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Fighting fire with fire: development of intranasal nalmefene to treat synthetic opioid overdose

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List of abbreviations

AE, adverse event

AUC_{0-t}, area under the concentration time curve from time zero to the last measurable concentration

BMI, body mass index

C_{max}, maximum plasma concentration

DDM, dodecyl maltoside (Intravail®)

ECG, electrocardiogram

IM, intramuscular

IN, intranasal

LC-MS/MS, liquid chromatography-tandem mass spectrometry

m/z, mass divided by charge

P_aCO₂, partial pressure of carbon dioxide

P_aO₂, partial pressure of oxygen

T_{max}, time to reach C_{max}

t_{1/2}, elimination phase half-life

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Abstract

The dramatic rise in overdose deaths linked to synthetic opioids (e.g., fentanyl, carfentanil) may require more potent, longer duration opiate antagonists than naloxone. Both the high affinity of nalmefene at μ opiate receptors and its long half-life led us to examine the feasibility of developing an intranasal (IN) formulation as a rescue medication that could be especially useful in treating synthetic opioid overdose. In this study, the pharmacokinetic properties of IN nalmefene were compared with an intramuscular (IM) injection in a cohort of healthy volunteers. Nalmefene was absorbed slowly following IN administration, with a median T_{max} of 2 h. Addition of the absorption enhancer dodecyl maltoside (Intravail®) reduced T_{max} to 0.25 h and increased C_{max} by ~2.2-fold. The pharmacokinetic properties of IN nalmefene (3 mg) formulated with dodecyl maltose has characteristics consistent with an effective rescue medication: its onset of action is comparable to an IM injection of nalmefene (1.5 mg) previously approved to treat opioid overdose. Furthermore, the C_{max} following IN administration is ~3-fold higher than following IM dosing, comparable to previously reported plasma concentrations of nalmefene observed 5 min. following a 1 mg IV dose. The high affinity, very rapid onset, and long half-life (>7 h) of IN nalmefene present distinct advantages as a rescue medication, particularly against longer-lived synthetic opioids.

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Introduction

A Government Accountability Office report released in October, 2018 declared the opioid crisis a public health emergency (U.S. Government Accountability Office, 2018). The most visible manifestation of this crisis is the rising number of opioid overdose deaths and the dramatic spike in fatalities linked to fentanyl and related synthetic opioids. Thus, based on 2017 estimates (NIDA, 2018) synthetic opioids (“synthetics”) were linked to more than half of the estimated 49,000 opioid-related deaths, far surpassing fatalities attributed to either heroin or prescription opioids. There are multiple factors responsible for the dangers posed by synthetics including very high potencies, rapid onset of action, long half-lives, and ease of synthesis; this latter property translates to a low cost of goods relative to heroin and prescription opioids (reviewed in Skolnick, 2018). Furthermore, the piperidine-based structure of fentanyl is highly mutable. More than 1400 fentanyl analogs have been described in the patent and scientific literature, and a dozen or more are available on the illicit market (Misailidi, et al., 2018), adding another layer of complexity for both detection and interdiction by law enforcement.

Naloxone is currently the only FDA approved treatment for suspected or confirmed opioid overdose. The efficacy of naloxone at reversing the pharmacological actions of opioids, including synthetics like fentanyl, has been well established in both the emergency department and operating room (Glass et al., 1994; Kaplan, et al., 1999; reviewed in Boyer, 2012). There are two FDA approved naloxone products (an auto-injector and a nasal spray) that are primarily used by first responders (e.g., police, emergency medical service technicians, bystanders) to treat overdose victims (Skolnick, 2018). However, both anecdotal reports (Mattingly, 2017; Zezima, 2018) and clinical case studies (Sutter et al., 2017; Uddayasankar, et al., 2018) indicate

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overdose with synthetics such as fentanyl and carfentanil often requires more naloxone than the standard unit doses (2 mg IM/4 mg IN) generally available to first responders. Some authors (Li, et al., 2018) have recommended parenteral naloxone doses of up to 12-15 mg to successfully reverse a synthetic overdose. While each overdose situation is unique (Skolnick, 2018), the current NIDA position states: “Overdoses of fentanyl should be treated immediately with naloxone and may require higher doses to successfully reverse the overdose” (NIDA, 2016). Moreover, the short half-life of naloxone ($t_{1/2}$ 1.3-2.4 h) (Ryan and Dunne, 2018) can complicate the management of overdose with long-lived synthetics, including fentanyl (Ahonen, et al, 2000; Kharasch, 2015).

In response to the increasing number of overdose deaths linked to synthetics, NIH leadership recently called for the development of “...stronger, longer-acting formulations of antagonists” (Volkow and Collins, 2017). At face value, the opiate antagonist nalmefene fulfills these criteria. Thus, multiple studies (Emmerson, et al., 1994; Toll, et al., 1998; Cassel, et al., 2005) have demonstrated the affinity of nalmefene is ~5x higher than naloxone at both native and recombinant μ opioid receptors. The half-life ($t_{1/2}$) of parenterally administered nalmefene, is ~ 8.2-8.9 h (Dixon, et al. 1986), comparable to the half-lives of synthetics like fentanyl (7-8 h) and sufentanil (6-9 h) (Ahonen, et al., 2000; Kharasch, 2015). In addition, the efficacy of nalmefene in treating opioid overdose has been established. Thus, parenteral nalmefene was FDA approved (1995) to treat opioid overdose, but withdrawn from the market in 2008 due to low sales, with no significant safety issues (Federal Register, 2017). Here, we describe a pilot study in healthy volunteers demonstrating the feasibility of developing an intranasal nalmefene formulation to treat opioid overdose.

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Materials and Methods

Study Details: The study was approved by the MidLands Independent Review Board (Overland Park, Kansas); all subjects gave written informed consent before participation. The study was conducted at Vince & Associates Clinical Research (Overland Park, KS) and carried out in accordance with the International Conference on Harmonization for Good Clinical Practices guidelines. This trial was registered as NCT03129347 (www.clinicaltrials.gov).

Participant Characteristics: Male and female volunteers aged 18-55 years, with body mass index (BMI) 18-32 kg/m² were eligible for participation. Subjects were currently not taking either prescription or over-the-counter medications; nonsmokers and subjects who smoked 20 or fewer cigarettes per day were enrolled. Screening procedures conducted within 21 days of study initiation included the following: medical history, physical examination, evidence of nasal irritation, 12-lead electrocardiogram, complete blood count, clinical chemistry, coagulation markers, hepatitis and human immunodeficiency screening, urinalysis, and urine drug screen. Female subjects were tested for pregnancy at screening and admission to the clinic. Subjects were excluded if they had either abnormal nasal anatomy or symptoms (e.g., runny nose, nasal polyps), an upper respiratory tract infection, used opioid analgesics for pain relief within the previous 14 days, or in the judgment of the investigator, had significant acute or chronic medical conditions. Subjects were required to abstain from alcohol from admission to the end of the last blood draw of the study, from nicotine and from caffeine-containing products and food for at least 1 hour prior to and 2 hours after dose administration, and from caffeine-containing products and food from midnight the day prior to and 4 hours after nalmefene

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dosing. On days of dosing, a subject's vital signs were required to be within the normal range before receiving nalmeferene, defined as: systolic blood pressure > 90 mmHg and \leq 140 mmHg; diastolic blood pressure > 55 mmHg and \leq 90 mmHg, resting heart rate > 40 beats per minute (bpm) and \leq 100 bpm, and respiratory rate > 8 respirations per minute (rpm) and \leq 20 rpm.

Study Design: The study was an inpatient, double-blind (for IN administration), randomized, 4-period, 4-treatment, 6-sequence, crossover. Subjects were randomly assigned to 1 of 6 sequences to ensure at least 2 subjects in each sequence. On the day after clinic admission, participants were administered the study drug in randomized order with a 4-day washout period between doses. Subjects remained at the clinic for 17 days until all 4 treatments were administered. They were contacted 3-5 days after discharge by a follow-up telephone call. Subjects fasted overnight before each dosing day and received one of the following 4 treatments:

Treatment A: 3 mg IN (one 0.1 mL spray of a 30 mg/mL nalmeferene solution in one nostril)

Treatment B: 3 mg plus 0.25% dodecyl maltoside (DDM) IN (one 0.1 mL spray of a 30 mg/mL nalmeferene solution containing 0.25% DDM in one nostril)

Treatment C: 1.5 mg IN (one 0.1 mL spray of a 15 mg/mL nalmeferene solution in one nostril)

Treatment D: 1.5 mg IM (1.5 mL of a 1.0 mg/mL nalmeferene solution)

The IN treatments were randomized while the IM dose was the last treatment for all subjects.

The high dose (3 mg) of nalmeferene was selected based on the relative bioavailability (~50%) of the structurally related molecule, naloxone (Krieter, et al., 2016), and the FDA guidance on parenteral dosing of nalmeferene that produces a maximum reversal of a suspected opioid

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overdose (Food and Drug Administration, 1995). In Phase I studies, IV doses of up to 24 mg have been well tolerated in normal volunteers (Dixon et al, 1986).

Study details: IN devices were coded so neither the staff nor the subjects knew the treatment administered. IN nalmefene was administered in the supine position, and subjects remained in this position for approximately 1 hour after dosing. Subjects were instructed not to breathe when the drug was administered to simulate an opioid overdose with a patient in respiratory arrest. Nasal passages were examined by medical personnel for irritation using a 6-point scale at pre-dose and at 5 minutes and 0.5, 1, and 4 hours post-dose. Nasal irritation was scored as follows: 0 (normal appearing mucosa, no bleeding); 1 (inflamed mucosa, no bleeding); 2 (minor bleeding that stops within 1 minute); 3 (minor bleeding taking 1 to 5 minutes to stop); 4 (substantial bleeding for 4 to 60 minutes, does not require medical intervention); and 5 (ulcerated lesions, bleeding that requires medical intervention). Sense of smell was evaluated using "Sniffin' Sticks" (US Neurological LLC, Poulsbo, Washington) at screening and admission, pre-dose, and 4 hours post-dose during Periods 1-3, and prior to discharge; correct identification of 10 or more odors out of 12 constituted a normal smell test. Subjects were required to identify 10 of 12 odors correctly to be admitted to the study. A finding of a subject identifying less than 10 odors during the course of study was reported as an adverse event (AE) of a reduced sense of smell. Twelve-lead ECG's were collected pre-dose and at 1 and 8 hours post-dose. Venous blood samples (4 mL) were collected for the analyses of plasma nalmefene concentrations pre-dose and at 2.5, 5, 10, 15, 20, 30, 45, and 60 minutes and 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 48, 60, and 72 hours post-dose using Vacutainer® tubes containing sodium heparin. Plasma was stored at < -60°C until analyzed.

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Study Drugs: Nalmefene hydrochloride (cGMP grade) was purchased from Rusan Pharma Ltd. (Mumbai, India). The IN and IM solutions were formulated by the VACR pharmacy staff.

Nalmefene was dissolved in 0.1 M citrate buffer, pH 4.0 for all IN formulations. Nalmefene was dissolved in saline for injection for IM administration, the pH was adjusted to pH 3.9 using dilute HCl, and the solution checked for sterility and pyrogenicity prior to administration. This IM formulation is identical to that listed for Revex® (nalmefene hydrochloride injection) (FDA, 1995). An Aptar multi-dose device (Aptar, Louveciennes, France) used for IN administration consisted of a pump and a 10-mL brown glass bottle. Based on solution weights taken before and after dose administration and the analytically determined concentrations of nalmefene, Treatments A, B, and C delivered mean doses (SD) of 2.97 ± 0.12 mg, 2.96 ± 0.15 mg, and 1.50 ± 0.11 mg, respectively. Plasma concentrations from 3 subjects (2 receiving 1.5 mg IN nalmefene and 1 receiving 3 mg nalmefene + DDM) were not used in the analysis because the amount of solution delivered by these devices was ≤ 0.057 mL.

Bioanalytical Methods: Plasma nalmefene concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Plasma samples (0.050 mL) were added to individual wells of a 96-well plate along with 0.050 mL acetonitrile containing the internal standard (0.5 ng nalmefene- d_3) followed by 0.5 mL acetonitrile. After vortex mixing, the plate was centrifuged for 5 minutes at 4°C, then 0.50 mL of supernatant was transferred into a new 96-well plate and evaporated to dryness. It was reconstituted with 0.20 mL methanol:0.1% formic acid in water (15:85) and submitted to LC-MS/MS analysis.

Nalmefene was analyzed using an AB Sciex API-5000 LC-MS/MS system (Framingham, Massachusetts) with an atmospheric pressure chemical ionization source operated in the

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positive ion mode. The mobile phase consisted of a gradient increasing from 0.2% formic acid in water:acetonitrile (9:1) to acetonitrile:methanol (1:1) at a rate of 0.5 mL/min through an Asentis Express C18 2.7 μm , 50 x 2.1 mm column. Nalmefene eluted at approximately 0.90 minutes. Ions monitored were m/z 340.1 and 268.1 for nalmefene and 343.2 and 268.1 for the internal standard. The calibration curves (peak area ratios) were linear ($r^2 > 0.994$) over the concentration range of 0.200 ng/mL to 20.0 ng/mL; the lower limit of quantitation was 0.200 ng/mL. The interday precision of the calibration curves and quality control samples ranged from 2.38 to 5.61%, and the accuracy ranged between -1.20 to 1.11% during the analysis of the samples.

Data Analyses: The safety population included all subjects who received at least 1 dose of nalmefene; the pharmacokinetic population included all subjects who received at least 1 dose of nalmefene with sufficient data to calculate meaningful pharmacokinetic parameters.

Pharmacokinetic parameters were calculated using standard noncompartmental methods and a validated installation of WinNonlin[®] Phoenix, version 6.3 (Certara, Princeton, NJ). Descriptive statistics were calculated with R Software version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Values of peak plasma concentrations (C_{max}) and the time to reach C_{max} (T_{max}) were the observed values obtained directly from the concentration-time data. The terminal elimination half-life ($t_{1/2}$) was estimated by linear regression analysis. The area under the concentration time curve from time zero to the last quantifiable concentration (AUC_{0-t}) was determined by the linear up/log down trapezoidal method. Within an ANOVA framework, comparisons of ln-transformed dose-normalized PK parameters were performed using a mixed effects model where sequence, period, and treatment were the independent factors. The 90%

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confidence interval (CI) for the ratio of the geometric least squares means of C_{\max} and AUC_{0-t} were constructed for comparison of the three IN treatments to the IM formulation. The 90% CIs were obtained by exponentiation of the 90% CIs for the differences between the least squares means based upon an ln scale. Pharmacokinetic comparisons were performed using a mixed effects model where sequence, period, and treatment were independent factors. All analyses of demographic and safety data were performed using SAS[®] statistical software, version 9.3 (SAS Institute, Inc., Cary, NC).

Results

Participant Characteristics: Ten male and four female participants (Table 1) received at least one dose of nalmefene; 10 subjects completed all 4 treatments and follow-up procedures. One subject withdrew consent after all 3 IN doses but before the fourth (IM) dose was administered. Another subject withdrew consent for personal reasons 6 hours after being administered the last dose (IM) of nalmefene. Two subjects left the study 24 hours after the last dose: one withdrew for personal reasons and the other was removed for disruptive behavior.

Pharmacokinetics: Following IN administration of 3 mg nalmefene, plasma concentrations were quantifiable for most subjects starting at 15 minutes post-dose. When 0.25% DDM was added to the formulation, plasma concentrations were quantifiable in the majority of the samples by 5 minutes post-dose (mean 0.93 ng/mL) (Fig. 1, inset). At 15 minutes, nalmefene concentrations following 3 mg IN with 0.25% DDM were approximately 12-fold higher than in its absence (4.57 vs 0.392 ng/mL) (Fig 1, inset). Addition of DDM also reduced the median T_{\max} from 2 hours to 15 minutes and increased C_{\max} more than two-fold (4.45 versus 1.99 ng/mL) (Table 2). The $t_{1/2}$ estimates of nalmefene were between 6.6-7.8 hours following IN and 8 h following IM

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administration; addition of DDM did not appear to alter the $t_{1/2}$ of IN nalmefene (Table 2). Six hours after IN administration of the 3 mg dose, plasma concentrations of nalmefene (in either the presence or absence of DDM) were ~0.9 ng/mL. By comparison, 6 hours after a 4 mg dose of IN naloxone, plasma concentrations were ~0.15 ng/mL (Krieter et al, 2016). IN nalmefene exhibited dose proportionality when the dose increased from 1.5 to 3 mg as evidenced by a doubling of both C_{max} and AUC_{0-t} (Table 2). The relative bioavailability of nalmefene after IN administration, when corrected for the dose, was 64-66% based on C_{max} when compared to the IM administration and 55-63% when AUC_{0-t} was used for this calculation (Table 3). There were modest differences between males and females in some of the pharmacokinetic parameters of nalmefene (Table 4). However, the small sample size of this pilot study precludes any definitive conclusions regarding sex-related differences in the pharmacokinetic properties of nalmefene following both IN and IM dosing.

Safety: Ten subjects experienced at least one AE classified as at least possibly related to nalmefene; all were mild in severity. The AEs reported by more than one participant were nausea (5), vomiting (3), dizziness (3), headache (2) and hyperhidrosis (2). There were no clinically significant laboratory values, and there were no apparent effects of IN nalmefene on the sense of smell (data not shown).

Discussion

Synthetic opioids present multiple challenges for first responders attempting to rescue overdose victims. High potency synthetics (fentanyl and other synthetics identified in overdose victims can be ≥ 2 orders of magnitude more potent than morphine [Burns, et al., 2018; Misailidi, et al., 2018]) may require very high doses of the competitive antagonist, naloxone (Li,

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et al., 2018; Sutter, et al. 2017; Uddayansankar et al. 2018), the only drug currently approved to treat opioid overdose, in order to effect a successful rescue. Not only are these high potencies problematic, but the long half-lives of fentanyl (7-8 h) and analogs such as sufentanil (6-9 h) (Ahonen, et al., 2000; Kharasch, 2015) and carfentanil (~5.7 h) (Uddayasankar, et al., 2018) further complicate rescue with naloxone. Thus, therapeutically effective plasma concentrations of naloxone ($t_{1/2}$ 1.3-2.4 h; Ryan and Dunne, 2018) may not be sustained in the presence of long duration synthetics, leading to a recurrence of symptoms (re-narcotization) including respiratory depression (Kaplan and Marx, 1993; Burns, et al. 2018) that complicates management of overdose and may require redosing with naloxone. Finally, synthetic opioids are orders of magnitude more lipophilic than morphine and related semi-synthetic opiates like oxycodone (Drewes, et al., 2012). This high lipophilicity favors rapid equilibration between plasma and CSF, resulting in a rapid onset of action. While this property is highly valued in an analgesic, it also results in a rapid onset of respiratory depression, effectively reducing the window for rescue.

Based on its high affinity and long half-life relative to naloxone (Table 5), we hypothesized that nalmefene could be useful as an IN rescue medication especially well-suited to treat synthetic opioid overdose. Moreover, because parenteral nalmefene was previously approved to treat opioid overdose (FDA, 1995), development of an IN product is substantially de-risked from both a safety and regulatory standpoint compared to a new chemical entity. The structural similarity to naloxone led us to select nalmefene doses (1.5 and 3 mg) based on the hypothesis that its IN bioavailability would also be similar to naloxone (Krieter, et al., 2016). In order to limit the number of arms in this pilot study, we elected to determine if IN nalmefene

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exhibited dose proportionality and to examine the effects of a single concentration of DDM on one dose of IN nalmeferene. The concentration of DDM (0.25%) selected was based on previous studies (Pillion and Maggio, 2013) demonstrating an enhanced IN absorption of other small molecules. Furthermore, this concentration of DDM is used in an FDA approved IN sumatriptan product (Neurelis, 2019), which de-risks its use in a nalmeferene product from a regulatory perspective. The approval of Narcan® (naloxone) Nasal Spray for treating opioid overdose was based on achieving both an onset of action and maximum plasma concentration comparable to a previously approved parenteral dose (Krieter, et al., 2016). Because parenteral nalmeferene was previously approved for the management of known or suspected opioid overdose (FDA, 1995), by analogy, an IM injection was selected as an appropriate comparator for an IN nalmeferene product candidate. The choice of a comparator dose (1.5 mg, IM) was based on the FDA label for parenteral nalmeferene; for the management of known or suspected opioid overdose, the label states: “A total dose greater than 1.5 mg did not increase the therapeutic response” (FDA, 1995).

The rapid delivery of high plasma concentrations is a cardinal feature of an effective IN rescue product (Krieter, et al, 2016; FDA, 2017). In the absence of DDM, the onset of IN nalmeferene is too slow to be useful as a rescue medication (Figure 1; Table 2). However, DDM, a member of a class of alkylsaccharide transmucosal absorption enhancers (reviewed in Pillion and Maggio, 2013), reduced the median T_{max} of IN nalmeferene to a value (0.25 h) comparable to IM administration (0.33 h) (Table 2). In an overdose rescue, the first few minutes are critical, perhaps more so when rapid onset synthetics are involved. It is notable that the T_{max} of IN nalmeferene indicates that its onset is more rapid than the FDA approved 4 mg dose of IN

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naloxone (4 mg), with a reported T_{max} of 0.5 h (Table 5). Moreover, a comparison of drug exposure at early time points (FDA, 2017) demonstrated that in the presence of DDM, the AUC_{0-t} values (t=5-20 min post-dose) of IN nalmefene were higher than the reference IM dose (Table 2); exposures were statistically significantly higher following IN dosing at 10, 15, and 20 min. ($p \leq 0.026$, ANOVA). While ~3-fold higher than following IM administration (1.5 mg), the C_{max} value produced by IN nalmefene (3 mg) in the presence of 0.25% DDM is in the range of plasma concentrations observed 5 minutes after a 1 mg IV dose in young and elderly males (3.7 and 5.8 ng/mL, respectively) (FDA, 1995). Despite the dramatic effects of DDM on both the C_{max} and T_{max} of IN nalmefene (Table 2; Fig 1), overall exposure as measured by AUC_{0-t} was increased by only ~20%, indicating the principal effect of DDM was to increase the rate of absorption. This latter observation is consistent with the hypothesis that DDM and related alkylsaccharides act as absorption enhancers by transiently opening tight junctions between cells in the nasal epithelium (Maggio and Pillion, 2013).

While multiple studies have reported nalmefene has a higher affinity than naloxone at both native and recombinant μ opioid receptors (Emmerson, et al., 1994; Toll, et al., 1998; Cassel, et al., 2005), there is no compelling evidence this translates to a clinically significant advantage in a hospital setting (Glass, et al., 1994; Kaplan, et al., 1999). However, in a study attempting to model overdose rescue in a non-hospital setting, Yong, et al., (2014) compared the effects of bolus IM injections of either nalmefene or naloxone to reverse carfentanil-induced loss of righting reflex and respiratory depression. Rats were administered 10 μ g/kg of carfentanil (IV) and 5 minutes later, were administered either nalmefene (9.4-150 μ g/kg) or naloxone (150 μ g/kg). Nalmefene, at doses as low as 9.4 μ g/kg significantly reduced the

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duration of the loss of righting reflex, and at doses between 9.4-18.8 $\mu\text{g}/\text{kg}$ reduced the duration of loss of righting reflex to the same extent as 150 $\mu\text{g}/\text{kg}$ of naloxone. At a dose of carfentanil (20 $\mu\text{g}/\text{kg}$, IV) that depressed respiration, nalmefene (37.5-150 $\mu\text{g}/\text{kg}$) produced a near complete to complete reversal within 10 min., restoring both P_aO_2 and P_aCO_2 to pre-carfentanil values. In contrast, naloxone (150 $\mu\text{g}/\text{kg}$) produced a partial, albeit significant reversal of respiratory depression. While the use of a single dose of naloxone limits interpretation of these results, these data are consistent with a higher potency of nalmefene to reverse the pharmacological effects of carfentanil closely linked to overdose.

Along with therapeutic advantages, a high potency, long duration opioid antagonist like nalmefene has the potential to produce a protracted withdrawal in opioid dependent individuals. There is one clinical report comparing nalmefene and naloxone in patients admitted to emergency departments with suspected narcotic overdose. In this double-blind study (Kaplan, et al., 1999), 156 patients in 9 centers were randomized to receive IV doses of nalmefene (1 or 2 mg) or naloxone (2 mg) every 5 minutes as needed for up to 4 doses. Most patients received a single dose of study drug, and in those patients with a confirmed opioid overdose, both drugs produced rapid and robust reversals of respiratory depression. Adverse events in opioid-positive patients were present in all three treatment arms: 12.5% (3/24) in the naloxone group, 10% (3/30) in the 1 mg nalmefene group, and 26.1% (6/23) in the 2 mg nalmefene group, respectively. While the incidence of adverse events was highest in the 2 mg nalmefene group, the overall difference among treatment arms was not significant ($p>0.27$), and no significant overall time-treatment interactions emerged. (Kaplan, et al, 1999). It is difficult to extrapolate findings from an emergency department setting to that envisioned for

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the field use of an IN nalmeferene product by first responders. Nonetheless, the overall incidence of adverse events reported in the Kaplan, et al. (1999) study is lower than recent case report data provided by both first-responders and community-based organizations using a 4 mg naloxone nasal spray (Avetian, et al., 2018). Withdrawal symptoms (including nausea, vomiting, irritability, sweating, muscle cramps, piloerection and diarrhea) precipitated by an overdose rescue are unpleasant and distressing, but not life threatening (Boyer, 2012). Given the alarming rise in synthetic opioid-related fatalities over the past 5 years (NIDA, 2018), the potential for iatrogenic withdrawal symptoms is medically justified weighed against the risk of a fatal overdose.

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Authorship Contributions

Participated in research design: Crystal, Gyaw, Krieter, Skolnick

Responsible for study conduct: Gyaw, Krieter

Performed data analysis: Krieter, Skolnick

Wrote or contributed to the writing of the manuscript: Crystal, Gyaw, Krieter, Skolnick

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Footnotes

Disclosures: P.S. and R.C. are employees of Opiant Pharmaceuticals, Inc. P.K. and S.G. are employees of the National Institutes of Health.

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Figure Legend

Figure 1. Mean plasma concentrations of nalmefene following single intranasal and intramuscular administration. Doses were as follows: 3 mg IN (closed circles), 3 mg plus 0.25% (w/v) DDM IN (open circles), 1.5 mg IN (triangles), and 1.5 mg IM (half-filled circles). Inset: Mean plasma concentrations of nalmefene between 2.5 min. and 2 h post-dose.

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References

Ahonen J, Olkkola K, Hynynen M, Seppala T, Ikavalko H, Remmerier B, and Salmenpera M (2000) Comparison of alfentanil, fentanyl and sufentanil for total intravenous anesthesia with Propofol in patients undergoing coronary artery bypass surgery. *Brit. J. Anesthes.* **85**:533-540.

Avetian G, Fiuty P, Mazzela S, Koppa D, Yeye V, and Hebbar, P (2018) Use of naloxone spray 4 mg in the community setting: a survey of use by community organizations. *Curr Med Res Opin* **34**:573-576.

Boyer EW (2012) Management of opioid analgesic overdose. *N. Engl. J. Med.* **367**:146-155.

Burns SM, Cunningham CW, and Mercer, SL (2018) DARK classics in chemical neuroscience: fentanyl. *ACS Chem. Neurosci.* **9**: 2428-2437.

Cassel J, Daubert J, DeHaven R (2005) [³H]Alvimopan binding to the μ opioid receptor: comparative binding kinetics of opioid antagonists. *Eur. J. Pharmacol.* **520**:29-36.

Dixon R, Howes J, Gentile J, Hsu H-B, Garg D, Weidler D, Meyer M and Tuttle R. (1986) Nalmefene: intravenous safety and kinetics of a new opioid antagonist. *Clin. Pharmacol. Ther.* **39**:49-53.

Drewes AM, Jensen RD, Nielsen LM, Droney J, Christrup LL, Arendt-Nielsen L, Riley, J, and Dahan A (2012) Differences between opioids: pharmacological, experimental, clinical and economical perspectives. *Brit. J. Clin. Pharmacol.* **75**:60-78.

Emmerson P, Liu M-R, Woods J, and Medzihradsky F (1994) Binding affinity and selectivity of opioids at mu, delta and kappa receptors in monkey brain membranes. *J. Pharmacol. Exp. Ther.* **271**:1630-1637.

Federal Register (2017) Determination That REVEX (Nalmefene Hydrochloride Injection), 0.1 Milligram Base/Milliliter and 1.0 Milligram Base/Milliliter, Was Not Withdrawn From Sale for Reasons of Safety or Effectiveness.

<https://www.federalregister.gov/documents/2017/11/03/2017-23952/determination-that-revex-nalmefene-hydrochloride-injection-01-milligram-basemilliliter-and-10> (last accessed December, 2018).

Food and Drug Administration, 1995. Revex® (nalmefene hydrochloride injection). https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/020459s006lbl.pdf (last accessed December, 2018).

Food and Drug Administration, 2015. Narcan® (naloxone hydrochloride) nasal spray. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208411lbl.pdf (last accessed March, 2018)

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Food and Drug Administration (2017) Draft guidance on naloxone hydrochloride.

<https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM554404.pdf> (last accessed December, 2018).

Glass P, Jhaveri R, and Smith L (1994) Comparison of potency and duration of action of nalmeferne and naloxone. *Anesth. Analg.* **78**:536-541.

Kaplan J and Marx J (1993) Effectiveness and safety of intravenous nalmeferne for emergency department patients with suspected narcotic overdose: a pilot study. *Ann. Emerg. Med.* **22**:187-190.

Kaplan J, Marx J, Calabro J, Gin-Shaw S, Spivey W, Gaddis G, Zhao N, Harchelroad F (1999) Double-blind, randomized study of nalmeferne and naloxone in emergency department patients with suspected narcotic overdose. *Ann. Emerg. Med.* **34**:42-50.

Kharasch, E (2015) Opioid half-lives and hemlines: the long and short of fashion. *Anesthesiol.* **122**: 969-970.

Krieter P, Chiang N, Gyaw S, Skolnick P, Crystal R, Keegan, F, Aker, J, Beck, M, and Harris, J (2016) Pharmacokinetic Properties and Human Use Characteristics of an FDA-Approved Intranasal Naloxone Product for the Treatment of Opioid Overdose. *J. Clin. Pharmacol.* **56**:1243-1253.

Li K, Armenian P, Mason J, and Grock A (2018) Narcan or narcan't: tips and trick to reversing opioid toxicity. *Ann Emerg Med.* **72**:9-11.

Lynn R, and Galinkin, J (2018) Naloxone dosage for opioid reversal: current evidence and clinical implications. *Ther. Adv. Drug. Saf.* **9**:63-88.

Maggio E, and Pillion D (2013) High efficiency intranasal drug delivery using Intravail® alkylsaccharide absorption enhancers. *Drug Deliv and Transl Res* **3**:16-25.

Mattingly, J., 2017. Synthetic heroin is too powerful for the overdose antidote.

<https://www.bloomberg.com/news/articles/2017-08-16/heroin-era-antidotes-can-t-handle-overdoses-in-age-of-synthetics> (last accessed December, 2018).

Misailidi N, Papoutsis I, Panagiota, N, Artemisia D, Spiliopoulou C, and Athanaselis S (2018) Fentanyl continue to replace heroin in the drug arena: the cases of ofcfentanil and carfentanil. *Forensic Toxicol.* **36**:12-32.

NIDA (2016) Fentanyl. <https://www.drugabuse.gov/publications/drugfacts/fentanyl> (last accessed Dec. 2018).

NIDA (2018) Overdose death rates. <https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates> (last accessed Dec. 2018)

JPET #256115

Neurelis (2019) Neurelis announces first product approved using the company's Intravail® platform. <https://www.neurelis.com/neurelis-news/neurelis-announces-first-product-approved-using-intravail-press-release> (last accessed Feb. 2019).

Ryan S, and Dunne R. (2018) Pharmacokinetic properties of intranasal and injectable formulations of naloxone for community use: a systematic review. *Pain Manag* **8**:231-245.

Skolnick, P (2018) On the front lines of the opioid epidemic: rescue by naloxone. *Eur. J. Pharmacol.* **835**: 147-153.

Sutter M, Gerona R, Davis T, Roche B, Colby D, Chenoweth JA, Adams A, Owen K, Ford J, Black, H, and Albertson, T. (2017) Fatal fentanyl: one pill can kill. *Acad Emerg Med* **24**:106-113.

Toll L, Berzetei-Gurske I, Polgar W, Brandt, S, Adapa, I, Rodriguez L, Schwartz R, Haggart D, O'Brien A, While A, Kennedy J, Craymer K, Farrington L and Auh J (1998) Standard binding and functional assays related to medications development testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res Monogr* **178**:440-466.

Uddayasankar U, Lee C, Oleschuk C, Eschun G, Ariano R (2018) The pharmacokinetics and pharmacodynamics of carfentanil exposure: a case report. *Pharmacotherapy: J Human Pharmacol and Drug Ther.* **38**:e41-e45. doi.org/10.1002/phar.2117

U.S. Government Accountability Office (2018) Opioid Crisis: status of public health emergency authorities. <https://www.gao.gov/products/GAO-18-685R> (last accessed December 2018).

Volkow N, and Collins F, (2017) The role of science in addressing the opioid crisis. *NEJM* **377**: 391-394.

Yong, Z., Gao, X., Ma, W., Dong, H., Gong, Z., Su, R., 2014. Nalmefene reverses carfentanil-induced loss of righting reflex and respiratory depression in rats. *Eur. J. Pharmacol.* **738**, 153-157.

Zezima K (2018) Study: despite decline in prescriptions, opioid deaths skyrocketing due to heroin and synthetic drugs. <https://www.washingtonpost.com/amhtml/news/post-nation/wp/2018/04/10/study-despite-decline-in-prescriptions-opioid-deaths-skyrocketing-due-to-heroin-and-synthetic-drugs/> (last accessed December, 2018).

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	All	Female	Male
N	14	4	10
Mean age, years (range)	32.9 (18-55)	30.8 (26-36)	33.8 (18-55)
Race			
White	6	3	3
Black/African American	8	1	7
Ethnicity			
Hispanic or Latino	2	0	2
Not Hispanic or Latino	12	4	8
Mean weight, kg (range)	79.9 (64.1-101.3)	67.4 (64.1-69.9)	84.9 (70.0-101.3)
Mean BMI, ^a kg/m ² (range)	26.4 (20.4-32.2)	25.4 (22.4-28.5)	26.8 (20.4-32.2)

Table 1: Subject demographics

^aBMI, body mass index

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Parameter (Units) ^a	3 mg IN (N=14)		3 mg IN/0.25% DDM (N=13)		1.5 mg IN (N = 11)		1.5 mg IM (N=13)	
C _{max} (ng/mL)	1.99	(51.3)	4.45	(65.7)	0.961	(43.8)	1.53	(43.5)
C _{max} /D (ng/mL/mg)	0.662	(51.3)	1.48	(65.7)	0.641	(43.8)	1.02	(43.5)
T _{max} (h)	2.00	(0.33- 3.00)	0.25	(0.17-1.00)	2.00	(1.00-2.07)	0.33	(0.25-8.00)
AUC _{0-t} (ng·h/mL)	12.7	(68.1)	15.2	(71.8)	5.58 ^d	(57.9)	10.6	(45.7)
AUC _{0-2.5 min}	0.00	(0.00)	0.004	(0.006)	0.00	(0.00)	0.00	(0.00)
AUC _{0-5 min}	0.00	(0.00)	0.029	(0.037)	0.00	(0.00)	0.008	(0.013)
AUC _{0-10 min}	0.005	(0.010)	0.257	(0.239)	0.00	(0.00)	0.072	(0.053)
AUC _{0-15 min}	0.026	(0.036)	0.596	(0.506)	0.006	(0.006)	0.168	(0.106)
AUC _{0-20 min}	0.226	(0.202)	0.924	(0.708)	0.023	(0.019)	0.278	(0.170)
AUC _{0-t} /D (ng·h/mL/mg)	4.24	(68.1)	5.06	(71.8)	3.72	(57.9)	7.07	(45.7)
t _{1/2} (h)	7.87 ^b	(40.8)	7.11	(45.5)	6.59 ^c	(53.3)	8.01 ^d	(39.2)

Table 2 Pharmacokinetic parameters of nalmefene following intranasal and intramuscular administration

Abbreviations: %CV, percent coefficient of variation; AUC_{0-t}, area under the plasma concentration-time curve from time zero to the last measurable concentration; AUC_{0-t}/D, AUC_{0-t} divided by the dose; AUC_{0-x}, AUC from time zero to x minutes; C_{max}, maximum plasma concentration; C_{max}/D, C_{max} divided by the dose; t_{1/2}, terminal half-life; T_{max}, time to C_{max}

^aGeometric mean values (%CV) for all except T_{max} which is median (minimum, maximum)

^bN = 13; ^cN = 10; ^dN=12

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Parameter (Units)	IN Administration (Test)	Comparison (IM as Reference)	Ratio (Test/Reference) of Adjusted Means ^a	90% CI for Ratio
C_{\max} /Dose (ng/mL/mg)	3 mg IN (Trt A)	A vs D	65.8	49.6-87.2
	3 mg/0.25% DDM IN (Trt B)	B vs D	135	100-180
	1.5 mg IN (Trt C)	C vs D	63.4	46.6-86.4
AUC _{0-t} /Dose (ng·h/mL/mg)	3 mg IN (Trt A)	A vs D	63.1	53.1-74.9
	3 mg/0.25% DDM IN (Trt B)	B vs D	69.5	58.1-83.2
	1.5 mg IN (Trt C)	C vs D	55.4	45.8-67.1

Table 3: Statistical summary of treatment comparisons

Abbreviations: AUC_{0-inf}/Dose, AUC per mg naloxone administered; C_{\max} /Dose, C_{\max} per mg naloxone administered; IM, intramuscular; IN, Intranasal; Trt, Treatment

^aGeometric least-squares mean ratio between treatments, expressed as a percentage of Reference (IM, Treatment D)

Parameter (Units) ^a	3 mg IN (A)		3 mg/0.25% DDM IN (B)		1.5 mg IN (C)		1.5 mg IM (D)	
	Female (N=4)	Male (N=10)	Female (N=4)	Male (N=9)	Female (N=3)	Male (N=8)	Female (N=4)	Male (N=9)
C _{max} (ng/mL)	2.20 (47.0)	1.90 (54.9)	5.51 (72.7)	4.04 (64.3)	1.19 (53.1)	0.888 (40.8)	1.13 (21.7)	1.75 (44.2)
T _{max} (h)	2.00 (2.00-2.00)	1.52 (0.33-3.00)	0.25 (0.25-0.25)	0.25 (0.17-1.00)	2.00 (1.00-2.07)	2.00 (1.00-2.00)	0.63 (0.25-8.00)	0.33 (0.15-2.00)
AUC _{0-t} (ng·h/mL)	14.6 (57.1)	12.0 (74.7)	18.4 (84.1)	13.9 (69.8)	7.38 (75.9)	5.03 (51.8)	14.0 (33.1)	9.38 (45.8)
t _{1/2} (h)	8.14 ^b (46.9)	7.79 (41.7)	9.22 (10.4)	6.34 (45.1)	7.54 (92.2)	6.23 ^c (41.3)	10.3 (43.2)	7.07 ^d (32.0)

Table 4: Pharmacokinetics of nalmefene in males and females following intranasal and intramuscular administration

Abbreviations: %CV, percent coefficient of variation; AUC_{0-t}, area under the plasma concentration-time curve from time zero to the last measurable concentration; C_{max}, maximum plasma concentration; t_{1/2}, terminal half-life; T_{max}, time to C_{max}.

^aGeometric mean values (%CV) for all except T_{max} which is median (minimum, maximum), ^bN=3; ^cN=7; ^dN=8

Parameter	Nalmefene	Naloxone
K _i (nM)	1.0 ¹	5.4 ¹
t _{1/2} (h)	7.11 ²	2.08 ³
t _{max} (h)	0.25 ²	0.5 ³
C _{max} (ng/mL)	4.45 ²	4.83 ³

Table 5 Nalmefene and naloxone: a comparison of affinities at μ opioid receptors and pharmacokinetic properties following intranasal administration. ¹K_i values were estimated using [³H]alvimopan binding to cloned human μ opioid receptors (Cassel, et al., 2005). The ~5-fold higher affinity of nalmefene compared to naloxone is consistent with both K_i values obtained (0.13 and 0.62 nM, respectively) using [³H]DAMGO as a radioligand in monkey brain membranes (Emmerson, et al., 1994) and pA₂ values of 9.38 and 8.51, respectively, in functional assays using guinea pig ileum and mouse vas deferens (Toll, et al., 1998).²Data from Table 2.

³Data from FDA, 2015 (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208411lbl.pdf)

