 and **Figure 2, C** and **D**, for U_2. Significant differences between the two opioids were observed for saccadic peak velocity from t = 2 to t = 30 min (U_1) and from t = 2 to t = 43 min (U_2), with both in favor of oliceridine, and for body sway from t = 2 to t = 62 min (U_1) and from t = 2 to t = 60 min (U_2), with both in favour of oliceridine.

Discussion

There is a wide variety of opioids available for clinical use,[3,4] with large differences in their pharmacologic properties such as pharmacokinetics, receptor kinetics, and pharmacodynamics. Concerning the latter, this relates to their intended effects, such as pain relief, as well as to the diverse range of adverse effects that opioids produce. For example, opioids vary in the degree of likability and consequently in their potential for abuse, the extent of respiratory depression, and possibly the level of neurocognitive defects.[3,4,11,13] All adverse effects require careful consideration when selecting an opioid for clinical use. The difficulty lies in determining the most appropriate opioid for specific objectives, such as managing postoperative pain.

FIGURE 1 Utility P(B) – P(H).

Shown are the oliceridine (blue lines) and morphine (red lines) utility functions ± 95% CI for U_1 = P(B) – P(H), where B = benefit (pain relief from cold pressor test), and H = harm as function of effect-site concentration (CE) or time t. (A) U_1(CE), where H = saccadic peak velocity. (B) U_1(CE), where H = body sway. (C) U_1(t), where H = saccadic peak velocity, after 3 mg oliceridine or 10 mg morphine. (D) U_1(t), where H = body sway, after 3 mg oliceridine or 10 mg morphine.

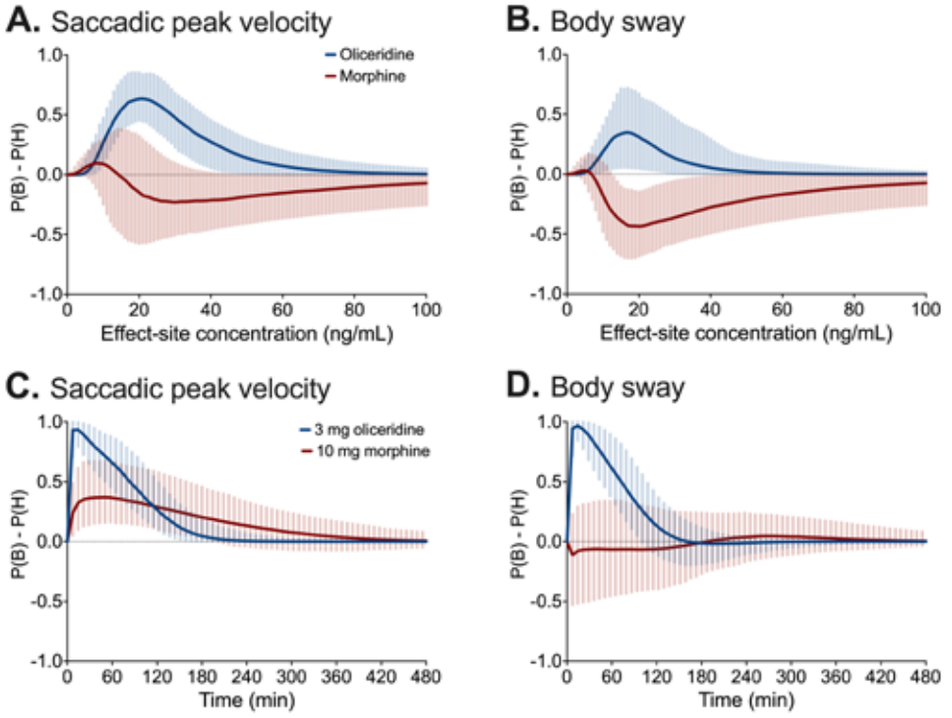
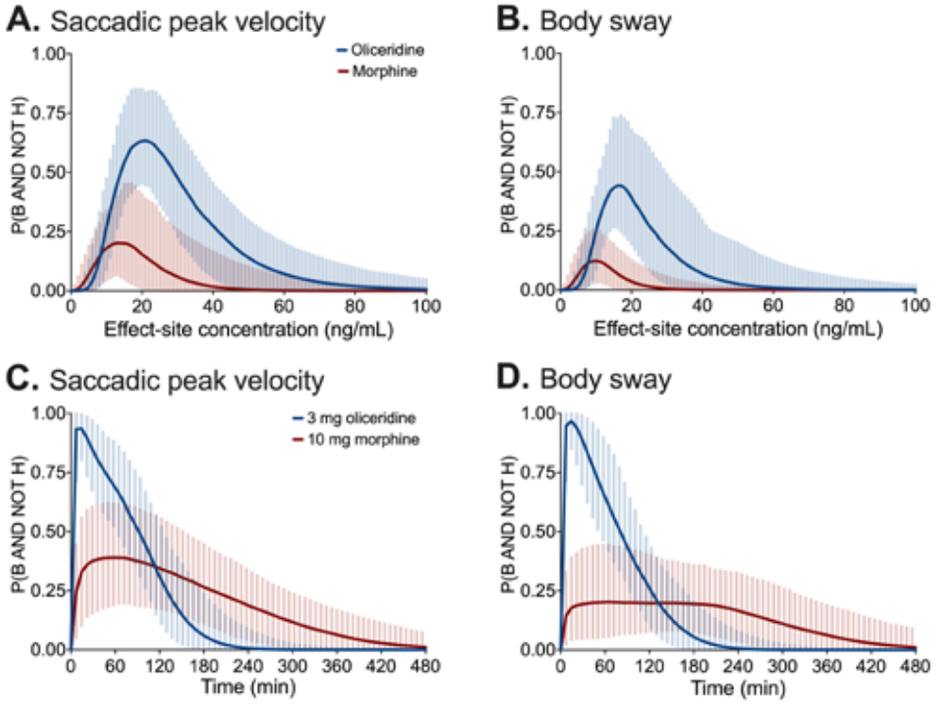


FIGURE 2 Utility P(B *and not* H).
Shown are the oliceridine (blue lines) and morphine (red lines) utility functions ± 95% CI for U_2 = P(B and not H), where B = benefit (pain relief from cold pressor test), and H = harm as function of effect-site concentration (CE) or time t. (A) U_2 (CE), where H = saccadic peak velocity. (B) U_2 (CE), where H = body sway. (C) U_2 (t), where H = saccadic peak velocity, after 3 mg oliceridine or 10 mg morphine. (D) U_2 (t), where H = body sway, after 3 mg oliceridine or 10 mg morphine.



Neurocognitive Tests

In our current study, our primary aim was to compare the negative impact on neurocognitive functions caused by oliceridine and morphine. To that end, we used two measurements from the NeuroCart neurocognitive test battery,[16] which provides objective neurophysiologic and subjective evaluations (such as memory and mood) of neurocognition. Specifically, our focus was on two objective measures of central nervous system function, i.e., saccadic peak velocity and body sway, which we consider pertinent to the postoperative period. Previous studies on the effects of the benzodiazepines on saccadic peak velocity revealed significant linear relationships between drug concentration and effect, although considerable interindividual differences were observed in the magnitude of the concentration–response slope.[17,24] After administration of temazepam, no correlations were observed between the slopes of the concentration–effect curves for subjective scores of alertness and saccadic peak velocity,[17] while after diazepam, peak saccadic velocity changes correlated with decreases in plasma cortisol levels.[24] These findings suggest that saccadic peak velocity serves as a reliable and objective measure to track the level of sedation. Brain areas involved in the saccadic peak velocity include the superior colliculus, substantia nigra, and amygdala.[16] We consider body sway a measure of balance, motor stability, and postural control, with the cerebellum and brainstem implicated in this measure.[16,25]

Oliceridine versus Morphine

Our pharmacodynamic analyses showed that the oliceridine and morphine C_{50} values for saccadic peak velocity were greater than those for antinociception: oliceridine, 90 ± 14 ng/ml (saccadic peak velocity) *versus* 12 ± 2 ng/ml (antinociception); and morphine, 54 ± 15 ng/ml (saccadic peak velocity) *versus* 23 ± 7 ng/ml (antinociception; **Table 1**). Since both opioids are used to manage pain, a combined analysis giving their utility is needed to understand efficacy and safety of these treatments. Here, we present two such analyses. First, we calculated the oliceridine/morphine therapeutic index ratio, which strongly favoured oliceridine for both neurocognitive endpoints (ratio values, 0.33 to 0.34; $p < 0.01$). The therapeutic index represents the ratio at which 50% effect is achieved and does not consider other parts of the concentration-effect curves.[26] As desired and adverse effects often originate from different brain areas, their concentration-effect relationships may diverge significantly.[26] To address this concern, we calculated the difference in predicted probabilities for benefit and harm across a wide concentration range (U_1). Ultimately, a therapeutic index must align with the utility, as they did in our study, but the utility is more broadly applicable. It offers the utility across a range of concentrations and allows assessment of a variety of functions, such as P(B *and not* H), which indicates the probability of desired effect without any accompanying harm.[27] In summary, our results indicate that oliceridine is a relatively more potent analgesic than morphine (as based on comparison of C_{50} values) and exhibits a reduced adverse effect profile on neurocognition compared to morphine.

Comparison with the Literature

Our current results agree well with earlier analyses performed by us.[7,8] For example, we analysed data from a study that compared antinociceptive (cold pressor test) and respiratory (isohypercapnic ventilation) responses from 30 healthy volunteers who had similar characteristics to those enrolled in the current study, after receiving intravenous oliceridine doses (1.5, 3, and 4.5 mg) and morphine (10 mg).[7] U_1 utility functions as functions of drug concentrations were constructed. The results were consistent with our current observations (fig. 1, A and B). However, in the current study, we noticed greater variability in the morphine U_1 for both neurocognitive endpoints and in the oliceridine U_1 for body sway. In a separate study,[8] we determined the C_{50} for respiratory depression from oliceridine and morphine in an elderly population ranging from 55 to 89 yr old. We observed an oliceridine/morphine ratio of 0.70, which corresponds well to values observed in this study for peak saccadic velocity (0.60) and body sway (0.56). Combined, these earlier and current data strongly suggest that the likelihood of benefit after oliceridine treatment outweighs that of harm when considering pain relief, as well as neurocognitive and respiratory effects. The opposite is true for morphine.

Mechanism

While our study was not designed to uncover underlying mechanisms, it is worth discussing potential reasons for the observed differences in drug effect.

DIFFERENCE IN BIAS TOWARD THE β-ARRESTIN PROTEIN PATHWAY

Morphine and oliceridine are both μ-opioid receptor agonists, but, unlike morphine, oliceridine possesses a bias toward G-protein signalling with reduced β-arrestin recruitment and receptor internalization.[5,6] The G-protein system is predominantly (but not exclusively) associated with analgesia, and the β-arrestin system is associated with opioid-related adverse events.[28] Although involvement of the β-arrestin protein in effects of opioids on neurocognitive function is theoretically possible, we found no evidence in the literature of such effects in young and healthy individuals. Furthermore, while β-arrestin expression is reduced in the aging brain, and β-arrestin gene overexpression is detected in age-related neurodegenerative disorders, these long-term changes do not reflect the acute effects induced by the two opioids in our study.[29,30] Therefore, no conclusions can be drawn regarding the potential positive or negative effect of β-arrestin activity on opioid-induced neurocognitive defects.

TOLL-LIKE RECEPTOR 4 (TLR4)

Apart from their effects on opioid receptors, opioids can interact with other receptors or neurotransmitter systems, leading to diverse physiologic effects. One such off-target interaction is with TLR4.[14,31–33] Opioids, particularly morphine, may cause the release of proinflammatory mediators from microglia cells through the activation of TLR4 expressed on these cells. Morphine-induced neuroinflammation is associated with effects that oppose the opioid system, such as hyperalgesia and tolerance, and possibly even cause opioid-induced respiratory depression.[14,31,33] Additionally, neurocognitive defects may arise from neuroinflammation and TLR4 activation.[14,33] Morphine, but not oliceridine, increases spinal expression of TLR4 in rats after surgery,[34] suggesting that oliceridine may induce less neuroinflammation and subsequently fewer neurocognitive defects.

It is important to note that the specific mechanisms underlying the observed differences require further research and investigation to gain a comprehensive understanding of the contrasting effects between oliceridine and morphine.

Study Limitations

We acknowledge that our analyses are on just two of the eight neurocognitive tests performed in this study.[16] We *a priori* carefully selected the most relevant endpoints for our purposes, aiming to obtain indications of drug effect on neurocognition specifically relevant to the postoperative period. The two tests we analysed, saccadic peak velocity (the primary endpoint of the study) as an index of sedation and body sway as an index of balance motor stability, provided similar results in terms of divergence between oliceridine and morphine on neurocognition. A final limitation may be that since tests were performed repetitively; one test may have influenced the other test, although we did not observe any indication for that in previous studies with the same tests.[35] In addition, as all neurocognitive tests (except the first postdose saccadic eye movement) were performed at least 30 min after the pain tests, such influences (if present) were assumed to be minimal.

Conclusions

Our analyses indicate that the biased ligand oliceridine has a superior utility on neurocognitive outcomes compared to morphine. This was true for peak saccadic velocity and body sway. The current findings agree with earlier data on respiratory depression and highlight the potential advantages of oliceridine over morphine in terms of safety and neurocognitive effects. Further research is warranted to delve deeper into the underlying mechanisms and validate our conclusions.

SUPPORTING INFORMATION

Supplemental document 1: Inclusion and exclusion criteria, https://links.lww.com/ALN/D300
Supplemental document 2: Methods and utility functions, https://links.lww.com/ALN/D301
Supplemental document 3: Supplemental figures 1 to 4, https://links.lww.com/ALN/D302

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CHAPTER 6

EFFECTS OF OLICERIDINE ON CENTRAL NERVOUS SYSTEM FUNCTIONING VERSUS MORPHINE: A RANDOMISED, PLACEBO-CONTROLLED, CROSSOVER STUDY IN HEALTHY PARTICIPANTS

Submitted

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Abstract

AIM Oliceridine, a G-protein biased μ-opioid agonist, has shown low incidence of opioid-related events and has been theorized to have less effect on the central nervous system (CNS) compared to traditional opioids like morphine. This study explored effects on CNS functioning of oliceridine, morphine and placebo in healthy participants.

METHODS This was a randomised, double-blind, placebo-controlled study in 23 healthy males and females, aged 18-55 years. Participants received three out of five possible treatments as intravenous bolus in a partial-block, cross-over design: placebo, oliceridine 1 or 3 mg, or morphine 5 or 10 mg. Neurocognition was measured using multiple CNS function tests. Saccadic eye movement peak velocity (SPV), a biomarker for sedation, was selected as the primary endpoint. The cold pressor pain test was included for the assessment of analgesic effects. Venous blood samples were obtained for pharmacokinetic profiling and safety parameters were recorded throughout the study.

RESULTS Oliceridine affected SPV significantly less compared to morphine (difference [95% CI]: -11.40 degrees/s [-21.19, -1.61], p=0.0236). In general, morphine had a more sustained effect on secondary endpoints. Oliceridine 3 mg induced superior analgesia on the cold pressor test compared to both morphine doses within the first hour post-dose, while mean effects of morphine lasted longer than those of oliceridine. Pharmacokinetic and safety profiles were consistent with earlier reports.

CONCLUSION Oliceridine demonstrated statistically significant smaller effects than morphine on a wide range of functional tests, including SPV, while demonstrating a statistically significant early analgesic effect compared to morphine. Results suggest a favourable risk-benefit profile of oliceridine compared to morphine in the treatment of severe pain requiring acute relief.

Introduction

Opioids are listed by the World Health Organization as essential medicines for several indications including their use intravenously for the management of severe acute pain during the post-operative period where pain relief can often not be adequately provided by non-opioid medications.[1,2] However, intravenous opioid medications carry the potentially life-threatening risk of respiratory depression, as well as significant adverse effects on gastrointestinal function and cognition that can complicate recovery during the post-operative period, increasing the risk of prolonged hospital stays.[3-5]

To address this unmet need for well-tolerated and effective therapies for the management of severe acute pain, there have been many developments, of which one notable paradigm is that of the 'biased ligands'.[6-10] Being structurally distinct from classic opioids such as morphine or synthetic opioids such as fentanyl, these novel analgesics stimulate only a part of the normal receptor coupling mechanisms, which may lead to improved clinical benefit with a reduced risk for the development of opioid-related adverse events.[8] Specifically, improved safety characteristics have been observed in animal models and are hypothesised to be due to varied mechanisms, including the biased pharmacologic action compared to conventional opioids.[6] Preferential intraneural activation of the G-protein signalling pathway while minimizing β-arrestin activity after binding to the μ-opioid receptor (MOP) is associated with less opioid-related adverse events affecting e.g., respiratory, and gastrointestinal function, while maintaining analgesic potency.[6,8,10] Oliceridine (Olinvyk®, Trevena, Inc., USA) is one such G-protein biased ligand and has been approved by the United States Food and Drug Administration for use in adults for the management of acute pain severe enough to require the use of an IV opioid and for whom alternative treatments are inadequate (Food and Drug Administration 2020). Previous studies have suggested that oliceridine may be associated with reduced respiratory depression over time compared to morphine.[11-14] However, there may be additional underlying mechanisms responsible for other potential clinical benefits, including pharmacokinetics (PK), pharmacodynamics (PD), and even having less off-target effects like impact on inflammation.[15] The latter effects may be particularly relevant to cognition since it has been theorised that persistent inflammatory signalling is an important contributor to the development of post-operative cognitive dysfunction.[16,17]

Postoperative recovery and patients' ability to function in daily life can be greatly impacted by the treatment they receive to manage pain.[18-20] Adequate analgesia is essential, but this may come at an intolerable cost with respect to side effects inherent with the use of opioids.[3,4] While many clinicians are aware of the respiratory depression and addiction risks,[21-23] the development or exacerbation of cognitive dysfunction, which may range from sedation to confusion or progress to delirium, is less well-recognized.[24-27] Cognitive dysfunction can therefore have potential implications for post-operative recovery and health outcomes, and some instances may result in deficits that persist beyond the immediate post-operative period. The mechanism of these cognitive complications is unclear, though it has been hypothesized that opioids, such as morphine, can bind to the toll-like receptor 4 (TLR4), and the subsequent neuroinflammatory response may contribute to these post-operative cognitive sequelae.[16,17] Rats treated with oliceridine demonstrate reduced levels of spinal cord TLR4 after experimental fracture compared to morphine-treated animals.[15]

The current study was designed to compare the effects of intravenous bolus doses of oliceridine and morphine on CNS functioning using the NeuroCart® test battery[28] in healthy participants. We hypothesized that oliceridine would demonstrate a reduced effect on neurocognitive function compared to IV morphine, at comparable levels of analgesia.

Materials and methods

Ethics and Registration

The study was conducted at the Centre for Human Drug Research (CHDR, Leiden, the Netherlands) after approval by the Medical Review and Ethics Committee of the BEBO foundation (Assen, the Netherlands) and the Central Committee on Research Involving Human Subjects (competent authority) in The Hague, The Netherlands (identifier NL79823.056.21). The study was conducted in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH GCP) and ethical principles as referenced in EU Directive 2001/20/EC. Prior to the first participant screened, the trial was registered with the ISRCTN registry (identifier ISRCTN13308001).

Trial Design

This was a single-centre, randomised, double-blind, placebo-controlled, dose-ranging, partial-block, crossover study in healthy participants. The study consisted of three identical treatment periods with at least 7 days between dosing days. Participants were admitted the evening prior to the experiment during each study period and discharged 12 hours after dosing. The treatments were randomly allocated, and each participant received three out of the five possible treatments in one of 20 treatment sequences (out of 60 possible sequences). The randomisation schedule was balanced so that all treatments and treatment combinations were equally represented. On each dosing day, treatment was administered intravenously as a 3 mL bolus over 60 seconds: oliceridine 1 or 3 mg (Trevena Inc., USA), morphine hydrochloride 5 or 10 mg (Centrafarm B.V., The Netherlands), or 5% dextrose as placebo (Baxter B.V., The Netherlands). The doses of oliceridine and morphine were selected based on the approximate equianalgesic effects (respective potency ratio 1:5 upon first dose[11,29]) and the expectation that these would yield measurable effects on the neurocognitive test battery, while being safe to administer in our clinic to opioid-naïve participants. In addition, the individual high doses of both drugs (oliceridine 3 mg and morphine 10 mg) represented the maximum single doses typically administered in clinic. There were no changes to trial design or endpoints after commencement of the study.

Study Participants

Before performing any study-related procedures, participants provided written informed consent, and a medical screening was performed within four weeks of the first study drug administration. Male and female healthy volunteers, aged ⩾18 and ⩽55 years with a body mass index ⩾18 and <32 /m² at screening, using effective contraception, were eligible. Exclusion criteria included poor metabolizers of CYP450 2D6 substrates (as defined after genotyping assessment at screening); any clinically significant medical conditions, in particular conditions that would affect sensitivity to cold (e.g., Raynaud's disease) or pain (e.g., allodynia); pain tolerance >80% on, or intolerability to, the cold pressor pain test as determined at screening; use of any medication within 14 days or five half-lives before dosing (excluding contraceptives); positive drug or alcohol breath test at screening.

All eligibility criteria are provided in the study protocol, which is available as Appendix A.

Pharmacodynamic and Pharmacokinetic Assessments

To evaluate the effect of oliceridine and morphine on neurocognition, measurements using the NeuroCart®,[28,30] a validated test battery developed at CHDR incorporating a wide range of CNS function tests, were performed twice pre-dose and at set times after dosing throughout the day.

A detailed overview of the experimental set-up and the timing of each study measurement is provided in the protocol (Appendix A). An overview of the measurements of CNS functioning that were performed is presented in **Table 1**.

Table 1 Overview of the measurements of CNS functioning.

Primary endpoint	
Saccadic peak velocity (Saccadic eye movement)	A parameter that is frequently used as a biomarker for sedation when assessing (side) effects of drugs involving the CNS
Secondary endpoints	
Saccadic- and smooth pursuit eye movements	Both tests frequently used for the assessment of (side) effects of drugs involving the CNS
Pupillometry	A measurement widely applied to measure MOP target engagement of opioids
Adaptive tracking test	A test of sustained attention also sensitive to impairment of eye-hand coordination
Body sway	A test that provides a measure of postural stability
Digit symbol substitution test	An assessment incorporating visual perception and other cognitive functions including attention, short-term memory, and psychomotor speed
Bond and Lader VAS	Questionnaire that is often used to quantify subjective drug effects regarding alertness, mood, and calmness
VAS Bowdle	A test that quantifies psychotomimetic effects thereby addressing various abnormal states of mind

Abbreviations: CNS = central nervous system, MOP = μ-opioid receptor, VAS = visual analogue scale

To allow for adequate comparison between oliceridine and morphine in terms of effects on CNS functioning, it was essential to choose equianalgesic dose levels. For the assessment of analgesia, the cold pressor pain test was included. The test is part of a validated test battery and has previously shown to be sensitive to opioids.[31-33] Briefly, pain detection (PDT) and tolerance thresholds (PTT) were determined by instructing the participants to place their non-dominant hand in a cold-water bath (1.0 ± 0.5°C) and indicate when they started to feel pain (PDT) and when they reached their pain tolerance (PTT) whence they withdrew their hand from the water bath. For safety reasons, a limit of 120 seconds was programmed after which the measurement automatically ended in the event that tolerance was not yet reached.

A training session was performed during screening to reduce possible learning effects of the neurocognitive test battery, and to exclude participants in accordance with the eligibility criteria. Assessments were performed with the participant sitting comfortably in a chair, legs raised, in a quiet room that was fitted with ambient lighting. Each participant was assigned to a separate room to minimise distraction.

Blood samples were scheduled at regular intervals up to 12 hours post-dose to characterise the PK profiles of oliceridine, morphine and morphine's main metabolite, Morphine-6-glucuronide (M6G), as specified in the protocol (Appendix A). To prevent contamination of the sample with administered drug, 4 mL venous blood was drawn from the arm opposite to where drug was administered. After collection in K2EDTA Vacutainer® tubes, samples were placed on ice until processing and freezer storage below -70 °C. Plasma concentrations were measured at Labcorp Bioanalytical Services, USA, using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods with LLOQs of 0.0500 ng/mL for oliceridine and 0.500 ng/mL for morphine and M6G.

Safety and Tolerability Assessments

Safety and tolerability were assessed through the recording of treatment emergent (serious) adverse events (TE(S)AE's) from time of first screening visit through the end of the last visit, clinical laboratory tests, measurement of vital signs including continuous oxygen saturation (SpO_2 values <94% were recorded as TEAE), electrocardiograms (ECGS), Columbia Suicide Severity Rating Scale (C-SSRS, licenced at the Research Foundation for Mental Hygiene, Inc., USA) and the Pasero Opioid-induced Sedation Scale (POSS), a validated tool used to record the clinician's assessment of patient sedation on a 5-point rating scale.[34,35] All measurements are described comprehensively in the protocol (Appendix A).

Sample Size and Randomisation

A sample size of 20 was determined to have a power of >0.85 to detect a difference in means of 55 (°/sec) on the primary endpoint (SPV), assuming a standard deviation of differences of 77.5 (°/sec), using a paired t-test

with a 0.05 two-sided significance level. Based on historic data at CHDR, the chosen doses of oliceridine and morphine were expected to have a similar or greater treatment effect with a smaller SD.

A randomisation schedule was generated by an independent statistician using SAS version 9.4, and the list was made available only to the pharmacy of Leiden University Medical Centre (Leiden, the Netherlands) for preparation of the masked infusion syringes with study treatments. In the order of successfully completed medical screenings, participants received a randomisation number sequentially.

Statistical Methods

The statistical analyses are described in the protocol and statistical analysis plan (Appendices A and B). In short, repeatedly measured pharmacodynamic (PD) parameters were analysed with a mixed model analysis of covariance with treatment, time, period, and treatment by time as fixed factors and participant, participant by treatment, and participant by time as random factors and the (average) baseline measurement as covariate. Single measured PD parameters were analysed with a mixed model analysis of variance, with treatment and period as fixed factors and participant as random factor. The following statistical contrasts were defined: each individual active dose versus placebo, and pooled oliceridine versus pooled morphine. No adjustments for multiple comparisons were applied. Additionally, a post-hoc analysis was performed, in which only the data up to 2.5 hours post-dose were included, to see if there were statistically significant PD effects during this period of time. PK plasma concentrations were summarised for all participants who received at least 1 dose of the medication and had an adequate number of pharmacokinetic samples collected. Summarised safety and tolerability data was descriptively reported.

All statistical programming was conducted using SAS 9.4 for Windows (SAS Institute Inc., USA) and programming for PK (including noncompartmental analyses) was performed with R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Austria).

Results

Between 04 February 2022 and 10 June 2022, a total of 73 participants were screened out of whom 23 were randomised (10 females). Baseline characteristics are presented in Table 2, and the participant disposition is summarised in Figure 1. In total, 19 participants (8 females) completed all three study visits as planned per protocol. Two of the initially enrolled participants did not complete all planned visits: one participant was discontinued after the first visit (treatment morphine 10 mg) due to symptomatic COVID-19 and another participant withdrew consent after the second visit (treatments oliceridine 3 mg and placebo); both were replaced. The first replacement participant withdrew consent after one visit (treatment oliceridine 3 mg) and a second replacement was scheduled who completed all visits. The other initial replacement withdrew consent after two visits (treatments morphine 10 mg and placebo) and was not replaced. No participants withdrew consent due to AE's.

TABLE 2 Participant Demographics.

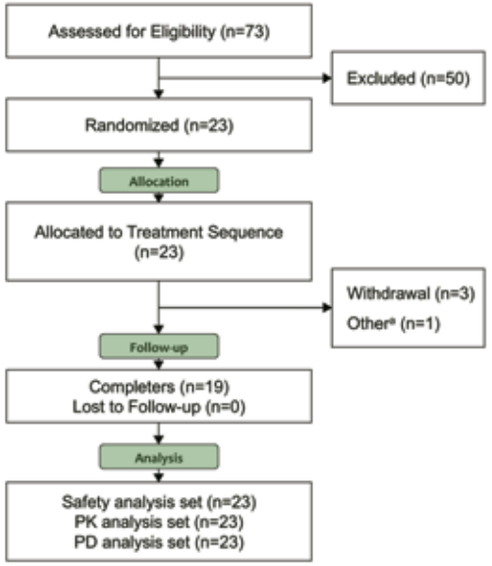
Characteristic	Category/Statistics	All Participants (N=23)
Sex	Female	10 (43.5%)
	Male	13 (56.5%)
Race	American Indian or Alaska Native	1 (4.3%)
	Black or African American	1 (4.3%)
	Mixed	1 (4.3%)
	Other[a]	1 (4.3%)
	White	19 (82.6%)
Age (years)	Mean (SD) Median [min-max]	31.6 (11.7) 26 [19-53]
Height (cm)	Mean (SD) Median [min-max]	176.5 (10.30) 173.3 [161.8-202.8]
Weight (kg)	Mean (SD) Median [min-max]	73.2 (13.0) 72.40 [54.80-104.7]
BMI (kg/m^2)	Mean (SD) Median [min-max]	23.26 (2.60) 23.4 [19.6-27.9]

Abbreviations: BMI = body mass index; max = maximum; min = minimum; SD = standard deviation. a. Other = Hispanic or Latino.

Pharmacodynamic Outcomes

On the primary study outcome, saccadic peak velocity (SPV), there was a statistically significantly reduced effect with oliceridine compared to morphine (estimate of the difference): -11.40 degrees/s (95% CI: -21.19, -1.61), p=0.0236 for the grouped treatment effect. A comparison of the respective low and high doses is shown in Figure 2. A significant difference between all individual treatments compared to placebo was observed.

FIGURE 1 Participant Disposition (CONSORT flow diagram).

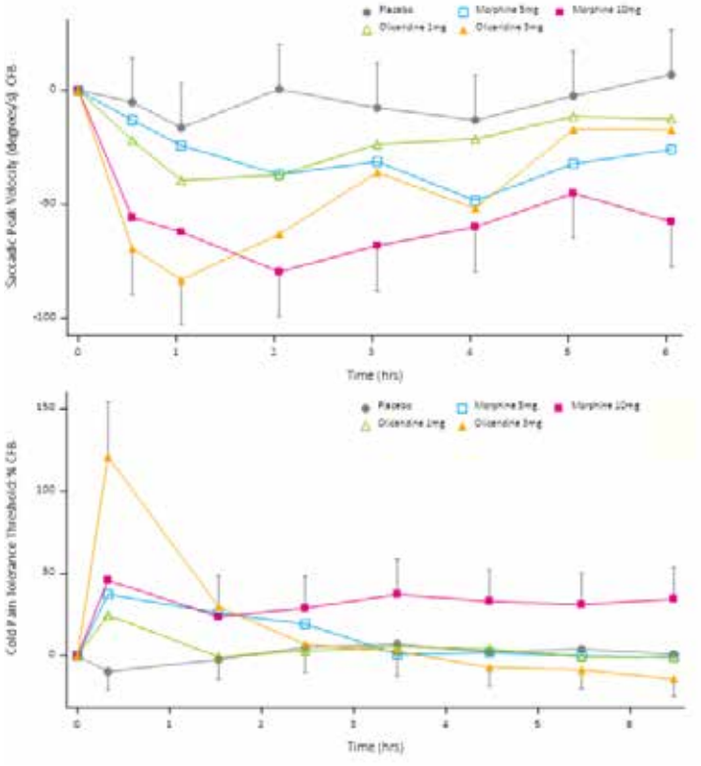


Abbreviations: CONSORT = Consolidated Standards of Reporting Trials; PD = pharmacodynamic; PK = pharmacokinetic.
a. Participant was discontinued per sponsor and investigator decision due to symptomatic COVID-19.

In general, on the secondary outcome measures, the effects of oliceridine and morphine on the parameters associated with CNS functioning tended to mirror those seen on SPV: morphine had a more pronounced and sustained effect compared to oliceridine, and the difference compared to placebo was observed to be statistically significant for both drugs, especially at the high doses. Results of all the pharmacodynamic measurements are shown in Appendix C.

Overall, the analgesic effects of the two treatments were similar, although the time course of analgesia differed slightly between the two treatments. For example, the magnitude of the effect of oliceridine on the cold PTT within the first hour post-dose was larger than that of morphine, but the mean effects of both dose levels of morphine lasted longer than those of oliceridine (Figure 2). Significant differences were observed between morphine 10 mg and placebo (estimate of the difference): 32.1% (95% CI: 17.7, 48.4), p=<.0001, as well as between grouped oliceridine treatments and grouped morphine treatments, favouring morphine (estimate of the difference): 12.1% (95% CI: 3.2, 21.9), p=0.0087. A significant treatment effect favouring oliceridine 3 mg versus placebo was observed in the post-hoc analysis of the timepoints up to 2.5 hours post-dose (estimate of the difference): 48.9% (95% CI: 30.7, 69.6), p=<.0001, and morphine 5 mg (estimate of the difference): 30.5% (95% CI: 14.4, 48.9), p=0.0002. The difference between morphine 10 mg and placebo remained statistically significant. There was no significant treatment difference between oliceridine grouped treatments and morphine grouped treatments in the post hoc analysis.

Figure 2 Estimated Means (95% CI error bars) Saccadic Peak Velocity (Panel A) and Cold Pain Tolerance Threshold (Panel B).



Abbreviations: CFB: change from baseline; CI = confidence interval.

Pharmacokinetic Outcomes

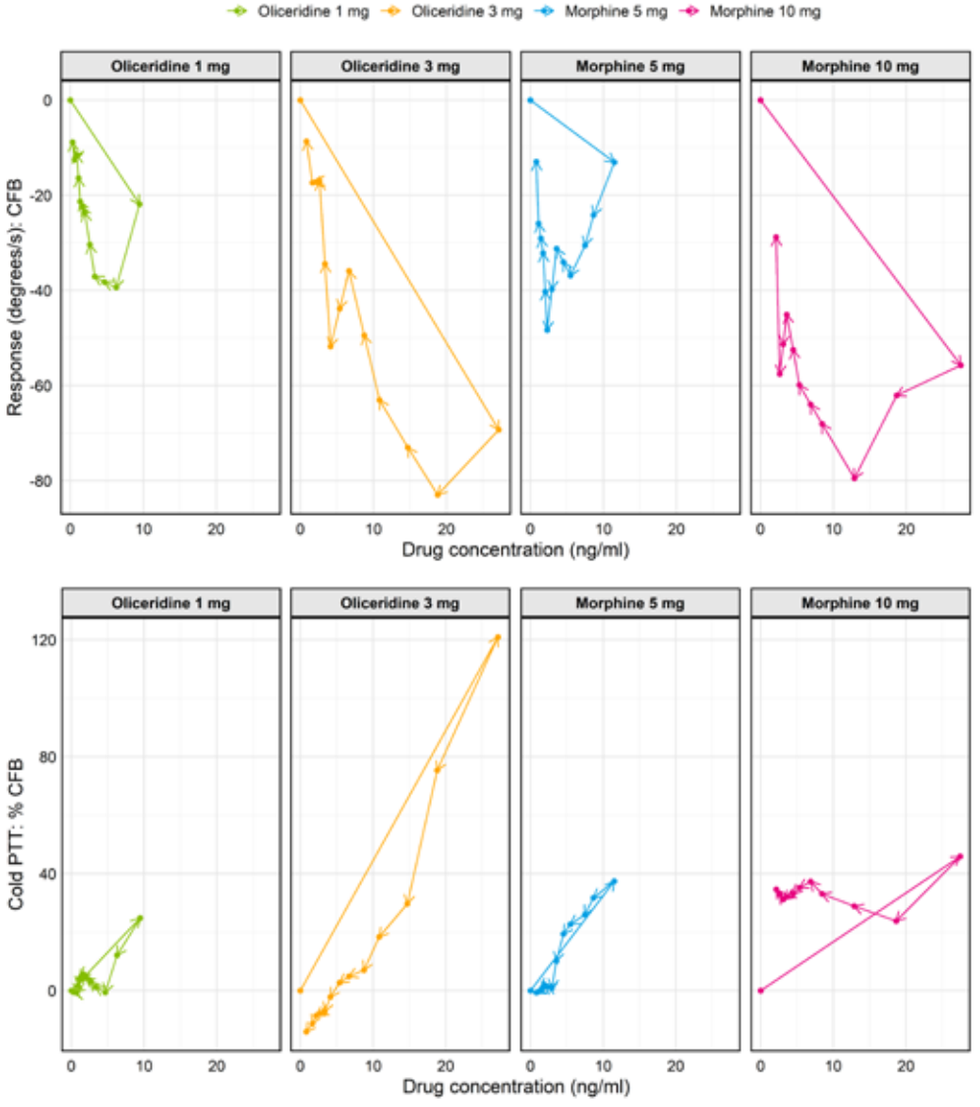
The PK results measured for oliceridine, morphine, and morphine's metabolite, M6G, were as expected based on the known profiles of these analytes. A summary of the exposure-related results is included in **Table 3**. The PK models as well as the individual PK data and mean plasma concentrations were previously published.[36] The hysteresis between the peak in plasma concentrations and the observed pharmacodynamic effects is illustrated in **Figure 3**. The PK/PD model accounting for an effect site compartment is described in a separate report.[36]

Table 3 Summary of Oliceridine, Morphine and Morphine's Metabolite M6G Pharmacokinetics.

Treatment	Parameter	Unit	N	Geometric Mean	SD	CV (%)
Oliceridine 1 mg	$AUC_{0\text{-}12h}$	h*ng/mL	11	21.106	4.1753	19.7
	C_{max}	ng/mL	11	15.918	4.3992	27.4
	$t_{½}$	h	11	1.642	0.5953	32.6
Oliceridine 3 mg	$AUC_{0\text{-}12h}$	h*ng/mL	12	63.521	9.5273	15.1
	C_{max}	ng/mL	13	46.481	12.1205	26.5
	$t_{½}$	h	12	1.755	0.2368	14.0
Morphine 5 mg	$AUC_{0\text{-}12h}$	h*ng/mL	12	36.760	8.2917	21.5
	C_{max}	ng/mL	12	56.938	16.4187	31.4
	$t_{½}$	h	8	1.664	0.2413	15.3
Morphine 10 mg	$AUC_{0\text{-}12h}$	h*ng/mL	13	82.839	14.0355	17.6
	C_{max}	ng/mL	13	103.459	60.5927	45.7
	$t_{½}$	h	13	2.270	0.8986	35.6
M6G (Morphine 5 mg)	$AUC_{0\text{-}12h}$	h*ng/mL	12	64.189	15.2833	24.0
	C_{max}	ng/mL	12	15.169	3.4032	21.9
	$t_{½}$	h	8	3.546	0.8130	21.2
M6G (Morphine 10 mg)	$AUC_{0\text{-}12h}$	h*ng/mL	13	145.261	35.3090	23.8
	C_{max}	ng/mL	13	33.744	8.2023	24.4
	$t_{½}$	h	11	3.079	0.6953	21.7

Abbreviations: $AUC_{0\text{-}12h}$ *= area under the concentration time curve from time 0 to 12 hours,* C_{max} *= maximum concentration,* CV *= coefficient of variation,* N *= number,* SD *= standard deviation,* $t_{½}$ *= half-life.*

Figure 3 Hysteresis of SPV (top panel) and Cold PTT (lower panel) per Treatment (Oliceridine 1 and 3 mg, and Morphine 5 and 10 mg).



Abbreviations: CFB *= change from baseline,* PTT *= pain tolerance threshold,* SPV *= saccadic peak velocity.*

Safety and Tolerability

Oliceridine and morphine were safe and generally well tolerated in healthy participants at the dose levels administered in this study. No SAE's were recorded during the study. In general, the incidences of individual types of TEAE's were lower in participants treated with oliceridine than in participants treated with morphine (Table 4). The most common TEAE's reported during the study were somnolence, nausea, and a decrease in SpO_2. The AE's in all participants were mild apart from 1 participant treated with morphine 5 mg who had 2 moderate AE's: nausea and syncope.

Table 4 Summary of Number (%) of Participants with AE's by SOC and Preferred Term.

SOC/ Preferred term	Placebo (N=13)	Oliceridine 1 mg (N=12)	Oliceridine 3 mg (N=13)	Morphine 5 mg (N=12)	Morphine 10 mg (N=13)
Gastrointestinal disorders	1 (7.7%)	1 (8.3%)	8 (61.5%)	6 (50.0%)	9 (69.2%)
Nausea	-	1 (8.3%)	8 (61.5%)	2 (16.7%)	8 (61.5%)
Vomiting	-	-	3 (23.1%)	3 (25.0%)	4 (30.8%)
Xerostomia	1 (7.7%)	-	3 (23.1%)	1 (8.3%)	5 (38.5%)
General disorders and administration site conditions	-	2 (16.7%)	6 (46.2%)	4 (33.3%)	9 (69.2%)
Fatigue	-	-	1 (7.7%)	-	3 (23.1%)
Feeling hot	-	-	1 (7.7%)	-	4 (30.8%)
Feeling of warmth	-	1 (8.3%)	3 (23.1%)	4 (33.3%)	1 (7.7%)
Tired and heavy	-	-	1 (7.7%)	-	2 (15.4%)
Investigations	-	-	4 (30.8%)	-	6 (46.2%)
SpO_2 decreased	-	-	4 (30.8%)	-	6 (46.2%)
Nervous system disorders	5 (38.5%)	10 (83.3%)	10 (76.9%)	7 (58.3%)	12 (92.3%)
Concentration impaired	-	1 (8.3%)	-	-	3 (23.1%)
Dizziness	-	1 (8.3%)	3 (23.1%)	1 (8.3%)	4 (30.8%)
Head pressure	-	-	-	1 (8.3%)	2 (15.4%)
Headache	1 (7.7%)	1 (8.3%)	2 (15.4%)	2 (16.7%)	1 (7.7%)
Lethargy	-	2 (16.7%)	1 (7.7%)	-	1 (7.7%)
Light headedness	-	3 (25.0%)	2 (15.4%)	1 (8.3%)	4 (30.8%)
Somnolence	5 (38.5%)	3 (25.0%)	6 (46.2%)	3 (25.0%)	7 (53.8%)
Psychiatric disorders	-	1 (8.3%)	3 (23.1%)	4 (33.3%)	2 (15.4%)
Visual hallucinations	-	-	-	-	2 (15.4%)

Abbreviations: AE = adverse event; SAE = serious adverse event; SOC = System Organ Class; SpO_2 = peripheral capillary oxygen saturation. Note: only AE's that occurred in >10% of participants for a given treatment are shown.

Concomitant metoclopramide was administered for nausea/vomiting once after treatment with morphine 5 mg and once after the 10 mg dose. SpO_2 decreases were only recorded in the higher dose groups and were more frequently recorded after administration of morphine 10 mg compared to oliceridine 3 mg (46.2% versus 30.8%, respectively), although no instances of apnoea were observed and the TEAE's resolved swiftly and without medical intervention. Investigator-scored somnolence was observed in more participants after treatment with morphine than after treatment with oliceridine regardless of dose level, as assessed with the POSS.

Discussion

In this study, we compared the CNS effects of a biased opioid ligand, oliceridine, to a classic opioid, morphine, using a wide range of CNS function tests in healthy, opioid-naïve males and females. On the primary outcome measure, saccadic peak velocity, a valid measure of sedation, oliceridine showed a statistically significantly reduced effect compared to morphine treatment. Both treatments showed comparable levels of analgesic effect measured by the cold pain test. These differences measured on the primary outcome were generally mirrored by similar treatment differences on the secondary outcome measures. A subset of data from this study was described in a recent publication, reporting the utility function analyses for oliceridine and morphine conveying the ratio between the probability of benefit, namely analgesia, and the probability of harm, for which two neurocognitive parameters were selected: saccadic peak velocity (SPV, chosen as a surrogate biomarker of sedation) and body sway (as surrogate of balance or motor stability).[36] The analyses showed that the clinical utility functions of oliceridine were clearly beneficial to those of morphine over the entire clinical concentration range. Taken together, these data suggest that, at comparable levels of analgesia, oliceridine shows a favourable risk-benefit profile compared to morphine on laboratory measures of neurocognitive function.

As intravenous opioids are commonly prescribed during postoperative recovery for the management of severe acute postoperative pain, it is important to understand the complete range of CNS side-effects that opioids can cause. The CNS test battery we used[28] includes a distinct set of neurophysiological and subjective assessments to objectively measure the effects of CNS-acting drugs, such as opioids.[30,28,37] The primary

endpoint defined for the study, SPV, was significantly less affected by oliceridine than morphine when comparing the grouped treatment effects of these two drugs. SPV has been demonstrated to provide an objective and sensitive measurement of sedation:[38,28,39] SPV could discern effects of temazepam 5 mg from placebo while this was only measured by self-reported outcome (through VAS tests) at a dose of 20 mg.[40] In the current study, the measured maximum decrease in SPV after the oliceridine 3 mg and morphine 10 mg doses was similar, with oliceridine exhibiting a more rapid onset of action and a shorter lasting effect (**Figure 2**). Based on literature and historical data (on file) that report larger effects on SPV after administration of different benzodiazepines and pregabalin, it is unlikely that the observed maximum effects are due to a ceiling effect on SPV.

The effect on cold pressor pain tolerance was larger after oliceridine compared to morphine. In line with earlier findings,[12] oliceridine produced significant analgesia within 30 min after dosing, although this effect did not persist as long compared to morphine treatment. Per oliceridine's posology, treatment is initiated with a 1.5 mg IV bolus dose followed by doses of 1-3 mg every 1-3 hours as needed with a maximum recommended dose of 27 mg per day. For morphine, a common adult intravenous dose is 10 mg administered approximately every 4 hours, generally not exceeding 100 mg per day. The results from our study match these dosing schemes and the reported potency ratio (although the morphine effect appeared to last longer than 4 hours). Taking into account the duration of the effect on SPV of both compounds, repeated dosing would presumably result in less sedated patients when being administered oliceridine compared to morphine.

In general, oliceridine had a smaller and shorter lasting effect on tests associated with alertness and body stability compared to morphine. Morphine has several active metabolites, of which M6G is one. M6G is a strong MOP agonist with higher affinity than morphine itself, and it has been suggested that M6G contributes to the analgesic effect after administration of morphine.[41,42] The presence of M6G may have been partly responsible for the sustained effect on multiple parameters observed for morphine (both dose levels) in this study.

Although morphine and oliceridine are both MOR agonists, oliceridine possesses a bias towards G-protein intracellular signalling with reduced β-arrestin recruitment after engaging with the MOP. The MOP G-protein signalling pathway is mainly associated with the desired effect, namely analgesia, while β-arrestin is hypothesised to predominantly be associated with multiple side effects such as respiratory depression.[6,8,10] However, no literature describing the link between β-arrestin and CNS side effects is available, and β-arrestin's role in these effects may well be unlikely. We have hypothesised that an off-target interaction of opioids (in particular morphine) with the TOLL-like receptor 4 (TLR4) can cause neuroinflammation[17,43,44] and thereby play a role in causing CNS dysfunction. This interaction with TLR4 on microglia, which has been demonstrated in particular with morphine, triggers proinflammatory mediator release, which can lead to neuroinflammation. This process can paradoxically induce hyperalgesia, tolerance, and potentially also adverse effects such as respiratory depression. Neurocognitive impairments may also stem from this neuroinflammation. Intriguingly, oliceridine does not seem to upregulate spinal TLR4 expression post-surgery in rats, unlike morphine, suggesting possibly reduced neuroinflammation and downstream neurocognitive issues.[15] Based on the results in our study, however, we cannot provide further insight into the underlying mechanisms.

The relatively small number of participants included in the study and the partial-block crossover design are possible limitations of this study, although the sample size did provide sufficient power to detect significant differences. We realise that the CNS tests included in this study measure the function of specific CNS functional domains and serve as an index of clinically relevant side effects (e.g., sedation, coordination, and bodily stability) but cannot directly be related to clinical practice.

In conclusion, we demonstrated that oliceridine had less impact on CNS functioning than morphine on a wide range of tests. Our observations are in line with previous studies that aimed to evaluate improved safety profile of the biased ligand oliceridine over the classic opioid morphine. Additional studies are required to confirm whether our findings would be duplicated in clinical practice.

SUPPORTING INFORMATION

APPENDIX A: Study Protocol
APPENDIX B: Statistical Analysis Plan
APPENDIX C: Results of all the Pharmacodynamic Measurements

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CHAPTER 7

A FIRST-IN-HUMAN CLINICAL TRIAL: ASSESSMENT OF SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF ALKS 6610

Submitted

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Abstract

AIM Opioids remain essential for pain management, but their adverse effects limit therapeutic use. Novel μ-opioid receptor (MOP) agonists with unique binding, pharmacokinetic or signalling properties are hypothesised to be effective and cause less side effects, in particular respiratory depression. ALKS 6610 is a selective, partial MOP agonist with potential signalling bias favouring the G-protein pathway over β arresting recruitment. This first-in-human (FIH) study evaluated the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALKS 6610 in healthy adults.

METHODS In a randomised, double-blind, placebo-controlled, single ascending dose study, 56 participants received single oral doses of ALKS 6610 (25–825 mg) or placebo (ratio 3:1). Safety, tolerability, PK, and PD (pupillometry and Bond & Lader visual analogue scale) were assessed. A pilot food-effect evaluation was also conducted at 150 mg.

RESULTS ALKS 6610 was generally well-tolerated up to 750 mg. The 825 mg dose caused dose-limiting nausea and vomiting. No respiratory depression or sedation was observed at any dose. PK analysis showed dose-proportional increases in exposure up to 750 mg (median t_{max} 2.25–3.5 hours, $t_{½}$ 9.8–16 hours). High-fat meals reduced exposure by 40%. Pupillometry confirmed MOP engagement with miotic effects at doses ⩾450 mg, exhibiting an apparent ceiling effect thereafter.

CONCLUSION Data from this FIH study suggests that ALKS 6610 engages the MOP as a partial agonist and demonstrated a favourable safety and tolerability profile up to 750mg, possibly with fewer respiratory and sedative side effects than classic opioids.

Introduction

Over the past decades, widespread use of opioids for managing acute and chronic pain has led to significant public health challenges including an epidemic of misuse, addiction, and overdose deaths.[1] Well-documented adverse effects associated with opioids such as respiratory depression, sedation, and opioid use disorder, complicate their therapeutic use.[2] Despite a notable decline in prescription opioid use in the United States since its peak in 2011,[3] millions of patients continue to be exposed to these serious health risks. Therefore, the development of safer opioids that offer effective pain relief with fewer side effects and reduced abuse potential remains critical.

The μ-opioid receptor (MOP) is a G-protein coupled receptor (GPCR), prevalent in all mammals, that is primarily involved in the modulation of pain. Drugs that act through the MOP remain a mainstay of pharmacotherapy for moderate-to-severe pain.[4] However, it is the activation of this receptor that is associated with the severe adverse effects caused by opioids. A recent approach aimed at creating safer opioids regards the designing of so-called 'biased ligands'. Biased ligands for the MOP that have a preference toward G-protein coupled intracellular signalling but low levels of β-arrestin recruitment are thought to retain analgesic properties, while reducing opioid-related side effects (in particular respiratory depression). This paradigm is based on preclinical studies in β-arrestin2 knockout mice[5] and further substantiated by recent clinical studies indicating advantages of the biased opioid oliceridine over morphine with respect to respiratory depression and central nervous system (CNS) functioning.[6,7] Additionally, opioid analgesics with slower or reduced brain exposure have been postulated to have a lower potential of abuse and addiction due to less rapid activation of brain circuits underlying reward and reinforcement.[8]

ALKS 6610 is a selective, partial MOP agonist with preliminary evidence of biased signalling toward the G-protein pathway and a predicted reduced rate of brain penetration. When studying ALKS 6610 pre-clinically, no β-arrestin2 recruitment was measured in *in vitro* assays for rat MOP signalling.

Here we present the results from a first-in-human (FIH) study that evaluated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of ALKS 6610 in healthy participants following administration of single ascending doses.

Methods

Trial design and participants

The study consisted of a randomized, double-blind, placebo-controlled, single ascending dose design with 7 cohorts of 8 participants each, and a pilot food effect evaluation. Participants who successfully completed a medical screening received single oral administrations of ALKS 6610 ranging from 25 to 825 mg or placebo (ratio 3:1) as part of the dose escalation study. The food effect evaluation consisted of an extended stay for a single cohort that included a second, double-blinded administration of the study drug (ALKS 6610 150 mg or placebo) in fed state.

The study enrolled healthy males and females (of non-childbearing potential) aged 18-60 years with a body mass index of 18-30 kg/m2. Exclusion criteria included: history of clinically significant illness or disease (including gastrointestinal surgery or conditions possibly affecting PK profiles); multiple cardiac criteria such as prolonged QTc interval or left bundle branch block demonstrated on electrocardiogram (ECG); presence of contraindications for opioid administration; history of substance use disorder per Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), or a positive drug test or alcohol test; history of lifetime suicidal ideation or behaviour, confirmed by responses to the Columbia Suicide Severity Rating Scale (C-SSRS).

Concomitant medications were prohibited from 2 weeks before administration of the first dose of study drug. Use of alcohol and caffeinated beverages or food was not allowed from 48 hours before each visit until discharge from the clinic. Participants refrained from using nicotine-containing products from 2 weeks before first dosing until the end of study participation. (Supplementary File 1)

In response to the COVID-19 pandemic, additional safety measures including pre-dose SARS-COV-2 PCR testing were put in place.

Setting and location of data collection

After approval of the protocol by the Medical Research Ethics Committee, Foundation Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands), this study was conducted at the Centre for Human Drug Research (Leiden, The Netherlands). The study was prospectively registered with the Dutch Trial Register (NL8337). Written informed consent was obtained for each participant prior to performing any study related measurements. The study was performed in accordance with the International Conference on Harmonisation guidelines on Good Clinical Practice guidelines, as laid down in the Declaration of Helsinki Declaration and its latest amendments.

Interventions

Participants were admitted to the clinic two days prior to dosing and underwent pre-dose safety assessments to reconfirm eligibility. Holter ECG recording with time-matched ECG tracings was started from 25-hours before and continued until 24-hours after study drug administration. The morning on the day of dosing, baseline safety, PK and PD measurements were performed, after which study drug was administered to participants in fasted state. Measurements were then repeated post-dose per predefined schedule up to 144-hours post-dose (Supplementary File 1 Table 5).

Safety – Safety and tolerability assessments included continuous monitoring for adverse events as classified according to MedDRA v21.1, vital signs, single ECGs and continuous cardiac Holter monitoring (Global Instrumentation, NY, USA), laboratory assessments, physical examinations and the C-SSRS questionnaire. Given the known side effects of opioids, continuous pulse oximetry using a finger probe (Nellcor™, Medtronic, MN, USA) and frequent end-tidal CO_2 measured through a nasal cannula (Capnostream™ 35, Medtronic) were included as respiratory parameters, and the validated Pasero Opioid-Induced Sedation Scale (POSS) was used to record sedation as scored by the investigator.

Pharmacokinetics (PK) – Blood plasma and urine were collected to allow for PK characterization of ALKS 6610 and its inactive metabolite RDC-059525. Blood samples were drawn pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 6, 8, 10, 12, 14, 24 (day 2), 48 (day 3), 72 (day 4), 96 (day 5), and 144 hours (day 7) post-dose. Urine samples were collected for the following intervals: 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, and 72-96 hours post-dose. Plasma and urine concentrations were analysed by Ardena Bioanalysis (Assen, the Netherlands) using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods.

Pharmacodynamics (PD) – Further measurements selected for this trial aimed to confirm target receptor engagement, as well as exploring the PD profile early on in the drug development process. Pupil diameter was

measured as the biomarker effect resulting from drug activity at the MOP.[9] Participants were seated in a chair while resting their head in a support system, in a room with dim lighting set at LUX of 30-125. A picture was taken from both eyes simultaneously (Canon EOS 1100D, Tokio, Japan) and the pupil-iris diameter ratio was automatically calculated at each timepoint (Qpupil, Jasper Janssen, LUMC, the Netherlands). Pupillometry was performed twice pre-dose and after each PK blood draw (separated by at least 3 minutes).

Additionally, the Bond & Lader visual analogue scale (B&L VAS) was included to measure subjective drug effects regarding alertness, mood, and calmness.[10,11] The B&L VAS has been frequently used in drug development to quantify psychotropic side-effects through a self-rated questionnaire consisting of 16 analogue scales from which the three affective dimensions are derived. This assessment was taken twice pre-dose and at 1, 2, 4, 6, 8, 24, 48, 72, and 96 hours post-dose.

Food effect – Participants in cohort 3 first completed their inpatient stay as part of the ascending dose design, before remaining in clinic for an additional week to take part in the pilot food effect evaluation. These participants received the same dose of ALKS 6610 or placebo twice, 7 days apart. Within 30 minutes prior to the second study drug administration, participants finished a standardized a high-fat breakfast. The rest of the measurements was identical to the study period with initial dosing.

Dose selection

Doses were determined from nonclinical toxicology studies with appropriate scaling. The dose range selected for this study were within the boundaries of exposures supported by the one-month toxicology studies.

Statistical methods

The study was double-blinded, and participants were randomly assigned to treatments. The randomization code was produced by a qualified vendor not involved in study execution (K&L), and only made available to the pharmacist preparing the study drug and the individuals responsible for preparing blinded summaries, graphs, and listings to support the dose decisions. The investigational drug and matching placebo were indistinguishable and were packaged in the same way.

The primary objective of the study was to evaluate safety, tolerability, PK and PD of ALKS 6610 after single ascending oral administration. All data were descriptively reported, presented as mean ± SD.

A mixed effects model was used to obtain preliminary information on the effect of a high-fat, high-calorie meal on the plasma PK of ALKS 6610 and RDC-059525. No formal sample size calculations were performed. Cohort size and the ratio active drug versus placebo was consistent with typical FIH dose-escalation study designs to evaluate initial safety, PK, and PD effects.

Noncompartmental analysis and programming of PK tables and figures was conducted with R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019). All listings, summaries, and statistical analyses were generated using SAS® version 9.4 or higher (SAS Institute Inc., Cary, NC, USA).

Results

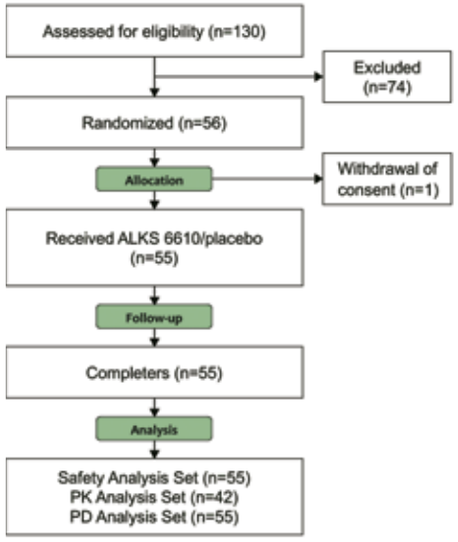
The study was performed from January 2020 to October 2020 during which a total of 130 participants were screened of whom 56 were randomized and 55 received study drug (**Figure 1**). One participant who was randomized to the placebo group withdrew consent prior to dosing, did not receive study drug and was subsequently not included in the analysis. Participant demographics and other baseline characteristics are summarized in **Table 1**.

Safety and tolerability

An overview of adverse events that occurred in at least 2 participants in any treatment can be found in **Table 2**. No serious adverse events occurred. Adverse events were reported in 73.8% (31/42) of participants who received ALKS 6610, with mostly mild gastrointestinal adverse events (nausea and vomiting) of which the frequency increased in a dose-dependent manner, and dizziness especially in the highest dose tested. Most or all participants receiving the two highest doses, 750 and 825 mg, experienced nausea and vomiting. The highest dose tested of 825 mg was assessed as not tolerated, with 2 participants experiencing moderate vomiting.

There were no clinically meaningful differences observed between any ALKS 6610 dose and placebo regarding vital signs, respiratory effects, laboratory assessments, physical examinations, C-SSRS, and POSS.

Figure 1 Participant disposition (CONSORT flow diagram).



Abbreviations: PD = pharmacodynamic, PK = pharmacokinetic.

Table 1 Participant baseline characteristics.

	Placebo	ALKS 6610						
		25 mg	75 mg	150 mg	225 mg	450 mg	750 mg	825 mg	All
	(N=13)	(N=6)	(N=6)	(N=6)	(N=6)	(N=6)	(N=6)	(N=6)	(N=42)
AGE (YEARS)									
Mean (SD)	30.4 (13.46)	36.8 (14.40)	34.2 (13.72)	26.2 (6.55)	27.3 (6.06)	38.3 (14.90)	39.3 (17.91)	30.7 (12.14)	33.3 (12.91)
SEX, N (%)									
Male	11 (84.6)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	5 (83.3)	4 (66.7)	5 (83.3)	38 (90.5)
Female	2 (15.4)	-	-	-	-	1 (16.7)	2 (33.3)	1 (16.7)	4 (9.5)
RACE, N (%)									
White	10 (76.9)	5 (83.3)	3 (50.0)	4 (66.7)	5 (83.3)	3 (50.0)	4 (66.7)	5 (83.3)	29 (69.0)
Black or African American	-	-	2 (33.3)	1 (16.7)	-	1 (16.7)	1 (16.7)	1 (16.7)	6 (14.3)
Asian	-	1 (16.7)	1 (16.7)	1 (16.7)	-	1 (16.7)	-	-	4 (9.5)
Other	3 (23.1)	-	-	-	1 (16.7)	1 (16.7)	1 (16.7)	-	3 (7.1)
WEIGHT (KG)									
Mean (SD)	74.8 (11.95)	75.2 (10.69)	79.6 (6.73)	75.6 (6.97)	69.7 (9.36)	73.2 (10.14)	82.9 (16.36)	75.4 (6.71)	75.9 (10.14)
HEIGHT (CM)									
Mean (SD)	180.0 (10.88)	179.2 (8.30)	178.6 (5.58)	183.1 (6.85)	175.0 (4.15)	175.9 (15.08)	174.9 (12.20)	180.0 (6.91)	178.1 (8.93)
BMI (kg/m^2)									
Mean (SD)	23.2 (2.39)	23.8 (2.53)	24.4 (3.20)	22.6 (1.47)	22.7 (2.81)	23.7 (2.37)	26.8 (3.12)	23.3 (1.86)	23.9 (2.71)

Abbreviations: BMI = body mass index; SD = standard deviation.

TABLE 2 Adverse events in ⩾2 of Participants in Any Treatment Group by Preferred Term.

SOC / PT	Placebo, n (%)				ALKS 6610, n (%)									
	All Cohorts Day 1 (N=13)	Cohort 3 Day 1 (N=2)	Cohort 3 Day 8 (N=2*)	All Cohorts Overall (N=13)	25 mg (N=6)	75 mg (N=6)	150 mg Day 1 (N=6)	150 mg Day 8 (N=6*)	225 mg (N=6)	450 mg (N=6)	750 mg (N=6)	825 mg (N=6)	All ALKS 6610 (N=42)	
													Day 1	Overall
Adverse event	6 (46.2)	1 (50.0)	2 (100.0)	7 (53.8)	2 (33.3)	4 (66.7)	2 (33.3)	3 (50.0)	5 (83.3)	5 (83.3)	6 (100.0)	6 (100.0)	30 (71.4)	31 (73.8)
Nausea	-	-	-	-	-	-	1 (16.7)	-	2 (33.3)	2 (33.3)	6 (100.0)	6 (100.0)	17 (40.5)	17 (40.5)
Vomiting	-	-	-	-	-	-	-	-	-	1 (16.7)	6 (100.0)	4 (66.7)	11 (26.2)	11 (26.2)
Somnolence	-	-	-	-	1 (16.7)	2 (33.3)	-	1 (16.7)	2 (33.3)		2 (33.3)	2 (33.3)	9 (21.4)	10 (23.8)
Dizziness†	-	-	-	-	-	1 (16.7)	1 (16.7)	-	-	1 (16.7)	1 (16.7)	3 (50.0)	7 (16.7)	7 (16.7)
Headache	-	-	-	-	1 (16.7)	1 (16.7)	-	-	2 (33.3)	1 (16.7)	0	1 (16.7)	6 (14.3)	6 (14.3)
Constipation	-	-	-	-	-	-	-	-	-	-	2 (33.3)	2 (33.3)	4 (9.5)	4 (9.5)
Feeling of relaxation	-	-	-	-	-	-	-	1 (16.7)	-	2 (33.3)	-	1 (16.7)	3 (7.1)	4 (9.5)
Dizziness postural	1 (7.7)	-	-	1 (7.7)	-	-	-	1 (16.7)	-	1 (16.7)		1 (16.7)	2 (4.8)	3 (7.1)
Chest discomfort	-	-	-	-	-	1 (16.7)	-	-	1 (16.7)	-	-	1 (16.7)	3 (7.1)	3 (7.1)
Fatigue	1 (7.7)	-	-	1 (7.7)	-	1 (16.7)	-	-	-	1 (16.7)	-	1 (16.7)	3 (7.1)	3 (7.1)
Orthostatic hypotension	2 (15.4)	1 (50.0)	-	2 (15.4)	-	-	1 (16.7)	2 (33.3)	-	-	-	1 (16.7)	2 (4.8)	3 (7.1)
Catheter site pain	-	-	-	-	-	-	-	-	2 (33.3)	-	-	-	2 (4.8)	2 (4.8)
Dry throat	-	-	1 (50.0)	1 (7.7)	-	1 (16.7)	-	-	-	-	1 (16.7)	-	2 (4.8)	2 (4.8)
Hallucination, visual	-	-	-	-	-	-	-	-	1 (16.7)	1 (16.7)	-	-	2 (4.8)	2 (4.8)
Hepatic enzyme increased	1 (7.7)	-	-	1 (7.7)	-	1 (16.7)	-	-	1 (16.7)	-	-	-	2 (4.8)	2 (4.8)
Hiccups	-	-	-	-	-	-	-	-	-	-	1 (16.7)	1 (16.7)	2 (4.8)	2 (4.8)
Medical device site reaction	-	-	1 (50.0)	1 (7.7)	-	-	-	-	1 (16.7)	-	1 (16.7)	-	2 (4.8)	2 (4.8)
Presyncope	-	-	-	-	-	-	-	1 (16.7)	-	1 (16.7)	-	-	1 (2.4)	2 (4.8)
Tremor	-	-	-	-	-	-	-	-	-	-	1 (16.7)	1 (16.7)	2 (4.8)	2 (4.8)
Dizziness†	-	-	-	-	-	1 (16.7)	-	-	-	-	-	1 (16.7)	2 (4.8)	2 (4.8)

*Abbreviations: PT=preferred term; SOC=system organ class. *Participants in Cohort 3 received the same treatment on Day 1 and Day 8 (either placebo or ALKS 6610, i.e., no crossover). †Dizziness was reported under Vascular disorders SOC when it was observed and assessed to be likely due to orthostatic changes, and under Nervous system disorders SOC when it was assessed to be likely vestibular in nature by the Investigator. Note: The safety population is defined as all randomized participants who receive at least one dose of study drug. Adverse events were defined as events that newly occurred or worsened from the time of the first dose of study drug. Note: For participants who received doses on both Day 1 and Day 8 in Cohort 3, all adverse events are attributed to either dosing under the fasted condition (onset after the first dose and before/on the second dose), or under the fed condition (onset after the second dose). Note: If a participant experienced more than one adverse event in a category, the participant is counted only once in that category.*

PK

PK parameters of ALKS 6610 were evaluated throughout a 144-hour period. Mean plasma concentration-time profiles are displayed in Figure 2. PK parameters are presented in Table 3.

Following oral administration, ALKS 6610 was absorbed with a median t_{max} ranging from 2.25 to 3.5 hours. The peak concentrations (C_{max}) as well as exposures (AUCs) of ALKS 6610 increased dose-proportionally up to 750 mg dose level. No further increase in C_{max} and AUC of ALKS 6610 was observed when dose was increased from 750 mg to 825 mg. The highest exposure of ALKS 6610 was observed at the 825 mg dose, which resulted in a mean C_{max} of 1295.000 ng/ml and mean AUC_{0-last} of 11688.199 ng h/ml. The mean terminal half-life ($t_{½}$) 9.8 to 16.0 hours over 25 to 450 mg dose range. At the 750 mg and 825 mg dose levels, a mean $t_{½}$ of 24 and 27.2 hours, respectively, was observed.

Renal clearance was estimated to be 21.1% to 33.8%, suggesting renal elimination may be one of the prominent clearance pathways.

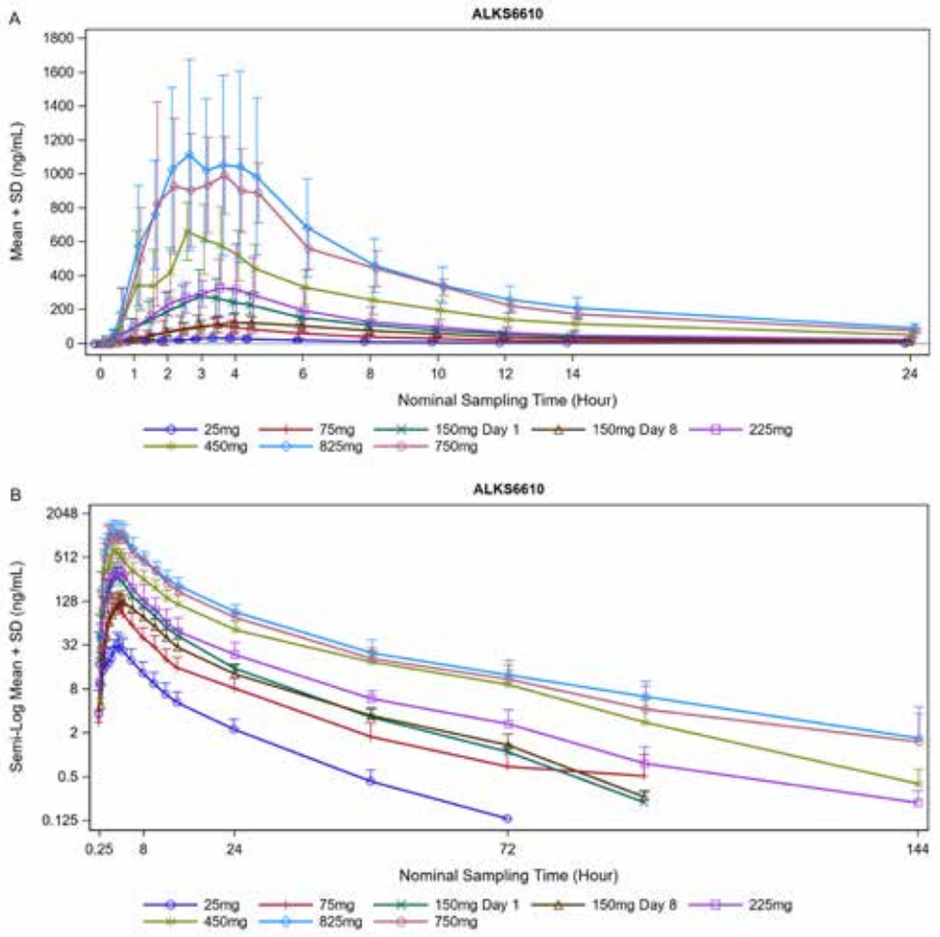
Administration of ALKS 6610 with high fat meal had no impact on median t_{max}, $t_{½}$ and Cl/F, but exposure of ALKS 6610 was reduced by 40%.

Table 3 Summary of Plasma PK Parameters of ALKS 6610 – PK Population[1].

Dose (mg)	25 mg (N=6)	75 mg (N=6)	150 mg (N=6)	225 mg (N=6)	450 mg (N=5)	750 mg (N=4)	825 mg (N=4)	150 mg-Fed (N=6)
Arithmetic Mean ± Standard Deviation [Coefficient of Variation]								
AUC_{0-inf} (ng·h/mL)	287.5 ± 105.1 [36.6%]	953.6 ± 376.9 [39.5%]	2372.5 ± 482.4 [20.3%]	3024.1 ± 1277.2 [42.2%]	6389.7 ± 2029.7 [42.2%]	10371.5 ± 1251.0 [12.1%]	11745.9 ± 4362.6 [37.1%]	1455.6 ± 425.0 [29.2%]
CL/F (mL/h)	97253.9 ± 34485.5 [35.5%]	90711.3 ± 38126.7 [42.0%]	65174.5 ± 11751.0 [18.0%]	83166.9 ± 26052.9 [31.3%]	83161.1 ± 26102.0 [31.4%]	75765.7 ± 4999.4 [6.6%]	92212.7 ± 32140.5 [34.9%]	112583.0 ± 40368.7 [35.9%]
C_{max} (ng/mL)	35.6 ± 14.1 [39.5%]	125.4 ± 58.9 [47.0%]	334.7 ± 113.4 [33.9%]	413.5 ± 225.5 [54.5%]	763.8 ± 164.4 [21.5%]	1152.0 ± 149.1 [12.9%]	985.0 ± 344.4 [35.0%]	140.3 ± 44.6 [31.8%]
$t_{½}$ (h)	9.8 ± 2.3 [23.6%]	150 ± 10.0 [66.4%]	11.2 ± 1.7 [15.4%]	13.7 ± 2.0 [14.6%]	15.9 ± 1.8 [11.0%]	27.2 ± 24.8 [91.0%]	23.9 ± 10.1 [42.5%]	13.2 ± 2.6 [19.6%]
Median (Min, Max)								
t_{max} (h)	3.50 (0.50, 4.50)	3.50 (2.50, 4.00)	3.00 (1.50, 4.00)	3.25 (2.00, 4.00)	2.50 (2.50, 4.00)	3.50 (1.48, 4.00)	2.25 (2.00, 4.50)	4.00 (3.50, 4.50)

*1. The PK population is defined as all subjects in the safety population who have sufficient PK data to derive at least one PK parameter. The PK population excluded 5 subjects who vomited. Abbreviations: 0-12= from time 0 to 12 hours after dosing; 0-24= from time 0 to 24 hours after dosing; AUC= area under the concentration–time curve; CI=confidence interval; CL/F= apparent total clearance following extravascular administration; C_{max} =maximum concentration observed; inf=from time zero to infinity; last=from time zero to time of last measurable concentration; $t_{½}$=terminal elimination half-life; t_{max} =time to attain C_{max}. Geometric coefficient of variation = square root of [exp(variance(parameter on log scale))-1] *100%*

Figure 2 Human Plasma Concentration (Mean + SD) of ALKS 6610 after Single Dose Administration by Nominal Sampling Time.

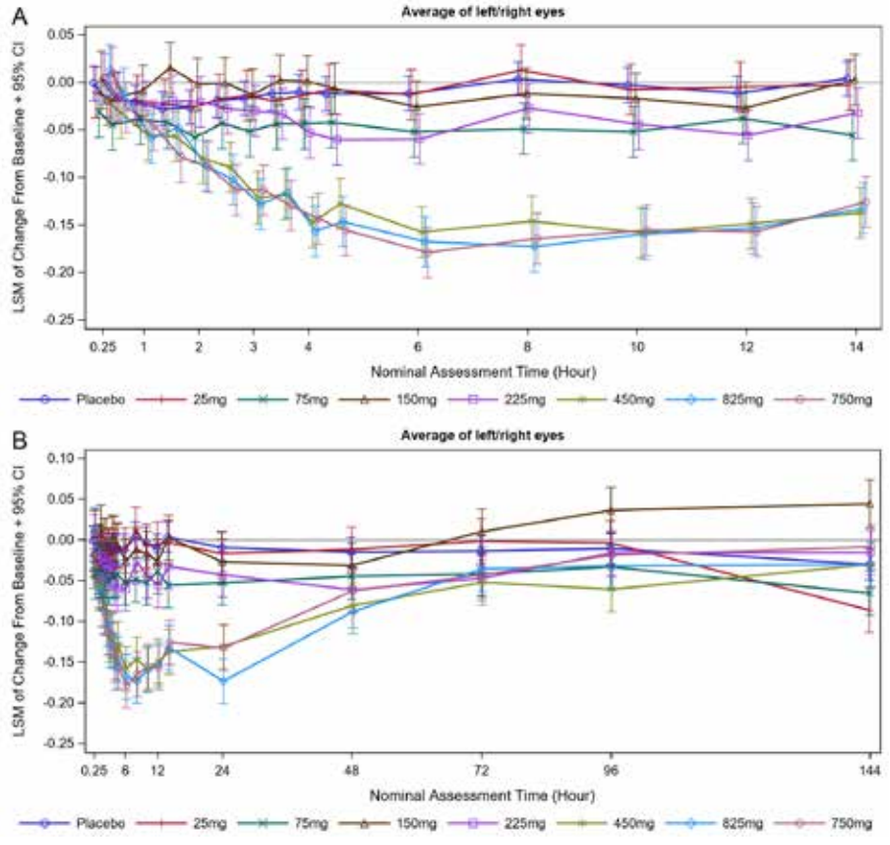


Abbreviations: SD = standard deviation. Note: Top panel (A) shows the PK data from 0-24 hours post-dose on a linear scale. The lower panel (B) shows the PK data from all timepoints up to 144 hours post-dose on a semi-log scale. Values below the lower limit of quantification (<LLOQ) have been set to zero in the calculation of the summary statistics.

PD

Pupil diameter decreased as plasma concentrations increased (Figure 3). No apparent dose-dependent effects on pupil diameter were observed at the lower dose levels up to 225 mg. For the three highest dose levels, 450, 750 and 825 mg, clear effects were observed with the maximum decrease in pupil/iris ratio at 8 hours post-dose (mean % change from baseline (SD): -32.848 (8.085), -33.296 (11.331), -33.407 (3.847) %, respectively. However, no dose-dependent differences were observed between those dose levels.There were no clinically relevant differences recorded for the B&L VAS.

Figure 3 LSM Change from Baseline (95% CI error bars) for Pupillometry.



Abbreviations: CI = confidence interval; LSM = Least Square Means. Note: Top panel (A) shows the pupillometry data from 0-14 hours post-dose. The lower panel (B) shows the pupillometry data from all timepoints up to 144 hours post-dose.

Discussion

Here we report on the findings in a FIH randomized controlled trial in which the safety, tolerability, PK and PD of the drug candidate ALKS 6610, a highly selective, partial MOP agonist (with potential biased signalling toward the G-protein pathway) was studied.

Although the human safety profile regarding the gastrointestinal (GI) tract and dizziness was as may be expected of any opioid (i.e., biased or not), ALKS 6610 did not result in clinically observed respiratory depression or sedation. Despite the simultaneous occurrence of nausea and dizziness in some cases, nausea was regarded as a stand-alone symptom rather than secondary to e.g., vestibular apparatus stimulation because of the difference in time of onset and duration. Overall, administration of ALKS 6610 was safe at all doses tested. Although dose escalation planned per protocol was to continue up to 1200 mg, this was halted after administration of 825 mg when that dose level was regarded not tolerable with two participants experiencing moderate symptoms of vomiting and the other four participants receiving active drug reported feeling nauseous for prolonged periods of time. Additionally, an apparent ceiling effect was observed for the pupillometry beyond the 450 mg dose. The final cohort was then administered a lower dose of ALKS 6610 750 mg, which was found to lead to milder GI symptoms. Reflecting on the dose selection in preparation of the study, the maximum tolerated dose level was lower than anticipated based on preclinical findings. However, it is important to note that no abnormalities were observed in safety parameters regarding the respiratory system (SpO_2 and $etCO_2$) and investigator-recorded sedation (POSS), which may be a result from the PK characteristics or pharmacological properties, including partial (not full) agonism or biased signalling at the MOP.

Human exposures increased proportionally to dose, and median t_{max} remained consistent across the tested dose range. ALKS 6610 was eliminated with a mean terminal half-life ($t_{½}$) ranging 9.8 - 16.0 hours over 25 to 450 mg dose range suggesting non-saturation of elimination pathways over this dose range. At 750 mg and 825 mg dose levels, mean $t_{½}$ of 24 and 27.2 hours, respectively, was observed, which could be due to high observed CV% (91% and 42.5%, respectively). The ratio of exposures of the main metabolite RDC-059525 ranged from 12.9% to 17.1% across all doses tested, suggesting non-saturation of metabolism pathways. Taken together, these findings supported the potential of ALKS 6610 to be an orally administered once daily treatment for relevant indications. The notable reduction in systemic exposure after co-administration of ALKS 6610 with a high fat meal suggested a clinically relevant decrease in absorption. However, the objective in this study was to obtain preliminary information on the effect of high fat food on PK, but not to perform an official FDA approved food interaction study. Renal clearance data suggest that renal excretion could play a prominent role in the elimination of ALKS 6610 and its metabolites.

Pupillometry is widely used as a biomarker for MOP target engagement.[12] miotic effects were observed in humans following PK, revealing central MOP engagement at lower equivalent doses. Between the 225 and 450 mg doses, a large increase in effect was observed, possibly due to a steep concentration-effect curve. The nonlinear effect profile may be attributed to high affinity receptor binding of ALKS 6610 to the MOP, or, hypothetically, intracellular events such as signal amplification may be also responsible. Another possible explanation of the assumed steep concentration-effect curve could be related to blood-brain barrier (BBB) penetration. Linear PK was observed in plasma, but no PK was measured in cerebrospinal fluid so disproportionality of CNS PK relative to increasing plasma levels cannot be excluded (e.g., due to saturation of the efflux transporter P-glycoprotein). Additionally, an apparent ceiling effect was observed from the 450 mg dose upwards, most likely the result of the partial agonist properties of ALKS 6610. This observation was not due a ceiling in the maximum possible test effect given the previous studies in which larger pupillary effects induced by other MOP agonists were recorded,[13] nor was it due to receptor desensitization or tolerance development as no repeated dosing was performed in this study and only opioid-naïve participants were included. Importantly, no ceiling was observed for GI related adverse events. This implies that those effects arose via a different pathway; presumably through the chemoreceptor trigger zone which forms a permeable BBB and detects emetic toxins in the blood.[14]

Lastly, we did not observe subjective drug effects in domains commonly linked with opioids after treatment with ALKS 6610, as measured with the B&L VAS. Although reports vary, previous studies testing therapeutically relevant doses of full MOP agonists such as morphine and methadone showed reduced alertness and/or increased calmness compared to placebo, as could be expected from drugs with CNS depressant

effects. The GI-related side effects at higher doses could have modulated the effects on the POSS and B&L VAS by causing sympathetic activation resulting in less sedation and might explain the (absence of) observed effects. However, this is unlikely given that morphine and other full MOP agonists also produce significant nausea in opioid naïve individuals,[15] so we therefore attribute the findings to ALKS 6610's partial agonist profile and its potentially biased agonism.

There are several limitations in the human trial worth addressing. In order to ensure that each participant was opioid-naïve, we did not implement a (partial) crossover design, but this meant that we knowingly introduced more statistical variability when comparing effects across cohorts. Secondly, we measured common opioidergic side effects using peripheral O_2 saturation, etCO_2 and sedation scales, but specialized studies aimed at evaluating effects on ventilatory function and neurocognitive functioning would be required to confirm our findings.[16] Furthermore, only pupillometry was included as a biomarker in the FIH trial, so no human data on the potential analgesic effects of ALKS 6610 are as of yet available, which would allow for a complete discussion on potential use in clinic. Finally, extrapolation of the results to the general population may be limited because the FIH study was performed in highly standardized setting with a study population excluding WOBCP and elderly, together forming a significant part of the target population.

In conclusion, our data suggests that ALKS 6610 engages the MOP as a partial agonist and is generally tolerated up to 750mg, possibly with fewer respiratory and sedative side effects than classic opioids.

SUPPORTING INFORMATION

S1 Study Protocol

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CHAPTER 8

ENA-001, A NOVEL POTASSIUM-CHANNEL BLOCKER, INCREASES SENSITIVITY TO HYPOXIA AND MITIGATES PROPOFOL-INDUCED RESPIRATORY EFFECTS IN HEALTHY VOLUNTEERS

Submitted

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Abstract

BACKGROUND Interference with normal ventilatory control is a common and potentially deleterious iatrogenic event during procedural sedation. Given that no specific antagonists are available for commonly used anaesthetics such as propofol, agnostic drugs that stimulate ventilation without influencing sedation or analgesia represent a major paradigm shift. ENA-001, which stimulates ventilation by acting at large conductance calcium-activated potassium channels (BK_{Ca2+}) expressed on type 1 carotid body cells, is being developed as such agnostic therapy for the treatment of respiratory depression in procedural patients.

METHODS In this randomized, double-blinded, placebo-controlled, crossover study, the effect of two continuous intravenous infusion doses of ENA-001 (6.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 18.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on propofol-induced respiratory depression was investigated during three separate visits in 14 healthy volunteers. At each visit, the acute hypoxic response (rise in minute ventilation per unit drop in arterial oxygen saturation) was assessed at multiple timepoints during concomitant treatment with (open-label, fixed sequence) placebo, low- and high- dose propofol infusion (targeting plasma concentrations of 600 and 1200 ng/mL, respectively) and under normo- and hypercapnic conditions.

RESULTS Administration of the ENA 001 high dose was found to significantly increase the hypoxic responsiveness compared to placebo (estimated difference [95% confidence interval]: 0.575 (0.407, 0.744), $p<0.0001$), and mitigate the respiratory effects induced by clinically relevant doses of propofol while maintaining a favourable safety profile.

CONCLUSION Data from this study support further development of ENA-001 as an agnostic respiratory stimulant for use in clinical practice.

Introduction

During procedural sedation, there is an increased risk of respiratory depression and airway obstruction due to drug interference with ventilatory control.[1] It is not possible to predict the onset, duration, or severity of deleterious respiratory events due to a number of contributing factors, including individual differences in drug sensitivity, level of sedation/pain management, pulmonary and central nervous system (CNS) dysfunction, underlying disease (e.g., pulmonary or cardiac disease) and concomitant medications. Therefore, the appropriate use and management of medication during procedural sedation is crucial to ensure patient safety and optimize recovery outcomes.[2] A strategic paradigm aiming to minimize the risks associated with these medications and improve patient outcomes includes the use of agnostic respiratory stimulants.[3]

Ventilation is controlled largely in the brainstem with input from the cortex and peripheral nerves. Chemoreceptors exist in both the brainstem (these central chemoreceptors are sensitive to carbon dioxide) and in the carotid bodies (these peripheral chemoreceptors are sensitive to oxygen and carbon dioxide, among other stimuli).[4,5] Oxygen sensing originates in the type 1 glomus cells of the carotid bodies. Although the exact mechanism of oxygen sensing remains poorly understood, oxygen sensitive potassium channels (e.g., BK_{Ca2+}, TASK-1, and TASK-3) play a crucial role.[6–9]

ENA-001, previously known as GAL-021, is a fast acting and short-duration intravenous agent acting partially by blocking the large conductance calcium-activated potassium channels (BK_{Ca2+}, Slo, Maxi K channels) in the carotid body to stimulate respiration. It has been previously reported that ENA-001 produced clear ventilatory stimulation in healthy subjects and attenuated suppression of ventilation by the opioid alfentanil.[10–13] It remains unknown which effects ENA-001 has on the hypoxic ventilatory response in humans, and the ability to attenuate the blunting of hypoxic sensitivity by a non-opioid central depressant.

Propofol is the most widely used short acting anaesthetic agent. Accompanying the benefits of propofol during procedural sedation, are undesired ventilatory effects with reduced respiratory drive and diminished neuromuscular tone in the upper airways.[14,15] This study aimed to further evaluate the potential of ENA-001 through measuring sensitivi-

ty to hypoxia, and to investigate whether it can successfully mitigate propofol-induced depression of the ventilatory response to hypoxia, i.e., a hypoxic challenge.

Methods

Ethics

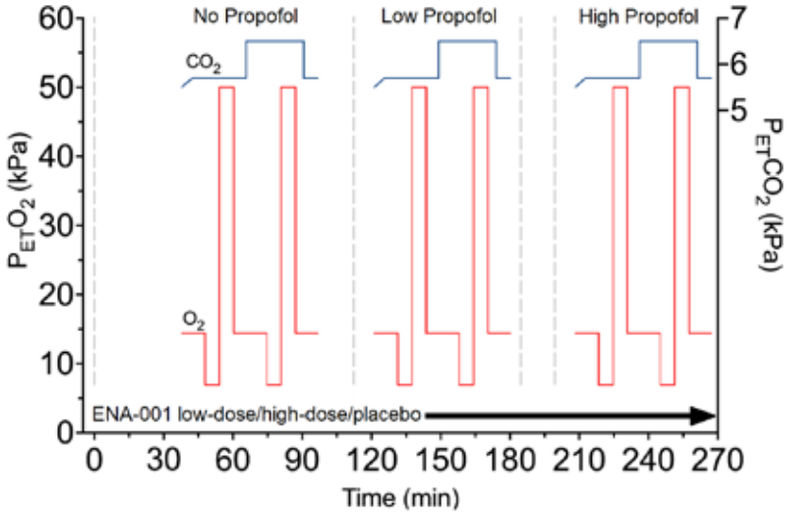
After approval of the protocol (EudraCT 2021-003013-19) by the Medical Review and Ethics Committee of the BEBO foundation (Assen, The Netherlands), the study experiments were performed at the Department of Anaesthesiology of the Leiden University Medical Centre (LUMC, The Netherlands) and all other activities regarding trial execution were performed at the Centre for Human Drug Research (Leiden, The Netherlands). The study was registered prospectively with the Dutch Trial Register, number NL9692. Before participation, all subjects gave written informed consent. The study was performed in accordance with the ethical principles for medical research involving human subjects of the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice (ICH GCP), and ethical principles as referenced in EU Directive 2001/20/EC.

Study design

This was a randomized, double-blinded, placebo-controlled, three-period crossover phase 1b clinical study. On each study day, placebo, low or high doses of ENA-001 were intravenously infused continuously for 270 minutes (see section Interventions). During study days, participants received concurrent intravenous placebo, low then high propofol dosages (open label, ascending order). The primary endpoint of the study was the ventilatory response to an acute hypoxic challenge (Acute Hypoxic Response; AHR):

AHR = (V_E in hypoxia – V_E in normoxia)/(SpO_2 in normoxia - SpO_2 in hypoxia), wherein V_E = minute ventilation in L/min and SpO_2 = arterial haemoglobin oxygen saturation via pulse oximetry reported as percentage. The AHR was measured during each propofol dosage at both normal and high end-tidal CO_2 concentrations ($P_{ET}CO_2$) (i.e., 6 short hypoxic episodes per study period). Study design is presented in **Figure 1**. Participants were discharged approximately 24 hours after study drug administration, and approximately seven days (minimum of three) were planned between study days. Participants were randomized to one of six sequences of study days (for placebo, low and high dose of ENA-001) according to a code that was generated by a study independent CHDR statistician using SAS version 9.4. The randomization code was made available to the pharmacy preparing the study drugs and an unblinded physician of the department of anaesthesiology to set the ENA-001 infusion pump.

FIGURE 1 Schematic representation of a treatment period.[22]
Shown in figure: continuous infusion of ENA-001 high/low dose or placebo, three dosages of placebo or propofol (separated by vertical dotted lines) and two hypoxic runs per dosage (red lines). Each hypoxic run consisted of a hypoxia/hyperoxic exposure (corresponding left Y-axis). During each dosage there were two runs, one at normocapnia followed by one at hypercapnia (blue lines with corresponding right Y-axis).



Study participants

Twelve healthy volunteers (aged 18-55 years; body mass index of 18-30 kg/m² and weight of 50-100 kg) with electrocardiogram (ECG) conduction intervals within gender specific normal ranges (e.g., QTcF of <430 and <450 msec for males and females, respectively, PR interval <220 msec) and vital signs within prespecified ranges (body temperature 35.5-37.5 °C, systolic BP 90-150 mmHg, diastolic BP 40-95 mmHg and pulse rate 40-100 beats/min) were recruited to participate in the study. Main exclusion criteria were a history of clinically significant medical condition or psychiatric diseases including anxiety disorder, or depres-

sion, or history of moderate-severe motion sickness, a history of alcohol or drug abuse, positive urine drug screen or alcohol breath test at screening or during any study period, regular smoking in the last year (i.e., >5 cigarettes per week), known difficult airway access, and participation in any other investigational study within three months prior to study drug administration. All sexually active participants who were not surgically sterile were required to use effective contraception throughout the duration of the study and for up to 90 days after study conclusion.

Interventions

PHARMACODYNAMIC ASSESSMENTS

Experiments were performed after a minimum of 8-hour overnight fasting. Prior to the experiments, an intravenous catheter was placed for study drug administration and an arterial catheter was inserted into the radial artery of the contralateral arm for blood sampling and continuous hemodynamic monitoring (HemoSphere, Edwards Life Sciences, Irvine, USA).

During the experiments, participants were placed in a semi-recumbent position. A facemask was fitted over nose and mouth, that was connected to a pneumotachograph pressure transducer system (Hans Rudolph Inc., Kansas City, USA) and a capnograph (ISA OR+, Masimo, California, USA) measuring ventilation and gas mixture on a breath-to-breath basis. Inspired gas mixtures of O_2, CO_2, and N_2 were delivered by the 2nd generation Leiden gas mixer controlled by ACQ/RESREG software (LUMC, The Netherlands). The fraction of CO_2 in the inspired gas mixture was adjusted using the dynamic end-tidal forcing technique[16] to attain target $P_{ET}CO_2$ before starting the hypoxic ventilatory assessments. Two hypoxic assessments were performed per propofol dosage. For the assessment at isocapnia, $P_{ET}CO_2$ was maintained constant at 0.3 kPa above resting values for several minutes. Subsequently, inspired O_2 was rapidly reduced in order to reach an end-tidal O_2 concentration ($P_{ET}O_2$) of 5.8 kPa, inducing a desaturation to a SpO_2 80-84% lasting 5-7 minutes. Thereafter, the inspired oxygen concentration was increased to 50 kPa for 5-10 minutes to rapidly re-establish normoxia and thereby mitigate the effect of hypoxia on the brain and carotid body[17], before repeating the hypoxic assessment at an incremental $P_{ET}CO_2$ of 1.3 kPa above resting values during the assessment at hypercapnia. All measured variables were visualized on a computer screen in real time during the experiments and saved to disk for further analysis.

DRUG ADMINISTRATION

The LUMC trial pharmacy prepared the study drugs. ENA-001 (Enalare Therapeutics Inc., Princeton, USA) was diluted in an infusion bag of Ringer's lactate and matching placebo consisted of sterile Ringer's lactate alone. Infusion bags were labelled with randomization and visit numbers in masked fashion to ensure blinding. Glass bottles of propofol 10 mg/mL MCT/LCT (Fresenius Kabi, The Netherlands) were dispensed ready for use. The drugs were administered using infusion pumps (Infusomat® Space, B Braun, Melsungen, Germany). The ENA-001 infusion scheme was based on the subject's weight and was similar to the dosing scheme of a previous study[10,11]: ENA-001 low dose 33.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min followed by a continuous infusion of 6.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 260 min; ENA-001 high dose 33.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 20 min followed by a continuous infusion of 18.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 250 min. To maintain the double blind, a study independent unblinded physician controlled the infusion pump during the loading dose period.

After the ENA-001 loading dose and the 'placebo-propofol' dosage, propofol was administered over a 155-minute period, designed to attain target plasma concentration of 600 and 1200 ng/mL during the propofol low and high dose, respectively. The regimen was as follows: 3 min at 239 $\mu g \cdot kg^{-1} \cdot min^{-1}$, followed by 6 min at 0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 61 min at 24 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (low-dose phase), a subsequent transition dose of 15 min at 47 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and subsequently 3 min at 239 $\mu g \cdot kg^{-1} \cdot min^{-1}$, followed by 6 min at 0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 61 min at 44 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (high dose phase).

Participants were pre-treated with 4 mg intravenous ondansetron as a prophylaxis for nausea.

BLOOD SAMPLING

For pharmacokinetic (PK) analysis of ENA-001 and propofol, arterial blood samples were obtained at multiple timepoints between t = 0-300 min, and venous samples were obtained hourly thereafter until t = 720 min, and at 840, 1320 and 1440 min. ENA-001 and plasma propofol concentrations were determined (Ardena, Assen, The Netherlands) using liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays validated over a range of 0.250 to 500 ng/mL for ENA-001 and 10.0 to 5000 ng/mL for propofol.

SAFETY AND TOLERABILITY ASSESSMENTS

Endpoints used to assess safety and tolerability included: treatment-emergent (serious) adverse events (TE[S]AE's), clinical laboratory tests, vital signs, ECG, physical examinations, and the Columbia Suicide Severity Rating Scale (C-SSRS). TE(S)AE's were recorded after obtaining informed consent until the last study visit and were classified according to MedDRA v24.1. Furthermore, plasma troponin levels evaluated at t = 24 h served as cardiac biomarker.

Statistical methods

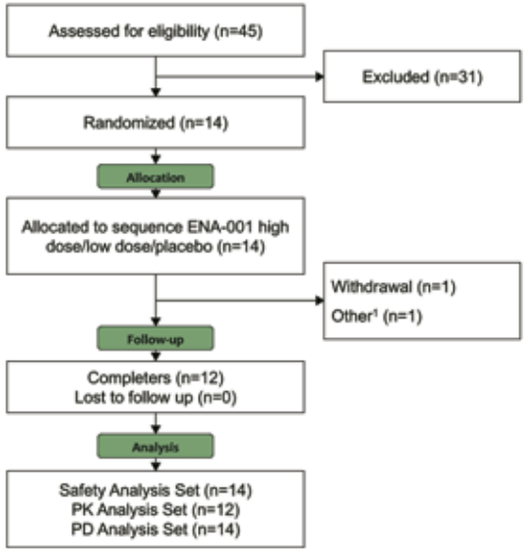
For the primary pharmacodynamic (PD) endpoint, the AHR (delta minute ventilation (MV) divided by the delta SpO_2), pre-hypoxic baselines and values during hypoxia were calculated. Pre-hypoxic baseline was defined as the minute median of MV and SpO_2 three minutes prior to hypoxia. Local regression was used to determine the maximum ventilation during each hypoxic measurement (A). The first timepoint that corresponded to the MV that was within 90% of A was identified and all data obtained during the following three minutes were selected; medians were then determined for ventilation and SpO_2 of this three-minute interval. AHR was analysed with a mixed model analysis of variance with the fixed factors treatment (Placebo, ENA-001 low, ENA-001 high), condition (No propofol Hypercapnia, no propofol Normocapnia, low propofol Hypercapnia, low propofol Normocapnia, high propofol Hypercapnia and high propofol Normocapnia) and treatment by condition, and the random factors participant, participant by treatment and participant by condition.

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and non-compartmental analyses were performed using the Phoenix WinNonlin version 8.3 (Certara USA, Inc., Princeton, NJ, USA).

Results

In total, 45 participants were screened for the study between 13-Sep-2021 and 29-Mar-2022, and fourteen participants were randomized. Two of the initially enrolled participants discontinued the study (one withdrew consent during the first treatment visit as a result of anxiety during the first hypoxic run, the other was excluded by the principal investigator after the first treatment visit because of restlessness during high dose propofol infusion causing ventilation mask leakage and data capture issues) and were replaced. Their data were used in the safety and PD analysis population, but no PK data were obtained for these participants. The CONSORT diagram summarizes participant disposition (**Figure 2**). Demographics and other baseline characteristics are presented in **Table 1**.

FIGURE 2 CONSORT flow diagram.



1. Participant was discontinued per investigator's decision. Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic.

Table 1 Participant demographics.

Characteristic	Category/Statistics	All Participants (N=14)
Sex	Female	6 (42.9%)
	Male	8 (57.1%)
Race	Asian	1 (7.1%)
	Black or African American	1 (7.1%)
	White	12 (85.7%)
Age (y)	Mean (SD)	27.1 (7.6)
	Median [min-max]	24 [18-41]
Weight (kg)	Mean (SD)	71.4 (11.7)
	Median [min-max]	71.80 [53.70-94.60]
Height (cm)	Mean (SD)	178.8 (9.71)
	Median [min-max]	179.0 [163.2-191.8]
BMI (kg/m^2)	Mean (SD)	21.94 (2.23)
	Median [min-max]	21.2 [19.2-26.2]

Abbreviations: BMI = body mass index; SD = standard deviation.

Pharmacodynamic assessments

The AHR was the primary PD outcome measure of the study. During all hypoxic exposures, target SpO_2 values were attained. Minute ventilation increased during hypoxia in all participants and during all treatments with greater increases during hypercapnia than during normocapnia. A dose dependent increase was observed for ENA-001. **Figure 3** shows the summary graphs of the mean ± SD of AHR, minute ventilation and SpO_2 per treatment. Administration of ENA-001 high dose resulted in a significant treatment effect versus placebo on AHR (estimated difference [95% confidence interval]): 0.575 (0.407, 0.744), p<0.0001, whereas ENA-001 low dose versus placebo was not significantly different 0.144 (-0.024, 0.312), p=0.0897. Analysis results for AHR are provided in **Table 2**. Propofol inhibited the AHR and had a greater effect on the hypercapnic response. However, concurrent administration of the ENA-001 high dose caused the AHR to remain comparable to pre-propofol values. In addition, the (physiological) increase in minute ventilation induced by hypercapnia during high dose propofol administration was more pronounced in the ENA-001 high dose group. Mean unclamped $P_{ET}CO_2$ values recorded for ENA-001 high dose were lower at timepoints prior to the hypoxic measurements at isocapnia compared to ENA-001 low dose and placebo.

Pharmacokinetic assessments

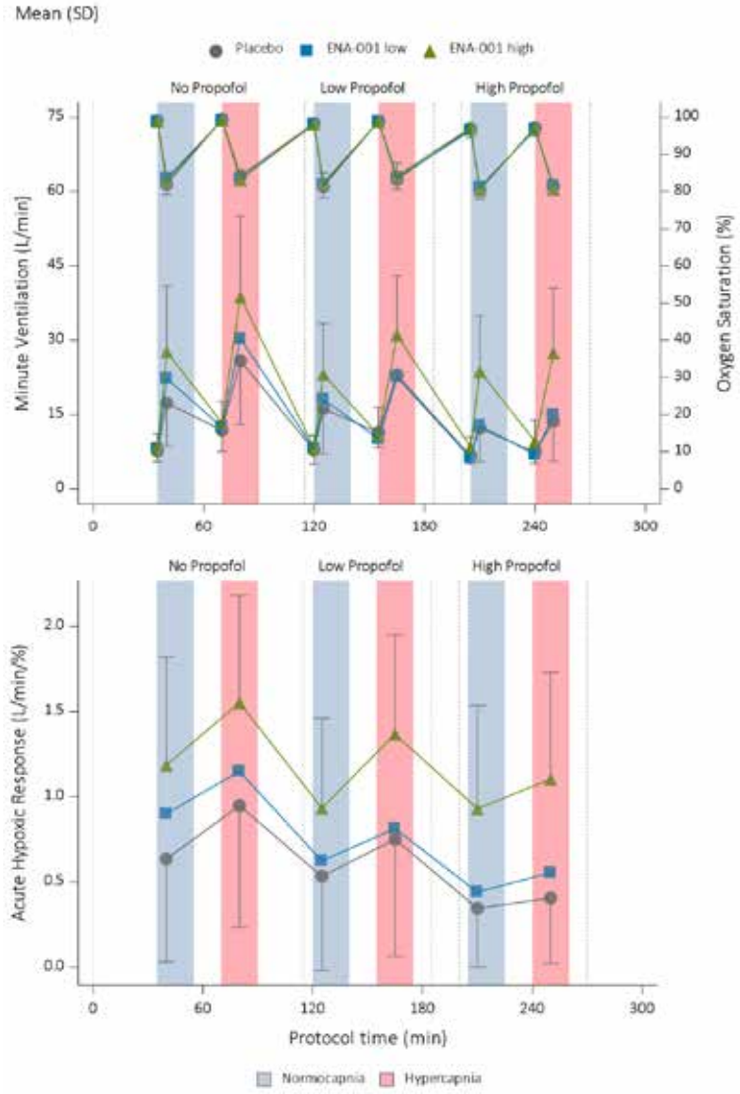
The PK of ENA-001 showed dose proportionality over the investigated dose range, and exposure levels were in the target range based on PK modelling that was performed in preparation of this study. Average plasma ENA-001 concentrations slightly increased during infusion and steady state concentrations were not reached with either ENA-001 low or high dose. No apparent effect of propofol on ENA-001 concentrations was observed.

Plasma propofol concentrations were within target range, although dose proportionality was not apparent, with relatively higher plasma concentrations recorded during the high dose infusion. Mean propofol concentrations were higher during infusion with either dose level of ENA-001 compared to those during placebo infusion (not statistically tested).

The pharmacokinetic parameters of both ENA-001 and propofol were in line with previously reported PK profiles of both molecules and are not discussed in this manuscript. A summary of the PK findings is available as supplementary file (S1).

FIGURE 3 Summary graphs of pharmacodynamic data.

Shown in figure: the mean (SD) of minute ventilation on the left Y-axis and corresponding SpO2 values on the right Y-axis (Panel A) at timepoints directly prior to and during each hypoxic run. Vertical blue shading indicates the hypoxic runs at normocapnia, red shading at hypercapnia. The propofol dosing blocks are separated by vertical dotted lines. Panel B shows the mean (SD) Acute Hypoxic Response in L/min per % desaturation.



Abbreviations: SD = standard deviation

TABLE 2 Analysis results Acute Hypoxic Response (L/min/%) under clamped $P_{ET}CO_2$ conditions.

			95% CI	
Treatment	Propofol and Hypoxia	LSM	Lower	Upper
Placebo	Overall	0.604	0.295	0.912
	No Propofol Normocapnia	0.635	0.294	0.975
	No Propofol Hypercapnia	0.949	0.608	1.289
	Low Propofol Normocapnia	0.534	0.194	0.874
	Low Propofol Hypercapnia	0.751	0.410	1.091
	High Propofol Normocapnia	0.346	0.006	0.686
	High Propofol Hypercapnia	0.407	0.066	0.747
ENA low dose	Overall	0.747	0.439	1.056
	No Propofol Normocapnia	0.901	0.560	1.241
	No Propofol Hypercapnia	1.150	0.809	1.490
	Low Propofol Normocapnia	0.624	0.284	0.965
	Low Propofol Hypercapnia	0.813	0.473	1.154
	High Propofol Normocapnia	0.442	0.101	0.782
	High Propofol Hypercapnia	0.554	0.214	0.895
ENA high dose	Overall	1.179	0.870	1.488
	No Propofol Normocapnia	1.185	0.844	1.525
	No Propofol Hypercapnia	1.552	1.212	1.893
	Low Propofol Normocapnia	0.934	0.593	1.274
	Low Propofol Hypercapnia	1.368	1.027	1.708
	High Propofol Normocapnia	0.930	0.590	1.271
	High Propofol Hypercapnia	1.104	0.764	1.444

Abbreviations: CI = confidence interval; LSM = least square means; $P_{ET}CO_2$ = end-tidal carbon dioxide.

Safety assessments

No SAE's occurred during the study. The most common AE's during the study were infusion site pain and related AE's, which were only reported for treatment with ENA-001, although no dose-dependent increase in occurrence was observed after treatment with ENA-001. One participant reported nausea with symptoms of a vasovagal reaction within the first 10 min after the start of ENA-001 high dose infusion and was administered 0.5 mg of intravenous atropine. The infusion of ENA-001 was temporarily ceased and re-started after 9 minutes when blood pressure had normalized, and an additional 4 mg of ondansetron was administered. The incidence of all other AE's was *similar* after each of the three treatments indicating no trend could be observed.

Blood pressure, heart rate, and cardiac index increased during hypoxic measurements as a result of the cardiovascular response that accompanied the increased ventilatory response, but no clinically meaningful difference was observed after treatment with ENA-001 versus placebo. Mean changes in clinical laboratory values and ECG were *similar* across the three treatments and there were no consistent differences observed between treatments for use of concomitant medications or physical examination findings, nor any positive responses to the C-SSRS questionnaire in any of the participants at any time during the study.

A summary of the safety findings including overviews of TEAE's, vital signs and ECG values is available as supplementary file (S2).

Discussion

Interference with normal ventilatory control is a common iatrogenic event during administration of sedatives or anaesthetics such as propofol. Although specific antagonists are available for some drugs (e.g., flumazenil for benzodiazepines), there is a shortcoming regarding other drugs including propofol. Multiple agnostic respiratory stimulants are under development, older drugs such as doxapram have a narrow therapeutic index and no longer have a place in clinical practice (see Peppin[18] and van der Schrier[19] for historical overviews of stimulants). ENA-001 acts as a potassium channel blocker at the carotid body and is an intravenous agnostic respiratory stimulant with possible clinical applications in the treatment of respiratory depression in procedural sedation or in postoperative patients. This study aimed to investigate the fundamental pharmacodynamics of ENA-001 and its effect on propofol-induced respiratory depression as may occur during procedural sedation with propofol.

The primary endpoint of the study was the isocapnic AHR. Here we used the AHR as biomarker of integrated carotid body activity. A decrease of AHR was observed during propofol infusion, most notably during ENA-001 placebo infusion. Concurrent administration of 18.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ENA-001 (high dose) induced a significant increase in the AHR compared to ENA-001 placebo and restored the AHR to pre-propofol value. The ENA-001 low dose (6.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was less effective. This could be expected based on data from previous studies in which the ENA-001 low dose showed no/minimal stimulatory effects on minute ventilation[12] whereas the ENA-001 high dose produced clear ventilatory stimulation in healthy participants and significantly attenuated alfentanil-induced ventilatory suppression to a certain extent.[10,11] Minute ventilation only increased during hypoxic exposure (i.e., not during

breathing of ambient air), indicating that the administered doses of ENA-001 only bolster ventilation when the participants were exposed to hypoxia; i.e., reset the intrinsic ventilatory response otherwise blunted by the central depressant. However, the small decrease in $P_{ET}CO_2$ prior to clamping for each hypoxic measurement, which was consistently observed during administration ENA-001 high dose, indicates the activity of the peripheral and central chemoreflex loops that caused a new steady state in ventilation and $P_{ET}CO_2$.[20,21] A population pharmacokinetic/pharmacodynamic model analysis that included a subset of data from this study has been described in a recent publication.[22]

Despite the clear ventilatory response, administration of ENA-001 did not lead to a significant difference in the level of consciousness compared to placebo, as measured by the Bispectral index (unpublished data on file). Furthermore, the only difference in the cardiovascular response that was observed between treatments was due to physiological changes accompanying the larger AHR with ENA-001 high dose administration, in particular during hypoxic measurements with concurrent administration of propofol high dose. At clinically relevant levels of ENA-001, no analeptic side effects were observed.

The infusion regimens in the current study were designed to maintain stable ENA-001 and propofol (low and high dose) concentrations during the respective dosing intervals. However, ENA-001 did not reach steady-state levels and concentrations increased during continuous infusion until infusion was stopped. Plasma concentration differences were not reflected in safety parameters and the ventilatory response of ENA-001 was evident throughout the treatment period. No apparent effect of propofol on ENA-001 concentrations were observed.

The propofol dosing regimen was designed to attain target plasma concentration of 600 and 1200 ng/mL during the propofol low and high dose, respectively. Propofol levels were higher during ENA-001 infusion (no apparent effect of dose level), but no underlying mechanism causing this possible interaction between the two drugs was identified. Despite higher plasma propofol concentrations, however, the effect of ENA-001 on AHR was significant.

The presented safety data indicated that ENA-001 has a favourable safety and tolerability profile in healthy participants at the two doses administered during this study. Apart from mild pain/burning sensation at the infusion site, which was expected based on previous studies with ENA-001, no trend in AE's was observed.

We realize that the limited number of healthy participants included in our study may not be fully representative of real-world patients (e.g., procedural, elderly, multi-morbidity) nor do the experimental conditions (i.e., clamped gas mixtures and propofol as sole challenging agent) reflect the complete variability encountered in clinic. Nevertheless, the data from this study showed that treatment with high dose ENA-001 significantly increased the AHR compared to placebo, both with and without co-administration of clinically relevant plasma concentrations of propofol, without impacting the level of sedation. These findings support that ENA-001 is a respiratory stimulant with potential for use in clinical practice, inducing stimulation of ventilation without influencing the effect of other administered drugs.

APPENDICES

S1 Summary of Pharmacokinetic Findings
S2 Summary of safety Findings

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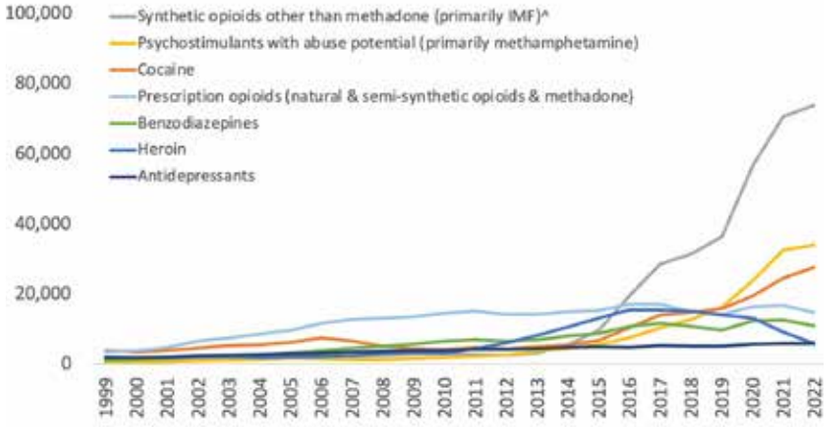
CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

Discussion

Nociception is crucial for protecting against potential harm, but safely relieving non-functional pain remains a universal goal. Opioids are central to severe pain management, yet potential for misuse, tolerance and life-threatening respiratory depression pose significant challenges. The grim reality is that the topic of this thesis has become increasingly relevant during the years during which we performed the research. Polysubstance and illicit synthetic opioid abuse currently exacerbate the opioid crisis (**Figure 1**), stressing the need for novel interventions.[1–4] Whether harmful effects occur despite adherence to prescribed regimens or as a result of overdose from illicit use, the pharmacology is the same. There has been recurrent excitement over new promising opioid therapies with minimal side effects. Although significant advancements are being made, the perfect treatment is yet to be discovered.

FIGURE 1 USA overdose deaths per drug category, 1999-2022.[5]



In this thesis I described the therapeutic potential of several opioidergic strategies and assessed treatments aimed at mitigating opioid-induced respiratory depression: the mu opioid receptor (MOP) partial agonist buprenorphine (**Chapters 2** and **3**), the dual enkephalinase inhibitor (DENKI) STR-324 (**Chapter 4**), two biased MOP agonists (Oliceridine **Chapters 5** and **6**, and ALKS 6610, **Chapter 7**), and finally the agnostic respiratory stimulant ENA-001 (**Chapter 8**) which stimulates the respiratory drive without counteracting desired analgesic effects like an opioid antagonist would. In the following discussion I review the studies we performed and discuss the implications of our findings for use of these therapies in clinic. Finally, I propose ideas for future research.

Partial agonism

Buprenorphine, a high-affinity MOP partial agonist, KOP antagonist and low-affinity NOP agonist, over 50 times more potent than morphine,[6] is primarily used as treatment for opioid use disorder in the USA. Although it has been reported to cause respiratory depression itself,[7] the hypothesis driving our study was that sustained high buprenorphine plasma concentrations competitively inhibit MOP binding of potent, short-acting full MOP agonist like fentanyl that can result in apnoea and death. Our study (**Chapters 2** and **3**) explored the pharmacodynamic interaction when intravenous fentanyl boluses were administered in the setting of ongoing MOP occupancy with sustained systemic concentrations of buprenorphine, focusing on peak ventilatory depression and apnoea. We designed a fentanyl challenge to observe the dose-dependent respiratory depressive effects up to the point of entire cessation of breathing, in order to mimic out of hospital opioid overdose. Although the cumulative dose of up to 1.8 mg/70 kg is roughly 6-9 times the induction dose used in general anaesthesia, this amount is found in 42% of the illicit pills tested by the US DEA.[8] As expected, we observed that buprenorphine produced a mild respiratory depressant effect prior to fentanyl exposure, but this reached a 'ceiling effect' due its partial agonist properties. Co-administered with placebo, fentanyl resulted in complete respiratory depression and prolonged apnoea, even when participants were not fully sedated. Participants experienced apnoea despite changes in oxygen and carbon dioxide that should normally drive compensatory ventilation, meaning they would have been at risk of death from opioid overdose if medical assistance would not be present or come too late. When the drugs were combined, buprenorphine produced a receptor binding-dependent reduction of fentanyl-induced respiratory depression. Clinically, this was particularly apparent in opioid-tolerant patients, but the pharmacometric model confirmed the presence of this effect in opioid naïve participants also. In the patients, buprenorphine plasma concentrations ⩾2 ng/mL clearly reduced the magnitude of respiratory depression induced by high-dose fentanyl. We note that these concentrations would not be tolerated by opioid naïve participants, given the dose limiting

side effects. However, the opioid-tolerant patients were more representative of the patients with opioid use disorder with an indication to be treated with buprenorphine, and we expect the observed PD interaction to translate to similar responses in clinical practice. Previous studies reported that steady-state concentrations of 1-5 ng/mL yielded 50-80% MOP occupancy, as measured by positron emission tomography with [^{11}C]-carfentanil radioligand in heroin-dependant participants.[9–11] Therefore, we attributed the protective effect to buprenorphine's high MOP affinity and its slow dissociation from this receptor. In addition to this protective effect when co-administered with full MOP agonists, findings from a recent meta-analysis also support the use of buprenorphine as an analgesic by itself for post-operative pain considering efficacy, safety profile and duration of action.[12] Although there certainly is reason to be optimistic about buprenorphine's potential, it is important to note that our results present a mean effects and individuals taking the drug might become apnoeic despite buprenorphine's partial agonism. Also, it is a strong opioid with dependency issues of its own as well as other side effects, such as severe nausea/vomiting and sedation. Currently, patients with OUD are most often treated with methadone in The Netherlands,[13] likely due to physicians' limited familiarity with the potential benefits of buprenorphine therapy. Based on our observations, I advocate for establishing a nationwide programme to improve the prescribing physicians' knowledge of the pharmacology and clinical applications of buprenorphine. In summary, we have demonstrated the potential of using a partial opioid agonist to prevent opioid induced apnoea (and resulting deaths), but buprenorphine should not be prescribed as if it were harmless.

Dual enkephalinase inhibition

In **Chapter 4**, we reported that STR-324 demonstrated a favourable safety and tolerability profile in healthy males up to 11.475 mg/h administered continuously for 48 hours. Contrary to preclinical findings, we did not observe consistent analgesic effects in this first-in-human trial. Given the number of parameters included and the fact that we did not correct for multiple testing, the sparse effects that we did observe were most likely due to chance. Anticipating the limited pharmacokinetic characterization of STR-324 due to its rapid metabolism, we used the main metabolite as a proxy to assist in dose escalation discussions. The short-lived nature of STR-324 and its rapid distribution outside the blood compartment aligned with preclinical findings and the absence of observed PD effects might be related to this. Alternatively, this might have been due to the evoked pain intensity not being sufficient to activate a measurable enkephalin response, or because prolonged exposure to a nociceptive stimulus is required to yield a clinically measurable effect of STR-324. Another aspect to consider, is the choice of pain models in our study. Analgesic activity of STR-324 was demonstrated in preclinical rodent models of postoperative- and neuropathic pain, albeit without dose dependency. In our study, we included pressure-, thermal-, electrical- and cold pressor pain tests. It would have been interesting to evaluate STR-324's effect on alternative pain models involving hyperalgesia such as ultraviolet B (UVB) irradiation or capsaicin application to the skin (e.g., forearm), as sensitivity to opioid receptor agonists has been reported.[14–17]

We included pupillometry to measure non-analgesic opioidergic effects (i.e., miosis). While a significant treatment effect versus placebo was observed on pupillometry, this was unlikely caused by MOP activation, and we attributed this to unexpected placebo group behaviour because pupil size did not decrease during the 48-hour administration of STR-324. Rather, pupil size remained increased throughout the measurement period during placebo infusion. Theoretically, the evoked pain could have elicited an adrenergic response resulting in mydriasis in the placebo group. The net physiological effect of adrenaline versus increased availability of enkephalins with STR-324 treatment would possibly lead to neutral pupils. However, the tests did not directly follow each other, and we have not observed mydriasis during placebo treatment in other studies using evoked pain tests. Additionally, the Bowel Function Index was included to evaluate any gastro-intestinal effects, complementing participants' reporting of adverse events. Although all commonly prescribed opioids cause constipation to some extent after even a short administration period, neither the 'single dose' 4-hour infusion nor the prolonged 48-hour infusion resulted in relevant findings. Lastly, we included the measurement of aminopeptidase N (APN) and Big Endothelin-1 (BigET-1) in plasma as exploratory biomarkers based on STR-324's expected mode of action, hypothesizing that a decreased activity of both could be measured. Surprisingly, we measured an *increase* in APN activity at the highest dose tested, and no change in BigET-1 was observed. The study was not powered to detect significant effects on these endpoints; thus, no definite conclusions can be drawn.

To summarise, STR-324 was found to be safe, but we didn't observe any PD effects in our tests. Inclusion of biomarkers such as experimental pain tests in early phase clinical trials offers critical insights that may guide drug development decisions. Systematically evaluating these parameters may help define dose range and characterise a drug candidate's (side) effect profile. While important, biomarkers primarily serve as models for clinically relevant endpoints; in healthy volunteers, they can only approximate specific aspects of a given disease. Negative PD findings in FIH studies don't always lead to discontinuation of a compound's development. A recent phase IIa proof-of-concept study evaluating STR-324's effect in patients who underwent laparoscopic surgery showed minimal analgesic potential, appreciably less than the comparator morphine. Enkephalinase inhibition may therefore still be considered an interesting pharmacotherapeutic avenue, but whether STR-324, or any other DENKI, will meet expectations in larger trials remains to be seen. Currently, the development of STR-324 has been terminated.

Preferential or 'biased' intracellular signalling

Since the observation that MOP agonists did not cause the typical respiratory depression in genetically modified mice that weren't capable of MOP β-arrestin signalling,[18] the concept of G-protein-biased MOP signalling has resulted in many efforts to design safer opioids. This thesis reports on two randomised placebo-controlled clinical trials in which such biased MOP agonists were evaluated. **Chapters 5** and **6** describe the study where we compared oliceridine, a novel opioid that is registered in the US, to morphine on a series of tests evaluating neurocognitive function. In **Chapter 7** the FIH trial evaluating the safety, tolerability, PK and PD of ALKS 6610, a selective, partial MOP agonist with biased signalling is discussed.

Attributed to its biased opioid properties, oliceridine has been reported to result in less side effects, including respiratory depression, while providing similar analgesia to full agonists such as morphine.[19–22] However, because intravenous opioids are widely used postoperatively for severe pain management, understanding their CNS side effects too is critical. Opioid-induced sedation, impaired cognitive functioning and physical instability can compromise intended convalescence and bear upon patients' health when administered these analgesics. The gap that novel opioids like oliceridine can fill is therefore clear, yet supportive evidence to demonstrate an improved CNS safety profile over classic opioids is currently lacking. In our study, we compared the effects of intravenous oliceridine (1 mg and 3 mg) with morphine (5 mg and 10 mg) on a range of neurocognitive tests in healthy opioid-naïve males and females. The primary outcome measure, saccadic peak velocity (SPV), an established biomarker of sedation, revealed a significantly reduced sedative effect for oliceridine compared to morphine at equi-analgesic doses. A finding that was substantiated by the results from a clinician-reported score of sedation. Comparable analgesic effects in the cold pressor test were observed for the two drugs, with a faster onset for oliceridine which was in line with earlier findings. The differences in sedation mirrored other neurocognitive biomarkers such as body sway, which was included as a surrogate for balance or motor stability. To optimally demonstrate oliceridine's favourable safety profile over morphine, we performed utility function analyses and showed it had a higher probability of benefit (pain relief) relative to harm (neurocognitive impairment) across clinically relevant concentration ranges. Although oliceridine's preferential activation of G-protein signalling and reduced β-arrestin recruitment are hypothesised to contribute to its reduced adverse respiratory effects, we questioned whether this could also explain the favourable CNS safety profile. Although β-arrestin's role in CNS side effects of opioids remains unclear, off-target interactions with TOLL-like receptor 4 (TLR4), particularly with morphine, may trigger neuroinflammation, contributing to CNS dysfunction.[23–25] Unlike morphine, oliceridine does not appear to upregulate TLR4 in animal models, potentially reducing neuroinflammation and associated cognitive impairments.[26]

Given that ALKS 6610 was administered to humans for the first time in our study, described in **Chapter 7**, the primary focus was on the safety and PK profiles. However, by integrating PD biomarkers alongside safety and PK parameters, the study investigated whether promising preclinical findings could be translated across species, providing a model for biomarker-driven early-phase drug development, similar to our study with STR-324. ALKS 6610 demonstrated a typical opioid safety profile, including GI side effects and dizziness, but without signs of respiratory depression (i.e., changes in breathing frequency, SpO_2, $etCO_2$ during rest) or sedation over a broad dose range. Nausea increased dose-dependently, with doses up to 750 mg generally well-tolerated. While 1200 mg was the highest planned human dose based on preclinical findings, escalation beyond 825 mg was halted due to vomiting and

prolonged nausea in all participants who received active dose in that cohort. Based on preclinical findings, extensive cardiac monitoring was included in the study. A possible dose-dependent increase in QTcF was observed, which was more apparent at the two highest doses of 750 mg and 825 mg, with maximum mean QTcF change from baseline of 12.3 ms and 22.7 ms, respectively.[27] Although these presumably regard supratherapeutic dose levels (no analgesic data is available), the finding poses a possible limitation for clinical use given the results regard means and clinically relevant QTcF prolongation might occur at lower dose levels. Pupillometry confirmed central MOP engagement, with a large increase in effect between the 225 mg and 450 mg, possibly due to a steep concentration-effect curve, and an apparent ceiling effect from the 450 mg dose upwards. The nonlinear effect profile could have stemmed from the high-affinity MOP binding, or potentially from intracellular processes such as signal amplification. Alternatively, blood-brain barrier (BBB) dynamics could have been involved. While plasma PK showed linearity, cerebrospinal fluid PK was not assessed, leaving the possibility of disproportionate CNS exposure relative to plasma levels, potentially due to saturation of efflux transporters like P-glycoprotein. The attenuated effect above the 450 mg dose was most likely caused by ALKS 6610's partial agonism. Other possible explanations such as a ceiling effect in the measurement scale, receptor desensitization or tolerance development could be excluded. Notably, the GI related adverse events showed a dose dependent increase up to the highest dose tested, implying different pathways underlying these effects. This discrepancy is presumably due to the chemoreceptor trigger zone, a part of the CNS that is in contact with the central blood compartment (i.e., permeable BBB), and can therefore detect emetic toxins in the blood. PK translated well across species (only human PK included in this thesis), with dose-proportional exposure and stable t_{max}. No further data on this drug candidate was collected, due to discontinuation of its development.

Although G-protein biased MOP signalling has been considered an important new strategy in developing safer opioids for the past two decades, this paradigm has yet to fully live up to that promise. It is important to acknowledge that developing new therapies is costly and takes time, but oliceridine has been the only biased opioid to be market approved to date and that drug is not devoid of respiratory side effects either. The concept remains debated, with recent studies questioning whether OIRD is indeed mediated through β-arrestin 2 signalling and proposing instead that any improved safety profile should rather be attributed to lower intrinsic efficacy (i.e., partial agonism).[28–30] Preclinical results are diverse, with some studies finding a decrease in OIRD while others observed improved analgesia but an exacerbated opioid side effect profile in mice that only signal through G-protein upon MOP activation.[31] Further research showed that low intrinsic efficacy alone could not fully explain the improved safety profile[32] and oliceridine has demonstrated less respiratory depression at equianalgesic dose levels of morphine in human studies.[33–35] Our findings in a small group of healthy study participants suggest that oliceridine also has an improved therapeutic index compared to morphine regarding CNS function. It may be a safer alternative for pain management with fewer neurocognitive side effects, and these results warrant further study in patients. As an individual drug, oliceridine definitely deserves a prominent place in the analgesic armamentarium and although I support the availability of oliceridine to clinicians in Europe, there are no indications for its registration in the EU. As physicians gain experience with biased opioids in clinical practice, it will likely encourage further research in this direction. However, the question remains whether the favourable benefit-harm ratio is the result of biased signalling, and future research will need to investigate the underlying mechanism. For ALKS 6610, we did not observe respiratory depression and sedation, supporting the case for biased opioids in the clinic. However, it is important to note that no data on analgesic effects are available, and we might not have picked up minor changes in ventilatory or neurocognitive function. For this drug candidate too, another mechanism than biased agonism, for example lower intrinsic efficacy, cannot be excluded as possible cause for the observed superior safety profile. In conclusion, based on the superior risk-benefit profile of oliceridine compared to morphine and no OIRD or sedation having been observed for ALKS 6610, biased opioid agonism might still be regarded a promising pharmacotherapeutic paradigm but more understanding of the complex signalling dynamics is required to fully endorse the concept.

Agnostic respiratory stimulation

In **Chapter 1**, we discussed several examples of agnostic respiratory stimulants that have the potential to reduce adverse respiratory effects while preserving the analgesic function of opioids. In **Chapter 8** we

present the results of a study evaluating ENA-001, a respiratory stimulant that acts partially by blocking the large conductance calcium-activated potassium channels (BK_{Ca2+} earlier named Slo, Maxi K channels) in the carotid body. In this placebo-controlled trial, we investigated the acute ventilatory response to hypoxia (AHR) in participants receiving two separate dose levels of ENA-001, under normo- and hypercapnic conditions, and both without and in conjunction with two dose levels of propofol. Propofol has been shown to negatively affect metabolic ventilatory control, blunting the HVR and potentially leading to conditions such as hypercapnia, bradypnea, or apnoea at high doses.[36–40] We observed that ENA-001 could effectively restore the HVR impaired by propofol. Specifically, an ENA-001 concentration of 1.5 µg/ml was required to counteract the respiratory depressive effects of propofol at a steady concentration of 2 µg/ml.[41] This propofol concentration is within the range commonly used to maintain adequate anaesthesia depth (based on e.g., bispectral index). Our findings supported the hypothesis that ENA-001 has the potential to mitigate the impact of centrally acting HVR depressants, both during normocapnia and hypercapnia, across the tested propofol concentration range. These results complement earlier observations where similar ENA-001 (previously GAL021) concentrations attenuated alfentanil-induced respiratory depression.[42] Of note, ENA-001 did not result in a relevant increase in respiration without exposure to hypoxia, and no neurological or cardiovascular side effects were observed. The mechanistic model based on the study data (not included in this thesis), indicated that ENA-001 acts on the multiplicative component of the HVR for oxygen and carbon dioxide through the BK-channels in the carotid body. Our observation that ENA-001 can modulate peripheral chemoreceptors to improve respiration, regardless of the chemical inhibiting central respiratory function and without inducing untoward effects of CNS stimulation, strengthens the case for agnostic respiratory stimulants as a potential therapy to prevent or treat OIRD. But ENA-001 may also prevent or treat hypoxemic episodes from other causes, for example apnoea in preterm infants. The respiratory stimulant doxapram, currently used alongside caffeine for this purpose, for example in neonates, shows conflicting clinical results and frequently causes analeptic side effects in adults.[43,44] ENA-001 may offer a more effective alternative. Lastly, intravenous administration is efficient when ENA-001 is given e.g., post-operatively. However, it would be interesting to investigate whether an intramuscular formulation could be developed, which may be more suitable in an outpatient setting. If possible, depot or extended-release mechanisms could be designed so that ENA-001 could be administered to patients with OUD and may help prevent OIRD. Still, first observations suggest that ENA-001 is short acting, not different to naloxone.

Future perspectives

The findings discussed in this thesis warrant further investigation in larger clinical studies. Given that buprenorphine plasma concentrations of ⩾2 ng/mL in our study were consistent with those achieved with injectable extended-release buprenorphine formulations (as discussed in **Chapter 1**), a respiratory study enrolling patients being treated with buprenorphine implants (e.g., Subutex) could be designed. Also, it would be interesting to investigate fentanyl's effect on analgesia when co-administered with buprenorphine. Worldwide, many patients take buprenorphine, and it is expected that this number will increase in the coming years due to an increasing number of patients who are on medication assisted treatment of opioid use disorder. In clinical practice, full MOP agonists like fentanyl and its analogues are often used as analgesics in the emergency and operating room. Patients who are being treated with buprenorphine for OUD will have sustained high plasma concentrations of buprenorphine, which might limit the analgesic efficacy of fentanyl when this drug is administered in-hospital. There is literature that describes the absence of a ceiling effect in analgesia for buprenorphine in healthy volunteers, but no such data are available for the high doses that were required to mitigate fentanyl-induced respiratory depression, and these effects have not been studied well in the target population consisting of opioid tolerant patients.[45,46] A possible study could use human evoked pain models in a small group of patients and serve as a proof-of-concept to get an indication whether buprenorphine limits the analgesic effect of fentanyl. Additionally, assessments of CNS functioning could easily be incorporated into this trial design by including several measurements to evaluate e.g., sedation, alertness, postural stability, and mood. While buprenorphine demonstrated favourable results partly due to its long half-life, the drug is eventually metabolised. Researchers are experimenting with deuterated buprenorphine (BUP-D2), prolonging its half-life without altering pharmacodynamics in rats.[47,48] Perhaps this altered molecule may prove of even greater value.

As mentioned, it would also be interesting to investigate whether our findings on oliceridine's CNS safety profile translate to relevant clinical outcomes such as a decrease in number of falls and/or subsequent injuries during opioid therapy. Future research should aim to clarify the mechanism underlying the differential effects of opioids on neurocognitive function and motor stability. Over the next decade, I anticipate advances in our understanding of the signalling dynamics of various (novel) opioids, providing a better explanation for current observations than biased signalling alone. Multifunctionality of drugs (i.e., targeting >1 receptor type) may be a key consideration here. I expect to see a rise in (pre)clinical development of this type of drug, of which several examples are discussed in **Chapter 1**. We recently performed a study comparing oxycodone to cebranopadol, a dual nociceptin/orphanin FQ peptide (NOP)-MOP agonist (not included in this thesis). Cebranopadol demonstrated potent and long-lasting analgesic effects, with 25% less respiratory depression at equianalgesic doses and a significantly slower onset of action (approximately 1 hour) compared to oxycodone.[49] Additionally, cebranopadol was associated with fewer respiratory adverse effects. I expect it will be worthwhile to follow the various efforts being made in this pharmacotherapeutic area.

Another mechanistic paradigm might be related to pharmacogenetics. Advancements in this field are being made regarding many different diseases, including those affecting the CNS (e.g., Parkinson's). The main focus in pharmacogenetics regarding opioidergic treatment has been on understanding how genetic variations affect individual responses to opioids (e.g., CYP2D6 variability, OPRM1 polymorphisms).[50,51] Illustrative of the relevance of pharmacogenomics in opioid treatment is a study that observed analgesic responses of different strains of mice to 5 mg/kg morphine ranging from 80% to 0%.[52] Currently, opioid treatment is still often one-size-fits-all. Incorporating pharmacogenetic testing into clinical practice could be of added value thereby enabling tailored treatment choices based in part on genetic profiles, resulting in optimal pain management with minimal side effects. Mirroring that development, interest in drugs that selectively target certain MOP splice variants is likely to grow. One example is IBNtxA,[53] but more promising candidates may emerge as the intricate genetic system is further unravelled.

As of 2025, the promise of *artificial intelligence* (AI) deserves mentioning, in this case in search of a safer opioid. As the paradigm of structure-based drug discovery has been gaining momentum, drug discovery/repurposing has become a key application of deep learning in the field.[54,55] Using AI to process huge amounts of data incorporating the latest knowledge about physiology and disease mechanisms, will help to identify (un)known molecules that are potentially superior to available therapeutic ligands. But AI will also assist drug development in other ways, such as predicting blood-brain permeability,[56] or even to predict the outcome of clinical trials based on target choice and trial design (e.g., inClinico).[57] Furthermore, taken together with the previous outlook regarding pharmacogenetics, I believe we are on the brink of entering a new era of highly personalized opioidergic treatments based on genetic and biometric data that will optimise drug selection and dosing, thereby improving efficacy and patient safety.

Lastly, despite the potential benefits of each pharmacotherapeutic strategy, it is also necessary to underscore the importance of education and prevention in the context of limiting opioid-induced harm. This applies to clinical practitioners prescribing opioids, as well as patients taking them. The tragic opioid crisis has created a worldwide awareness of the risks associated with this drug class, which have led to many educational efforts to prevent opioid abuse. An example of a prevention measure is the Opioid Risk Tool,[58] which includes questions about personal or family history of substance abuse and psychological disease. This validated screening tool assesses opioid abuse risk in primary care patients, assisting clinicians to identify those who may require closer follow-up when prescribing opioids.

Conclusions

We evaluated the effects of a MOP partial agonist (buprenorphine), a novel DENKI (STR-324), two biased MOP agonists (oliceridine and ALKS 6610), and an agnostic respiratory stimulant (ENA-001). Our findings offer promising avenues for improving the risk-benefit profile of opioid-based pain treatments. However, we emphasise the need for further research into diverse strategies to mitigate severe side effects and improve patient safety. Each pharmacological approach discussed in this thesis presents distinct advantages and limitations. While significant advancements have been made to address opioid-related side effects, truly safer opioid analgesia remains to be discovered, thus the pharmacological quest to strike gold continues.

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APPENDICES

Nederlandse samenvatting

Pijn is een complex fenomeen met zowel fysiologische als psychologische dimensies en kan een aanzienlijke impact hebben op kwaliteit van leven. Effectieve pijnstilling is daarom een kerndoel binnen de geneeskunde, waarbij opioïden door hun sterke analgetische potentie een centrale rol vervullen. Het gebruik van opioïden gaat echter gepaard met ernstige risico's zoals afhankelijkheid en dosisafhankelijke bijwerkingen, waaronder respiratoire depressie. Het sterk toegenomen gebruik in de afgelopen decennia heeft geleid tot wijdverbreid misbruik en de huidige opioïdcrisis, met name in de Verenigde Staten. Deze situatie benadrukt de noodzaak van nieuwe farmacologische strategieën die het evenwicht tussen effectiviteit en veiligheid verbeteren. Hoofdstuk 1 schetst de huidige stand van zaken, variërend van nieuwe analgetica en aanpassingen in formulering tot geneesmiddelen die bijwerkingen kunnen tegengaan. In dit proefschrift worden verschillende strategieën onderzocht: partiële agonisten, biased liganden, endogene opioïden en respiratoire stimulantia.

Wij onderzochten of de partiële mu-opioïd receptor (MOP)-agonist buprenorfine bescherming kan bieden tegen fentanyl-geïnduceerde respiratoire depressie, de belangrijkste oorzaak van mortaliteit ten gevolge van opioïd intoxicatie. In een gerandomiseerde, placebo-gecontroleerde studie met gezonde vrijwilligers (n=14) en opioïd-tolerante patiënten (n=8) combineerden we continue infusie van buprenorfine of placebo met oplopende fentanylbolussen. De resultaten toonden dat buprenorfine zelf enige, maar geen volledige respiratoire depressie veroorzaakte, en dat hoge plasmaconcentraties de ernst van fentanyl-geinduceerde effecten aanzienlijk verminderden. Tijdens buprenorfinebehandeling was de reductie in ventilatie significant kleiner en de kans op apneu en desaturatie sterk verlaagd (odds ratio apneu 0.07 in OT-patiënten). PKPD-modellering liet zien dat plasmaconcentraties ⩾2 ng/mL voldoende waren om fentanyl grotendeels te verdringen van de MOP en respiratoire depressie te beperken, zelfs bij hoge doses. Deze combinatie van klinische en gemodelleerde data levert het eerste bewijs dat buprenorfine, mits in stabiele spiegels aanwezig, bescherming kan bieden tegen een fentanyl-overdosis.

STR-324, een dual enkephalinase inhibitor (DENKI), werd in een eerste-in-mens studie geëvalueerd. Het betrof een dubbelblinde, placebo-gecontroleerde studie met een 4-uurs (n=30) en 48-uurs infusie (n=48) bij gezonde vrijwilligers. STR-324 werd goed verdragen tot 11,475 mg/h; bijwerkingen waren gering in ernst en dosis-onafhankelijk. Door snelle afbraak kon STR-324 zelf nauwelijks worden gekwantificeerd; de belangrijkste metaboliet (STR-324M) vertoonde dosis-proportionele farmacokinetiek. Ondanks preklinische aanwijzingen werden in experimentele pijnmodellen geen consistente analgetische effecten gevonden. Ook exploratieve biomarkers (pupillometrie, Bowel Function Index, plasma-enzymen) lieten geen overtuigende farmacodynamische respons zien. Mogelijke verklaringen zijn de korte halfwaardetijd, onvoldoende activatie van endogene enkefalines of ongeschikte pijnmodellen. Een vervolgstudie bij postoperatieve patiënten liet slechts beperkte analgesie zien ten opzichte van morfine. De klinische ontwikkeling van STR-324 is inmiddels stopgezet, al blijft enkephalinase-inhibitie conceptueel interessant.

Sinds de observatie dat MOP-agonisten geen respiratoire depressie veroorzaakten bij muizen zonder β-arrestin-2, wordt G-proteïne-biased agonisme onderzocht als strategie voor veiligere opioïden.

In de eerste studie (Hoofdstukken 5 en 6) werd oliceridine, een in de VS geregistreerd opioïd, vergeleken met morfine in een dubbelblinde, cross-over studie bij 20 gezonde vrijwilligers. Hersenfuncties werden getest door meting van o.a. oogvolgbewegingen (saccadic peak velocity (SPV), primaire uitkomst) en lichaamsstabiliteit (body sway), en de invloed op pijndrempels werd bepaald m.b.v. de cold pressor test. Oliceridine veroorzaakte bij equi-analgetische doses significant minder sedatie en motorische instabiliteit dan morfine, met vergelijkbare analgesie maar snellere onset. Utility function analyses bevestigden een gunstiger balans tussen analgesie en centraal zenuwstelsel bijwerkingen. Mogelijke verklaringen betreffen G-proteïne-biased signalering en het ontbreken van TLR4-gemedieerde neuroinflammatie.

Hoofdstuk 7 beschreef de eerste toediening van ALKS 6610, een orale partiële MOP-agonist met biased signaaltransductie. In de dubbelblinde, placebo-gecontroleerde studie werd primair veiligheid en farmacokinetiek geëvalueerd, met toevoeging van farmacodynamische biomarkers. ALKS 6610 had een typisch opioïd-veiligheidsprofiel (GI-klachten, duizeligheid), maar geen aanwijzingen voor respiratoire depressie of sedatie tot 825 mg. Misselijkheid was dosisafhankelijk, en cardiologische analyses toonden dosisgerelateerde QTc-verlenging bij 750 en 825 mg. Pupillometrie bevestigde MOP-activatie met een plateau vanaf 450 mg, passend bij partiële agonistische eigenschappen.

Hoewel biased agonisme al decennia als beloftevol geldt, is klinisch bewijs beperkt. Oliceridine toonde in ons onderzoek een betere balans tussen analgesie en bijwerkingen dan morfine. ALKS 6610 liet geen respiratoire of sedatieve effecten zien. Deze resultaten ondersteunen een potentieel verbeterd veiligheidsprofiel, maar het is niet zeker dat dit aan biased agonisme te danken is, of bijvoorbeeld aan partiële agonisme.

Tenslotte onderzochten wij ENA-001, een respiratoir stimulantium dat werkt via blokkade van BKCa2+-kanalen in het carotislichaam. In een dubbelblinde, placebo-gecontroleerde studie werd de hypoxic ventilatory response (HVR) gemeten bij gezonde vrijwilligers, onder normo- en hypercapnische omstandigheden en met en zonder propofol. Propofol verzwakte de HVR aanzienlijk; ENA-001 herstelde deze dosisafhankelijk. Een plasmaconcentratie van 1,5 µg/ml neutraliseerde de effecten van 2 µg/ml propofol, een klinisch relevante dosering voor anesthesie. Eerdere data dat ENA-001 alfentanil-geïnduceerde respiratoire depressie kan tegengaan, werden hiermee aangevuld. ENA-001 verhoogde de ademhaling niet in afwezigheid van hypoxie en veroorzaakte geen neurologische of cardiovasculaire bijwerkingen. Deze bevindingen ondersteunen de potentie van 'agnostische' respiratoire stimulantia om OIRD te voorkomen of te behandelen, met mogelijke toepassingen binnen en buiten de operatiekamer.

Samenvattend hebben wij de effecten van een partiële agonist (buprenorfine), een nieuwe DENKI (STR-324), twee biased agonisten (oliceridine en ALKS 6610) en een agnostisch respiratoir stimulantium (ENA-001) geëvalueerd. Onze bevindingen wijzen op veelbelovende mogelijkheden. Tegelijkertijd benadrukken wij de noodzaak van verder onderzoek naar uiteenlopende farmacologische strategieën ter beperking van ernstige bijwerkingen en ter bevordering van patiëntveiligheid. Elke in dit proefschrift besproken benadering kent specifieke voordelen en beperkingen. Hoewel belangrijke stappen zijn gezet om opioïd-gerelateerde neveneffecten te verminderen, is een volledig veilig opioïd-analgeticum nog niet gevonden; de farmacologische zoektocht naar een 'gouden standaard' zet zich daarom onverminderd voort.

CURRICULUM VITAE

Laurence Maxim Moss was born in Amsterdam on November 8th 1990 and attended Grammar School at the Stedelijk Gymnasium Haarlem, graduating in 2009. He subsequently studied medicine at the Vrije Universiteit Amsterdam, where he obtained his medical degree in 2017. After a brief period in consultancy, he joined the Centre for Human Drug Research in Leiden as research physician and project leader. The research presented in this thesis was conducted during this period, alongside his contributions to multiple clinical trials across other therapeutic areas including dermatology, immunology and psychiatry. During his tenure at CHDR, he qualified as a board-certified Clinical Pharmacologist. In 2024 he worked as a physician at the Intensive Care Unit of Flevoziekenhuis in Almere. Since January 2025 he has been training as a resident (AIOS) in Anaesthesiology at the Leiden University Medical Centre.

Laurence currently lives in Bussum with his wife Titia, and their children Maurits and Vita.

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