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Enhanced Intranasal Absorption of Naltrexone by Dodecyl Maltopyranoside: Implications for the Treatment of Opioid Overdose

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Abstract

Based on its high affinity for μ opiate receptors and reported half-life after oral administration, the pharmacokinetic properties of intranasal naltrexone were examined to evaluate its potential to treat opioid overdose. This study was prompted by the marked rise in overdose deaths linked to synthetic opioids like fentanyl, which may require more potent, longer-lived opiate antagonists than naloxone. Both the maximum plasma concentration (C_{max}) and the time (T_{max}) to reach C_{max} for intranasal naltrexone (4 mg) were comparable to values reported for a Food and Drug Administration-approved 4-mg dose of intranasal naloxone. The addition of the absorption enhancer dodecyl maltoside (Intravail) increased C_{max} by ~3-fold and reduced the T_{max} from 0.5 to 0.17 hours. Despite these very rapid increases in plasma concentrations of naltrexone, its short half-life following intranasal administration (~2.2 hours) could limit its usefulness as a rescue medication, particularly against longer-lived synthetic opioids. Nonetheless, the ability to rapidly attain high plasma concentrations of naltrexone may be useful in other indications, including an as-needed dosing strategy to treat alcohol use disorder.

Keywords

dodecyl maltoside, intranasal, naltrexone, normal volunteers, opioid overdose, pharmacokinetics

It has been estimated that approximately 11.8 million individuals (4.4% of the adult U.S. population) misused opioids in 2016; 2.1 million of them had a *Diagnostic and Statistical Manual* 5th edition-defined opioid use disorder.[1] This widespread misuse of opioids has resulted in an increasing number of overdose deaths over the past 2 decades.[2,3] The most recent estimates indicate there were more than 49 000 opioid-related overdose deaths in 2017[4]; Ruhm[5] suggests such estimates have been systematically underreported by 20% to 35%.

Naloxone was approved by the Food and Drug Administration (FDA) in 1971 for the treatment of suspected or confirmed opioid overdose and is currently the only opioid antagonist approved for this indication. Originally available as an injection used by medical personnel in emergency departments and by emergency medical technicians,[6] the FDA approved both an autoinjector (2014)[7] and a nasal spray formulation (2015)[8] that can be used by individuals (eg police, emergency medical services, and friends and family of overdose victims) with little or no prior training. The basis for approval of this nasal spray was a rapid onset of action and the ability to achieve plasma concentrations equivalent to an approved dose of parenterally administered naloxone.[9]

Although the annual number of overdose deaths because of prescription opioids and heroin continues to increase,[4] 2012-2013 marked the beginning of a dramatic upsurge in overdose deaths related to illicit fentanyl and fentanyl derivatives. The most recent data available (2017) indicate ~55% of opioid-related overdose deaths involved fentanyl and related compounds, more than either prescription opioids or heroin.[4] The high potency,[10] low cost,[11] and relatively long elimination half-lives of fentanyl and several of its analogues ($t_{1/2} > 7$ hours)[12–15] compared with heroin and many prescription opioids all contribute to the dangers posed by these synthetic opioids.[16,17]

Reversal of the pharmacological actions of fentanyl by naloxone has been demonstrated in both the operating room and emergency department.[6,18,19] However,

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because each overdose situation is unique, complicated by factors including the quantity of opioid(s) taken, the victim's level of tolerance, and the presence of other drugs (such as benzodiazepines) that can potentiate opioid-induced respiratory depression, the dose of naloxone required for a successful rescue by first responders is empirical and symptom driven.[16] Both anecdotal and well-documented case reports suggest that synthetics may be "too strong" for naloxone[20–23]; some authors have recommended parenteral administration of up to 12-15 mg of naloxone to reverse an overdose associated with synthetics.[24] The current position of the National Institute on Drug Abuse states: "Overdoses of fentanyl…may require higher doses [of naloxone] to successfully reverse the overdose."[25] Prompted by the upsurge in overdose deaths related to synthetics, National Institutes of Health leadership recently called for the development of "…stronger, longer-acting formulations of antagonists."[10]

Naltrexone is a high-affinity opioid antagonist currently approved as both a tablet[26] and a depot injection[27] for the treatment of both opioid and alcohol use disorders. At face value, both its higher affinity (~5× higher)[28,29] and longer half-life (4 hours)[26] relative to naloxone ($t_{1/2}$, ~1.3-2.4 hours after parenteral and intranasal administration)[30] appear to fulfill the criteria of a best-in-class overdose reversal agent. Moreover, preliminary data[31] suggest that naltrexone is absorbed following intranasal administration. The objective of the present study was to determine the pharmacokinetic properties of intranasal naltrexone in healthy volunteers and compare this with parenteral and oral dosing. Because a rapid onset of action favors successful reversal of an opioid overdose, we also determined whether dodecyl maltopyranoside (DDM; Intravail), previously reported to enhance the intranasal absorption of small molecules,[32] modified the pharmacokinetic properties of intranasal naltrexone.

Methods

Study Participants

The study was approved by the MidLands Independent Review Board (Overland Park, Kansas); all subjects gave written informed consent before participation. The study was carried out in accordance with the International Conference on Harmonization for Good Clinical Practices guidelines[33] at Vince & Associates Clinical Research, Inc., Overland Park, Kansas. This trial was registered as NCT02750748 (www.clinicaltrials.gov).

Male and female volunteers, aged 18-55 years with a body mass index of 18-30 kg/m^2 participated in the study. Subjects who were not currently taking either prescription or over-the-counter medications and were nonsmokers or 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. Women were tested for pregnancy (using a serum pregnancy test) both at screening and on admission to the clinic. Subjects were excluded if they had either abnormal nasal anatomy or symptoms (eg, blocked and/or 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 beginning at admission to the end of the last blood draw of the study, from nicotine 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 4 hours after naltrexone dosing. On days of dosing, a subject's vital signs were required to be within the normal range before receiving naltrexone, defined as systolic blood pressure > 90 and ≤ 140 mm Hg, diastolic blood pressure > 55 and ≤ 90 mm Hg, resting heart rate > 40 and ≤ 100 beats per m, and respiratory rate > 8 and ≤ 20 respirations per minute.

Study Design

This pharmacokinetic study was an inpatient, open-label, 4-period, 4-treatment, 1-sequence crossover study involving 14 subjects conducted at Vince & Associates Clinical Research (Overland Park, Kansas). On the day after clinic admission, participants were administered the study drug with a 4-day washout period between doses. Subjects remained in the clinic for 13 days until all 4 treatments were administered; they received a follow-up call 3 to 6 days later. Subjects were fasted overnight before each dosing day and received 1 of the following 4 treatments:

A. 4 mg naltrexone HCl intranasally (one 0.1-mL spray of the 40 mg/mL formulation in 1 nostril).
B. 4 mg naltrexone HCl plus dodecyl maltopyranoside intranasally (one 0.1-mL spray of the 40 mg/mL plus 0.25% DDM formulation in 1 nostril).
C. 2 mg naltrexone HCl intramuscularly (1.0 mL of a 2 mg/mL normal saline for injection in the gluteus maximus muscle.
D. 50 mg naltrexone HCl tablet orally with 8 ounces of noncarbonated water.

The intranasal doses were chosen based on a pilot study using 2 and 4 mg naltrexone HCl delivered in 0.1 mL. The 50-mg tablet is reported to produce a

maximum plasma concentration in the same range.[34] Concentrations of DDM up to 0.5% (w/v) in intranasal formulations have been used in studies of peptide absorption[32]; the present study used a concentration of 0.25% w/v.

Intranasal naloxone was administered to subjects in a reclined position, and they remained in this position for approximately 1 hour after dosing. Subjects were instructed not to breathe when the drug was administered intranasally. The nasal passage was examined by medical personnel for irritation using a 6-point scale at predose and at 5 minutes and 0.5, 1, 4, and 24 hours postdose. 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. Twelve-lead electrocardiograms were collected predose and 1 and 4 hours postdose. Venous blood samples (4 mL) were collected for the analyses of plasma naloxone concentrations predose and 2.5, 5, 10, 15, 20, 30, 45, and 60 minutes and 2, 3, 4, 6, 8, 12, 16, 30, 36, and 48 hours postdose using Vacutainer tubes containing sodium heparin. The plasma was stored at −70°C until analyzed.

Study Drugs

Naltrexone HCl was purchased from Mallinckrodt Pharmaceuticals (St. Louis, Missouri), dodecyl maltopyranoside (DDM; Incalco Pharmaceuticals, San Luis Obispo, California) was supplied by Aegis Therapeutics LLC (San Diego, California), and naltrexone HCl 50-mg tablets were obtained from Sun Pharmaceutical (Cranbury, New Jersey). The intransal and intramuscular solutions were formulated by the pharmacy staff of VACR. Naltrexone for intranasal administration ± 0.25% DDM (v/w) was dissolved in water for injection at a concentration of 40 mg/mL; the pH of the solution was pH 5.6. The intramuscular formulation was made using sterile saline and was filtered through a 0.2-μm filter. It was checked for sterility and pyrogenicity before administration; the pH of the solution was 5.7. The Aptar multidose device (Aptar, Louveciennes, France) used for intranasal administration consisted of a pump and a 10-mL brown-glass bottle. After priming, the devices delivered a mean weight of 102.3 ± 0.91 and 101.8 ± 1.93 mg of the naltrexone solution per nominal 0.1-mL spray with and without DDM, respectively.

Analytical Methods

Plasma naltrexone and 6β-naltrexol concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. The concentration range for both analytes was 0.2 to 20 ng/mL. Plasma samples (0.15 mL) were mixed with 0.1 mL of 1% formic acid in water and 0.05 mL of acetonitrile:water (2:8) containing the internal standards (0.5 ng naltrexone-d_3 and 0.25 ng 6β-naltrexol-d_3) and added to individual wells of a preconditioned 96-well plate. The plate was washed sequentially with 1% formic acid in water, water, methanol:water (1:1), and methanol. The analytes were eluted using 4% ammonium hydroxide in methanol. After evaporation, the residue was dissolved in 0.15 mL of methanol:0.1% formic acid (8:92) and submitted to LC-MS/MS analysis. The AB MDS Sciex API-5000 LC-MS/MS system (Framingham, Massachusetts) with an atmospheric pressure chemical ionization source was operated in the positive ion detection mode. The mobile phase consisted of a gradient increasing from 93% mobile phase A (10 mM ammonium formate, pH 4.0)/7% mobile phase B (acetonitrile:methanol, 2:8) with a flow rate of 0.5 mL/min through a 2.1 × 50 mm Kinetex EVO C18 2.6-μm column (Phenomenex, Torrance, California). Naltrexone eluted at approximately 1.45 minutes; ions monitored were *m/z* 342.2 and 324.2 for naltrexone and 345.2 and 327.3 for its internal standard. 6β-Naltrexol eluted at approximately 1.60 minutes; ions monitored were m/z 344.2 and 326.2 for 6β-naltrexol and 347.1 and 329.3 for its internal standard. The interday precision of the calibration curves and quality control samples for naltrexone ranged from 2.09% to 8.64%, and the accuracy ranged between −5.88% and 3.00% during the analysis of the samples. The interday precision of the calibration curves and quality control samples of 6β-naltrexol ranged from 2.29% to 6.44%, and the accuracy ranged between −3.00% and 3.00% during the analysis of the samples. The lower limit of quantification (LLOQ) for both naltrexone and 6β-naltrexol was 0.02 ng/mL.

Data Analyses

The safety population included all subjects who received at least 1 dose of naltrexone; the pharmacokinetic population included all subjects who received at least 1 dose of naltrexone 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, New Jersey). Descriptive statistics were calculated with R Software version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Values of peak plasma concentration (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 ($AUC_{0\text{-}t}$) was determined by the linear-up/log-down trapezoidal method and extrapolated to infinity ($AUC_{0\text{-}inf}$) by adding the value of the last quantifiable concentration divided by the terminal rate constant (λz). The extrapolated percentage of $AUC_{0\text{-}inf}$ was less than 20% for all concentration profiles; therefore, only $AUC_{0\text{-}inf}$ is reported. The apparent total body clearance (CL/F) was calculated as the dose divided by $AUC_{0\text{-}inf}$. Pharmacokinetic comparisons were performed using a mixed-effects model in which 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, North Carolina).

In Vitro Permeability Study

In vitro permeability studies were conducted at Sekisui XenoTech LLC (Kansas City, Kansas). Madin-Darby canine kidney cells (MDCKII cells) were used to evaluate the bidirectional permeability of naltrexone. Cells were purchased from the Netherlands Cancer Institute. MDCKII cells were plated and maintained on 24-well transwell plates for 3 to 5 days prior to the experiment, when they formed a confluent monolayer with tight junctions. Culture medium was removed, and incubation medium (Hank's balanced salt solution with 25 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] and 25 mM glucose) was added to the cells. After approximately 10 minutes, the transepithelial electrical resistance (TEER) values were recorded, and cells were preincubated at 37°C for 30 to 60 minutes. Incubation medium containing naltrexone ± 0.25% DDM or control substrates (10 μM ^{3}H-mannitol and 10 μM ^{14}C-caffeine) was then added ($n = 3$). The total incubation volumes were 200 and 980 μL for the apical and basolateral chambers, respectively. Incubations were performed under 2 pH conditions: pH 7.4 on both the apical (A) and basolateral (B) sides or pH 5.5 on the apical side and pH 7.4 on the basolateral side. Samples (100 μL) were collected from the compartment of interest at 15, 60, and 120 minutes and replaced with 100 μL of incubation medium.

Samples were mixed with the internal standard (d_6-hydroxybuproprion) and analyzed by ultra-high-performance LC (HPLC)-MS/MS. The HPLC system consisted of a Phenomenex Luna C8 guard column (4.0 × 2.0 mm), a Waters Acquity UPLC BEH C18 analytical column (50 × 2.1 mm, 1.7 μm), and an ABS SCIEX 4000 QTrap mass spectrometer in the positive ionization mode. The mobile phase consisted of 10 mM ammonium acetate in water (mobile phase A) and 10 mM ammonium acetate in acetonitrile with 0.1% v/v ammonium hydroxide. The gradient ran from 30% to 80% mobile phase B from 0.5 to 2.4 minutes. The mass transitions (*m*/*z*) for naltrexone were 342.2 and 324.1; for the internal standard, the mass transitions (*m*/*z*) were 262.0 and 244.0. Calibration standards were based on analyte/internal standard peak area ratios. Radioactive samples were analyzed by liquid scintillation counting.

The apparent permeability (P_{app}) was calculated as:

$$\frac{dQ}{dT} \times \frac{1}{A_0 \times C_0}$$

where dQ is the quantity (pmol) of naltrexone transported, dT is the incubation time (in seconds), A_0 is the surface area of the porous membrane (0.33 cm^2), and C_0 is the initial concentration (pmol/mL) of naltrexone in the donor chamber. The efflux ratio is the ratio of P_{app} B to A/P_{app} A to B.

Results

Clinical Study

Subject Characteristics. The safety population included 5 women and 9 men who received at least 1 dose of naltrexone (Table 1). One subject developed moderate dizziness and mild hyperhidrosis 18 minutes after the first dose (4 mg naltrexone intranasally), 1 subject each was terminated before period 2 and period 3 because of elevated blood pressure prior to dosing, and 1 subject withdrew consent before dosing of period 3 for personal reasons. One subject withdrew consent 24 hours after dosing of the fourth period and did not respond to the follow-up call; the number of samples from this subject was sufficient to calculate PK parameters. Therefore, 9 subjects completed administration of all doses and follow-up procedures.

The PK population included all subjects who received at least 1 treatment of naltrexone with the exception of 1 subject for whom the last blood sample was collected 45 minutes after the first dose.

Pharmacokinetics of Naltrexone. Following intranasal administration of naltrexone (4 mg), the mean plasma concentration at the earliest point studied (2.5 minutes postdosing) was 0.117 ng/mL. The addition of 0.25% DDM to the formulation increased the mean concentration by ~10-fold (1.15 ng/mL). At 5 minutes postdose, the mean concentrations of naltrexone with and without DDM were 11.9 and 1.51 ng/mL, respectively, an ~8-fold difference (Figure 1).

The addition of DDM decreased the median T_{max} for intranasal naltrexone from 30 to 10 minutes and increased C_{max} more than 3-fold (Table 2). By comparison, the overall exposure of naltrexone (as measured by $AUC_{0\text{-}inf}$) increased by ~54%, indicating the principal effect of DDM was to increase the rate of absorption.

Table 1. Subject Demographics

	All	Women	Men
n	14	5	9
Age (years), mean ± SD (range)	34.9 ± 10.7 (22-54)	29.0 ± 4.8 (22-35)	38.2 ± 11.9 (22-54)
Weight (kg), mean ± SD (range)	78.0 ± 17.8 (55.2-113.7)	66.9 ± 13.5 (55.2-84.9)	84.2 ± 17.5 (61.2-113.7)
BMI (kg/m^2), mean ± SD, (range)	25.4 ± 3.1 (19.8-29.7)	25.7 ± 3.5 (21.3-29.7)	25.2 ± 30.1 (19.8-29.3)
Race			
White	5	2	3
Black/African American	9	3	6
Ethnicity			
Hispanic or Latino	2	1	1
Not Hispanic or Latino	12	4	8

BMI, body mass index.

Table 2. Pharmacokinetics of Naltrexone Following Intranasal, Intramuscular, and Oral Administration

PK Parameter[a]	4 mg Intranasally	4 mg Intranasally + DDM	2 mg Intramuscularly	50 mg Orally
N[b]	13	12	10	10
C_{max} (ng/mL)	5.4 (66.8)	15.7 (52.0)	4.1 (34.0)	9.3 (31.8)
C_{max}/dose (ng/mL/mg)	1.5 (66.8)	4.4 (52.0)	2.3 (34.0)	0.2 (31.8)
T_{max} (h)	0.5 (0.2-2.0)	0.2 (0.1-0.3)	0.3 (0.2-1.0)	0.5 (0.3-3.0)
$AUC_{0-\infty}$ (ng·h/mL)	12.0 (33.7)	18.5 (31.0)	12.3 (25.6)	26.9 (31.8)
$AUC_{0-\infty}$/dose (ng·h/mL/mg)	3.3 (33.7)	5.1 (31.0)	6.8 (25.6)	0.6 (31.8)
CL/F (L/h)[c]	330 (28.9)	214 (33.6)	154 (19.0)	1890 (41.4)
$t_{1/2}$, (h)	2.5 (14.9)	2.2 (14.9)	2.0 (15.5)	6.4 (36.6)

%CV, percent coefficient of variation; C_{max}, maximum plasma concentration; C_{max}/dose, C_{max} per milligram administered; T_{max}, time to C_{max}; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time zero to infinity; $AUC_{0-\infty}$/dose, $AUC_{0-\infty}$ per milligram administered; CL/F, apparent oral clearance; $t_{1/2}$, terminal half-life.

[a]Mean (%CV) for all except median (range) for T_{max}.

[b]Number of subjects in PK population.

[c]Dose corrected for HCl salt to obtain nominal values of 3.618 mg (intranasally), 1.809 mg (intramuscularly), and 45.23 mg (orally) naltrexone free base, respectively.

The mean C_{max} following the 2-mg intramuscular dose of naltrexone was lower than the intranasal dose of 4 mg; T_{max} was reached at 20 minutes. Mean plasma concentration 2.5 and 5 minutes after 2 mg naltrexone intramuscularly was 0.678 and 1.04 ng/mL, respectively.

Oral absorption of naltrexone was slower than after either intranasal or intramuscular administration; concentrations in most of the samples collected during the first 15 minutes were below the LLOQ. The median T_{max} was 30 minutes postdose. The mean C_{max} value was 9.34 ng/mL, lower than that observed after intranasal dosing with DDM despite the amounts of naltrexone administered: 50 mg orally compared with 4 mg intranasally.

The mean terminal phase half-life ($t_{1/2}$) of naltrexone averaged approximately 2-2.5 hours following intranasal and intramuscular administration and 6 hours when administered orally. Plasma naltrexone was measurable for most subjects through 12 or 16 hours after the intranasal or intramuscular dose and measurable through 30 to 36 hours after the oral dose.

When corrected for dose, the geometric mean ratio of C_{max} after the 4-mg intranasal dose was approximately 60% of the value after the 2-mg intramuscular dose (Table 3). By comparison, the addition of DDM increased this value to almost twice that of the intramuscular dose (Table 3). The geometric mean ratio of C_{max} after the oral dose was ~9% of the intramuscular dose.

The relative bioavailability of naltrexone after intranasal dosing with and without DDM was 76% and 48%, respectively, compared with intramuscular administration. The relative bioavailability for the oral dose was 9%.

The small number of subjects in this study precluded any definitive conclusions concerning potential sex differences in the pharmacokinetic profile of naltrexone.

Pharmacokinetics of 6β-Naltrexol. The mean C_{max} of 6β-naltrexol, a major metabolite of naltrexone, was approximately twice as high following the 4-mg intranasal dose (both with and without DDM) compared with the 2-mg intramuscular dose (Table 4, Figure 2). However, the dose-corrected C_{max} values following intranasal and intramuscular administration were similar. The C_{max} values following the 50-mg oral dose were

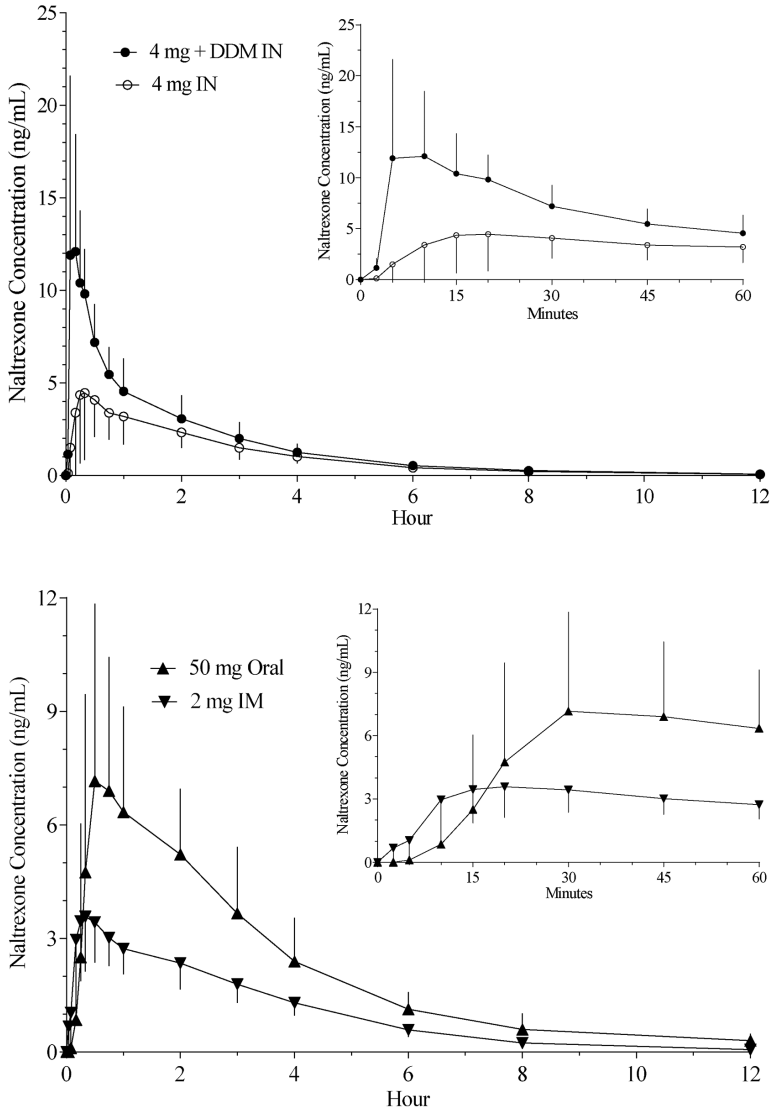

Figure 1. Plasma concentrations of naltrexone following intranasal, intramuscular, and oral administration of naltrexone HCl. Top: Subjects received 4 mg naltrexone intranasally with (closed circles) and without (open circles) 0.25% (w/v) DDM. Bottom: Subjects received 2 mg naltrexone by intramuscular injection (inverted triangles), and 50 mg orally (triangles). Insets: plasma naltrexone concentrations between 0 and 1 hour postdose. IN, intranasal; IM, intramuscular; PO, oral. Values represent mean ± SD.

more than an order of magnitude greater compared with either intramuscular or intranasal administration and 2- to 2.5-fold higher when corrected for dose. The dose-corrected values of $AUC_{0\text{-inf}}$ were similar for the intranasal, intramuscular, and oral routes of administration; similarly, $t_{1/2}$ was 12-14 hours for all 3 routes of administration.

DDM (0.25%) did not alter either the dose-adjusted C_{max} or $AUC_{0\text{-inf}}$ of 6β-naltrexol.

The small number of subjects in this study precluded any definitive conclusions concerning potential sex differences in the pharmacokinetic profile of 6β-naltrexol.

Safety. Ten subjects (71%) experienced at least 1 AE during the study that was considered to be related to naltrexone. The most common was dizziness (10%-25% for intranasal and intramuscular administration). All AEs were mild, with except for 1 moderate case of dizziness that occurred 18 minutes after the 4-mg intranasal dose; this symptom resolved within a few sminutes. No abnormal nasal irritation scores were noted during the study.

In Vitro Studies

Measurement of the bidirectional permeability of naltrexone across MDCKII cells was examined at pH 7.4 on both the apical and basolateral sides and when the pH was lowered to pH 5.5 on the apical side, more closely reflecting the pH of the nasal cavity.[35] When the pH was maintained at pH 7.4 on both

Table 3. Naltrexone Statistical Summary of Treatment Comparisons (Intranasal and Oral Versus Intramuscular Administration)

	Geometric Mean						
Parameter (Units)	4 mg Intranasally	4 mg Intranasally + DDM	50 mg Orally	2 mg Intramuscularly (Reference)	Comparison (Intramuscular Reference)	Ratio (Test/Reference) of Adjusted Means[a]	90%CI for Ratio
C_{max}/dose (ng/mL/mg)	1.26	3.93	0.193	2.09	4 mg IN	60.4	45.4-80.2
					4 mg IN + DDM	188	161-221
					50 mg PO	9.30	7.60-11.3
$AUC_{0-\infty}$/dose (ng·h/mL/mg)	3.16	4.96	0.581	6.56	4 mg IN	48.2	41.4-56.2
					4 mg IN + DDM	75.7	68.0-84.2
					50 mg PO	8.90	7.30-10.7

C_{max}, maximum plasma concentration; C_{max}/dose, C_{max} per milligram administered; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time zero to infinity; $AUC_{0-\infty}$/dose, AUC per milligram administered; IM, intramuscularly; IN, intranasally; PO, orally; CI, confidence interval.
[a]Geometric least-squares mean ratio between treatments, expressed as a percentage of intramuscular administration (reference).

Table 4. Pharmacokinetics of 6β-Naltrexol Following Intranasal, Intramuscular, and Oral Administration

PK Parameter[a]	4 mg Intranasally	4 mg Intranasally + DDM	2 mg Intramuscularly	50 mg Orally
n[b]	13	12	10	10
C_{max} (ng/mL)	3.0 (32.2)	3.3 (23.7)	1.5 (26.8)	90.7 (30.3)
C_{max}/dose (ng/mL/mg)	0.8 (33.2)	0.9 (23.7)	0.8 (26.8)	2.0 (30.2)
T_{max} (h)	2.0 (0.8, 6.0)	0.8 (0.3, 4.0)	3.0 (0.8, 4.0)	0.6 (0.3, 3.0)
$AUC_{00\infty}$ (ng·h/mL)	44.0 (23.1)	46.3 (18.3)	27.1 (19.0)	675 (19.9)
$AUC_{0-\infty}$/dose (ng·h/mL/mg)	12.2 (23.1)	12.8 (18.3)	15.0 (19.0)	14.9 (19.9)
$T_{1/2}$ (h)	13.7 (22.7)	12.8 (14.6)	12.4 (13.2)	13.9 (15.9)

%CV, percent coefficient of variation; C_{max}, maximum plasma concentration; C_{max}/dose, C_{max} per milligram administered; T_{max}, time to C_{max}; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time zero to infinity; $AUC_{0-\infty}$/dose, $AUC_{0-\infty}$ per milligram administered, $t_{1/2}$, terminal half-life.
[a]Mean (%CV) for all except median (range) for T_{max}.
[b]Number of subjects in PK population.

the apical and basolateral sides (Table 5), the efflux ratios for naltrexone were <2, consistent with the lack of involvement of a transporter.[36] Permeability values were similar when the concentration of naltrexone was increased 50-fold to 500 μM. When the pH of the apical side was decreased to pH 5.5 to simulate the gradient between the nasal cavity and blood, the permeability of naltrexone in the apical-to-basolateral direction decreased, whereas it increased in the efflux direction to 12.1-18.2. The addition of 0.25% DDM reversed these changes, so that the P_{app} values were similar to those when the pH on both sides was 7.4, and the efflux ratio reduced to 1.56 (Table 5).

TEER is used as an in vitro measure to assess the "tightness" of cell junctions.[32] DDM reduced TEER from 135 to 58.7 $\Omega \times cm^2$ when both sides of the membrane were held at pH 7.4 and from 111 to 60.7 $\Omega \times cm^2$ when the apical and basolateral sides were maintained at pH 5.5 and 7.4, respectively. The permeability of ^{3}H-mannitol, used as a measure of the integrity of the cell junctions, ranged between 0.5 and 1.4×10^{-6} cm/s in the A-to-B and B-to-A directions for both pH conditions; ^{14}C-caffeine, which crosses the membrane passively, had a permeability value of 31-34×10^{-6} cm/s in both directions.

Discussion

Based on the rapid increase in overdose deaths linked to synthetic opioids, the development of more potent, longer-acting opioid antagonists is one facet of an overall strategy to address the opioid crisis.[10] We hypothesized that naltrexone could be a suitable candidate for development as a nasal rescue medication based on these criteria. Thus, the affinity of naltrexone is ~5-fold higher than naloxone at the μ-opioid receptors,[28,29] and the reported $t_{1/2}$ of orally administered naltrexone (4 hours)[26] is longer than the range of values (1.3-2.4 hours) reported for intranasal naloxone.[30] Although not previously approved for treating suspected or confirmed opioid overdose, its selectivity for opioid receptors coupled with the safety and tolerability profile of oral naltrexone[26] substantially derisks the safety of this molecule for single use in an overdose.

Rapid onset of action is also a cardinal feature of an effective opioid overdose reversal agent. In this study, we examined the effects of DDM, a member of a class of alkylsaccharide transmucosal absorption enhancers (reviewed in reference 32) on the pharmacokinetic properties of naltrexone. Alkylsaccharides such as

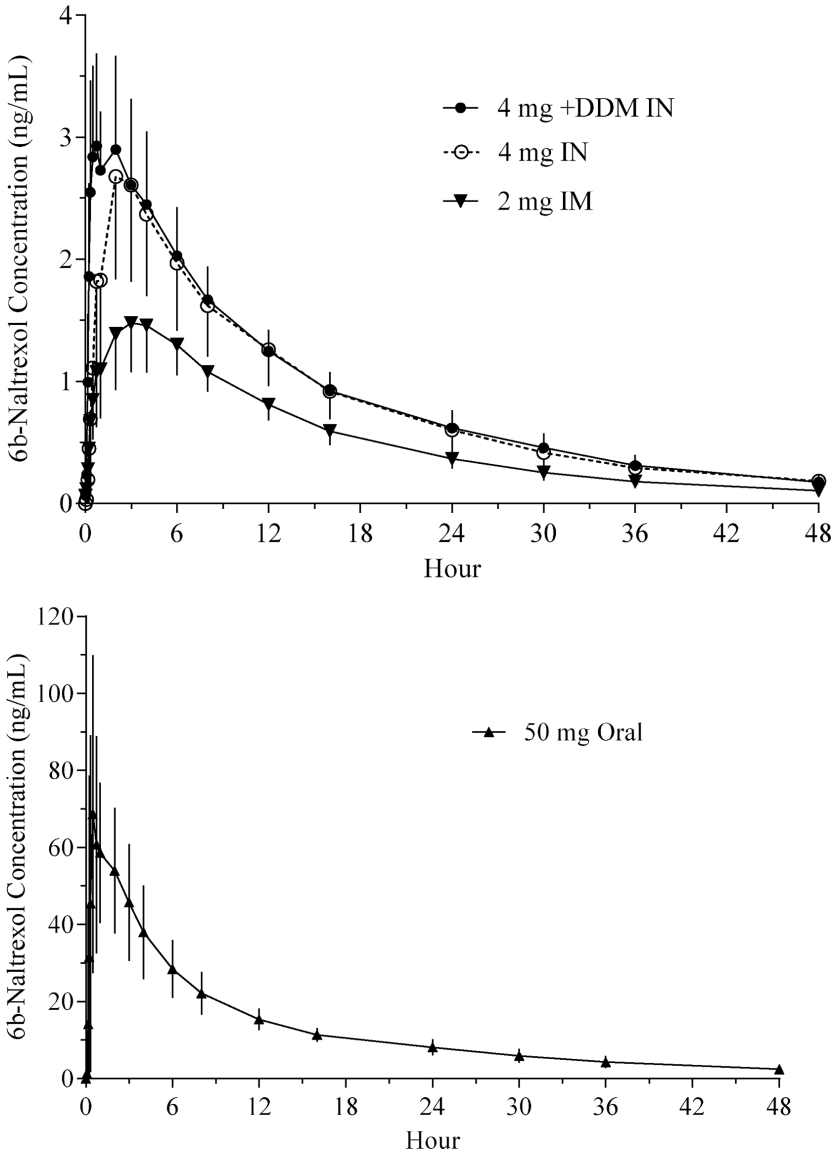

Figure 2. Plasma concentrations (SD) of 6β-naltrexol following intranasal, intramuscular, and oral administration of naltrexone HCl. Top: Subjects received 4 mg naltrexone intranasally with (filled circles) and without (open circles) 0.25% (w/v) DDM and 2 mg by intramuscular injection (inverted triangles). Bottom: Subjects received 50 mg naltrexone orally. IN, intranasal; IM, intramuscular; PO, oral. Values represent mean $\pm$ SD.

DDM have been demonstrated to enhance the nasal absorption of peptides and proteins (eg, calcitonin, insulin) and, more recently, low-molecular-weight compounds such as sumatriptan[32] and diazepam.[37] In the present study, the addition of DDM (0.25%) increased the C_{max} of intranasal naltrexone by approximately 3-fold (Table 2), accelerated T_{max} from 0.5 to 0.17 hours and increased its bioavailability from 48.2% to 75.7% relative to an intramuscular injection (Table 3). The concentration of DDM employed in this study was selected based on a concentration range (0.063%-0.5%) reported to enhance the effects of peptides and proteins,[32] and although its effects on the pharmacokinetic profile of naltrexone were dramatic, this concentration of DDM may not be optimum.

It has been hypothesized that alkylsaccharides such as DDM alter the pharmacokinetic properties of molecules by transiently opening tight junctions between cells in the nasal epithelium.[32] TEER in cell culture has been used as a measure of the integrity of tight junctions across cells and to examine the effects of putative absorption enhancers.[32] In DMCKII cells, 0.25% DDM reduced TEER by 56.5% when the pH was maintained at 7.4 on both the apical and basolateral surfaces and 45.4% when the apical side pH was reduced to 5.5, more closely resembling the environment of the nasal cavity.[35] These findings are consistent with observations using both a different cell type and assay conditions[32] that 0.1%-0.2% DDM results in large decreases in TEER.

Table 5. Bidirectional Permeability of Naltrexone Across MDCKII Cells

Concentrations	P_{app} ($\times 10^{-6}$ cm/s) A to B	P_{app} ($\times 10^{-6}$ cm/s) B to A	Efflux Ratio
Apical and basolateral pH = 7.4			
10 μM Naltrexone	24.3 (29.9)	36.1 (6.9)	1.49
500 μM Naltrexone	36.2 (8.3)	39.1 (2.8)	1.08
500 μM Naltrexone + 0.25% DDM	51.2 (27.0)	49.8 (7.2)	0.97
Apical pH = 5.5/basolateral pH = 7.4			
10 μM Naltrexone	5.13 (1.6)	62.3 (6.1)	12.1
500 μM Naltrexone	7.48 (7.0)	136 (2.9)	18.2
500 μM Naltrexone + 0.25% DDM	43.1 (2.09)	67.1 (6.3)	1.56

P_{app}, apparent permeability; efflux ratio, P_{app} of basolateral to apical direction divided by P_{app} of apical to basolateral direction.
P_{app} values are mean (%CV), n = 3.
^{3}H-Mannitol P_{app} = 0.5 to 0.7 $\times$ 10^{-6} cm/s in the 2 directions when pH is 7.4 on both sides and 1.4 to 1.0 $\times$ 10^{-6} cm/s when pH values are 5.5 and 7.4; ^{14}C-caffeine P_{app} = 31-34 $\times$ 10^{-6} cm/s in both directions at pH 7.4/7.4 and pH 5.5/7.4.

In vitro studies using DMCKII cells suggest that naltrexone was not actively transported across the apical or basolateral surfaces when the pH was maintained at 7.4 in both compartments (Table 5). However, when the pH on the apical side of the membrane was lowered to 5.5, the efflux ratio of naltrexone (both 10 and 500 μM) was markedly increased. This phenomenon could reflect a "trapping" of the ionized form of naltrexone, a weak base, in the acidic milieu of the apical compartment. This trapping phenomenon has been previously described with other lipophilic drugs in an acidic environment.[38] It is hypothesized that the ability of DDM to normalize the efflux ratio across this pH gradient could reflect increased permeability of the ionized form of naltrexone because of the transient reduction in the integrity of tight junctions produced by this alkylsaccharide in cell culture.[32] This hypothesis is consistent with the effect of DDM to markedly increase intranasal absorption of naltrexone (Figure 1).

DDM imparts several properties to intranasal naltrexone consistent with a highly effective overdose reversal agent including rapid delivery (T_{max}, 0.17 hours) of high plasma concentrations (C_{max}, 14.2 ng/mL). By comparison, the C_{max} and T_{max} values of a 4-mg naloxone nasal spray (currently the highest approved intranasal dose) are 4.83 ng/mL and 0.5 hours, respectively.[8] Because both naltrexone and naloxone are competitive μ-opioid receptor antagonists, it is hypothesized that the rapid delivery of higher plasma concentrations of a higher-affinity agent would be more effective at reversing an opioid overdose than the current standard of care. Nonetheless, the plasma half-life of intranasal naltrexone (2.2 hours) is essentially identical to naloxone (2.08 hours),[30] which could be viewed as a barrier to further development as a rescue agent, particularly in view of the long half-lives of synthetic opioids (eg, fentanyl, >7 hours; sufentanil, >6 hours; carfentanil, 5.7 hours),[12–15,23] now responsible for the majority of opioid overdose deaths.[4] Imaging studies using [^{11}C]carfentanil have demonstrated that oral naltrexone (50 mg) results in brain μ-opioid receptor occupancy that is significantly longer (eg, ~60% occupancy 23-33 hours postdosing)[39] than would be predicted from its plasma half-life.[39,40] By contrast, imaging studies in normal volunteers have demonstrated that occupation of μ-opioid receptors following either intravenous[41] or intranasal[42] naloxone ($t_{1/2}$, ~2 hours) mirrors its plasma half-life. Absent a clear stoichiometric relationship between occupancy of μ-opioid receptors and plasma concentrations of either naltrexone or its principal metabolite, 6β-naltrexol,[39] additional studies will be required to determine how the shorter plasma half-life of naltrexone observed after intranasal administration affects μ-opioid receptor occupancy in the central nervous system, which is a critical factor contributing to the duration of action of a rescue agent.[16,42] Rabiner et al[39] have hypothesized that the metabolite 6β-naltrexol, a long residence time of both naltrexone and 6β-naltrexol on μ-opioid receptors, and the trapping of these compounds in brain parenchyma could contribute to the anomalously long occupancy of μ-opioid receptors following oral naltrexone. Despite a high affinity at μ-opioid receptors[39] and long plasma half-life following intranasal administration (Table 4), it is unlikely that 6β-naltrexol significantly contributes to the pharmacological effects of naltrexone in the central nervous system. Thus, multiple reports,[43–45] including a clinical study employing an intravenous infusion of up to 20 mg of 6β-naltrexol,[43] concluded that this metabolite is peripherally restricted.

There are also other potential therapeutic uses for an intranasal opioid antagonist with this pharmacokinetic profile. Thus, the rapid onset and short half-life plasma of intranasal naltrexone appears be well suited for use in a "targeted dosing" strategy to treat alcohol use disorder. Operationally, intranasal naltrexone would be administered in response to high-risk situations, such as in the presence of alcohol-related cues or in anticipation of drinking. Although this technique is not widely employed, multiple studies have demonstrated targeted dosing of oral naltrexone reduces drinking behaviors.[46–48] Brain-imaging studies[39,40,49] have demonstrated that a standard 50-mg dose of oral naltrexone will produce a sustained high occupancy of μ-opioid receptors. However, consistent with a >10-fold lower affinity of naltrexone,[39] δ-opioid receptor occupancy is low (<25%) and highly variable among subjects at doses of naltrexone that result in a >90% occupancy of μ-opioid receptors.[49] Both preclinical and clinical studies have demonstrated that alcohol

releases endogenous opioids[50,51] that bind to δ-opioid receptors with high affinities.[49,52] Converging lines of evidence indicate that activation of δ-opioid receptors contributes to the reinforcing properties of alcohol.[49,53] Thus, the ability to rapidly achieve high plasma naltrexone concentrations via intranasal dosing while craving/anticipating alcohol may, through high occupancy of δ-receptors, increase its efficacy compared with once-daily dosing. This hypothesis merits clinical investigation.

Conclusions

In the presence of dodecyl maltoside, intranasal administration of naltrexone results in rapid and robust increases in plasma concentrations. However, its relatively short plasma half-life (~2.2 hours) could limit its usefulness as a rescue medication, particularly against longer-lived synthetic opioids such as fentanyl. Nonetheless, the ability to rapidly attain high plasma concentrations of this opioid antagonist may be useful for other indications, including an as-needed dosing strategy to treat alcohol use disorder.

Declaration of Conflicting Interests

P.S. and R.C. are employees of Opiant Pharmaceuticals, Inc. P.K., N.C., and S.G. are employees of the National Institutes of Health. P.S. was employed by the National Institutes of Health when the study was designed and executed.

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Data Accessibility

Investigators interested in a more detailed review of the data should contact Dr. Krieter at philip.krieter@nih.gov.

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