Inspire or expire: a matter of life or death

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CHAPTER 5

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A comparison of intramuscular (Zimhi) and intranasal naloxone (Narcan) in reversal of fentanyl-induced apnea: a randomized, crossover, open-label trial

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Abstract

Severe opioid-induced respiratory depression (OIRD) can be treated with intranasal (IN) or intramuscular (IM) naloxone. It is relevant to compare their efficacy and determine the optimal strategy to restore breathing activity following OIRD. Here we compare the required number of IM (5 mg/0.5 mL) versus IN (4 mg/0. 1mL) naloxone doses following 10 µg/kg intravenous fentanyl-induced apnea in opioid-naïve participants and chronic opioid users. After 2-min of apnea, IM or IN naloxone was given at 2-min intervals until return of adequate ventilation. If necessary, rescue intravenous naloxone was administered. In sixteen opioid-naïve participants, the required median IM naloxone doses was 1.5 (IQR 1-2) versus 2 (1-3) for IN naloxone (p=0.0002); one subject required rescue naloxone. Similarly, in six opioid users, IM was more effective than IN naloxone. Here we show the superiority of IM naloxone over IN naloxone in the number of doses required for full reversal of breathing following opioid-induced apnea.

Introduction

Opioid-induced respiratory depression (OIRD) followed by cardiac arrest, is the primary cause of death in opioid overdose cases, particularly in the US and Canada.[1-3] Potent opioids remain less of a problem in most European countries with some exceptions, e.g. the United Kingdom.[3] Currently, most opioid-related fatalities in community settings may be attributed to potent opioids, particularly fentanyl or its analogs, such as carfentanil.[1, 2, 4] The most effective treatment of OIRD is the administration of the opioid receptor antagonist naloxone.[2] Several naloxone formulations have been approved for use in both hospital and prehospital settings. In hospital, intravenous naloxone is typically administered to patients with an intravenous (IV) access line, while intranasal (IN) and intramuscular (IM) formulations are designed for community use during opioid overdose emergencies. These latter formulations can be administered by first responders, including bystanders.[2, 5]

As recently highlighted by the US Food and Drug Administration, there is an urgent need for experimental studies to determine the optimal naloxone dose for the rescue of OIRD in the community setting.[4] This is relevant for both IN and IM naloxone formulations. Several studies examined the use of IM and IN naloxone for managing opioid overdoses.[6-10] Most of these studies were conducted several years ago when heroin was the predominant cause of an opioid overdose, and studied relatively low doses of IM and IN naloxone. These trials were performed in real-world settings, often in supervised drug injection centers or in ambulances, and remain highly relevant. Generally, the studies concluded that while IN naloxone is effective, it has a slower onset of action compared to IM naloxone and frequently requires additional rescue naloxone doses. In the current opioid crisis, illicit fentanyl use has replaced heroin as the primary opioid of abuse,[3] which causes a more rapid and profound respiratory depression that may result in cardiac arrest secondary to hypoxia. As a result, it appears that higher and more concentrated doses of naloxone are required for the rapid and effective reversal of opioid respiratory effects.[4]

In this experimental study, we compared two approved relatively high-dose and high-concentration naloxone hydrochloride formulations: the 5 mg/0.5 mL intramuscular naloxone injector (ZIMHI) and 4 mg/0.1 mL naloxone nasal spray (Narcan).[11, 12] The study was conducted in healthy volunteers and self-reported chronic, daily opioid users, following the induction of apnea lasting at least 2-min using a fixed intravenous bolus fentanyl dose of 10 µg/kg. This experimental study performed in a highly controlled and monitored setting is an attempt to mimic a real-world community fentanyl overdose scenario and allows for the comparison of naloxone treatments following a fixed high dose of fentanyl. The primary endpoint of the

study was the number of IM and IN doses that were required to reverse OIRD to restoration of adequate breathing. We hypothesized that the IM formulation would require fewer doses than the nasal IN spray for reversal of OIRD.

Results

Opioid-naïve healthy participants

Twenty-nine healthy white individuals were assessed for eligibility. Eleven were excluded because of logistic reasons or exclusion criteria (see CONSORT flow diagram, Fig. 1). Eighteen healthy volunteers were randomized. One subject did not return for a second visit due to adverse effects experienced during the first session (persistent nausea and tiredness). The replacement of this subject did not develop apnea after fentanyl and the intervention (intranasal naloxone) was therefore not administered. Data from these two subjects were discarded and they were replaced by another subject. The demographics and study results of the 16 healthy volunteers who completed the study are given in Table 1. All volunteers completed the study without serious adverse events. Examples of the IM and IN reversal responses for a single subject and average reversal data are given in Figure 2. It shows the rapid induction of apnea following fentanyl administration and the differences in response following IM versus IN naloxone. In this particular subject one IM dose was sufficient to fully reverse respiratory depression within 2 min, whereas three IN doses were needed before adequate breathing was observed, with consequently a much slower return towards adequate baseline ventilation.

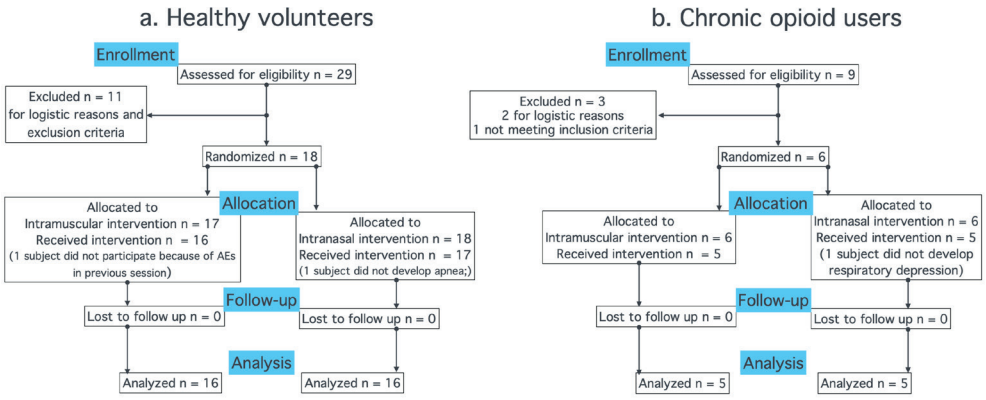

Figure 1. Consort flow diagrams.

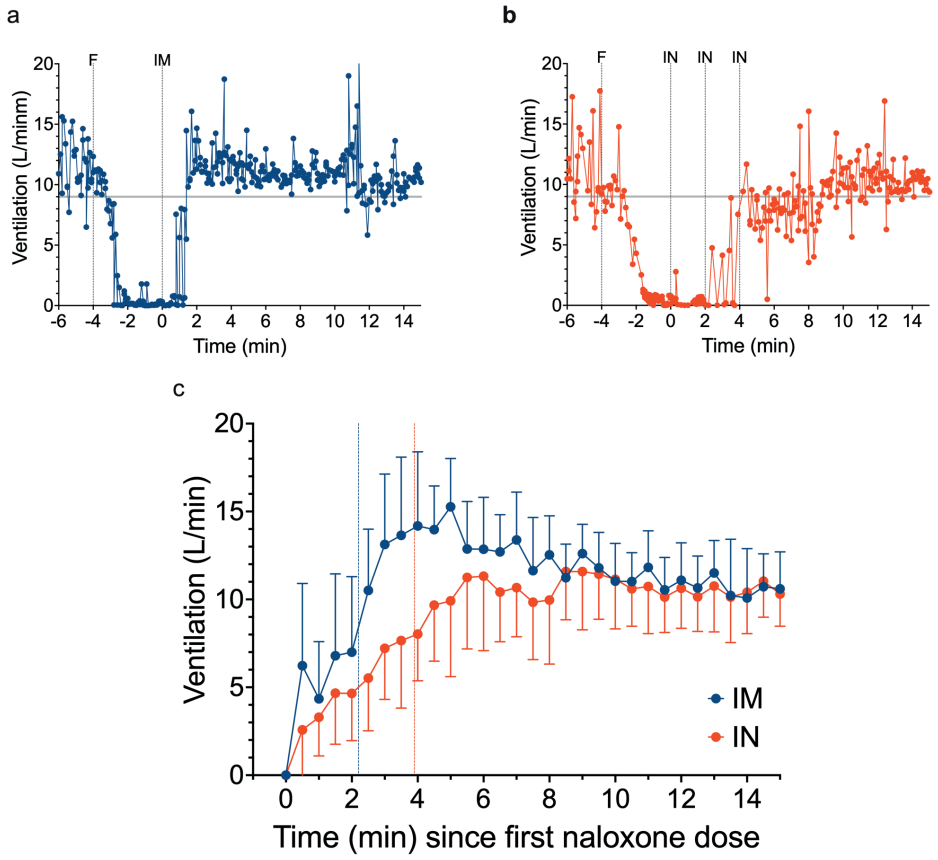

Figure 2. Examples of IM and IN naloxone-reversal of fentanyl-induced apnea.
a. Effect of a single dose of intramuscular naloxone (IM, 5 mg/0.5 mL) given at t = 0 min, following 2 min of apnea after an intravenous bolus dose of 10 µg/kg fentanyl (F) given at t = –4 min. b. Effect of three doses intranasal naloxone (IN, 4 mg/1 mL) following 2 min of apnea after an intravenous bolus dose of 10 µg/kg fentanyl (F = onset of the 90 s fentanyl administration). IN naloxone was given at t = 0, 2 and 4 min. The grey horizontal bar depicts 80% of adequate baseline ventilation. Each dot is one breath. Data are from a single healthy volunteer. c. Average ventilation data ± SD of the opioid-naïve individuals following apnea and reversal with intramuscular (blue) or intranasal (orange) naloxone. The horizontal lines depict the mean reversal times for the intramuscular dosing (blue) and intranasal dosing (orange). Source data are provided as a Source Data file.

Subject	Age (years)	Sex*	Weight (kg)	MME (mg)	IM doses	IN doses	IV rescue after IN (Yes/No)	Time to 80-100% reversal (min) IM	Time to 80-100% reversal (min) IN
A	25 – 29	M			1	2	No	1	2.2
B	20 – 24	F			2	3	No	1.8	3.1
C	20 – 24	F			2	2	No	2.4	2.8
D	25 – 29	M			2	4	No	3.1	3.7
E	25 – 29	M			2	3	No	3.1	4.5
F	20 – 24	M			1	3	No	1.7	4.3
G	20 – 24	M			2	2	Yes	2.3	4.1
H	20 – 24	M			1	2	No	1.7	1.7
I	20 – 24	F			1	2	No	2.2	3.8
J	20 – 24	F			1	3	No	2.5	9.2
K	20 – 24	F			1	3	No	1.6	4.4
L	20 – 24	M			2	2	No	2.5	2.9
M	20 – 24	M			2	3	No	3.9	4.6
N	25 – 29	F			2	2	No	1.4	2.8
O	20 – 24	M			1	2	No	2.7	2.3
P	20 – 24	F			1	2	No	1.1	2.9
Overall	23 (20–26)		72 ± 12	0				2.2 ± 0.8	4.0 ± 2.0
Median					1.5 (1)	2 (1)		2.3 (0.9)	3.4 (1.6)
Mode					1 50%)	2 (56%)			

Table 1. Participant characteristics and individual results of intramuscular (IM) and intranasal (IN) reversal of fentanyl-induced apnea in opioid-naïve healthy volunteers
F female; M male; MME morphine milligram equivalents; BMI body mass index; IV intravenous; Overall data are mean (range) or mean ± SD; Median data are with (interquartile range); Modes are given with (percentage) of the most frequent dose given. * self-reported.

Subject	Age (years)	Sex	Weight (kg)	MME (mg)	Opioids	IM doses	IN doses	IV rescue after IN (Yes/No)	Time to 80-100% reversal (min) IM	Time to 80-100% reversal (min) IN
Q	60 – 65	F	75 – 79	90	oxycodone	2	3	Yes	2.6	10.8
R	50 – 54	F	70 – 74	180	fentanyl	1	4	No	1.5	7.3
S	50 – 54	F	70 – 74	98	oxycodone	1	2	No	1.4	5.3
T	60 – 65	M	70 – 74	270	methadone	1	2	Yes	2.4	8.4
U	50 – 54	M	75 – 79	118	fentanyl	2	2	No	3.2	2.3
V	50 – 54	M	70 – 74	450	oxycodone	NA	NA	-	-	-
Overall	54 (52-63)		75 ± 2	201 ± 139					2.2 ± 0.8	6.8 ± 3.2
Median						1 (1)	2 (1)		2.4 (1.1)	7.3 (3.1)
Mode						1 (60%)	2 (60%)			

Table 2. Participant characteristics and individual results of intramuscular (IM) and intranasal (IN) reversal of fentanyl-induced apnea in chronic opioid users
F female: M male; MME morphine milligram equivalents; BMI body mass index; IV intravenous; NA not administered since no respiratory depression occurred after fentanyl; Overall data are mean (range) or mean ± SD; Median data are with (interquartile range); Modes are given with (percentage) of the most frequent dose given.

The mode (frequency) and median (interquartile range, IQR) number of IM naloxone doses to achieve return to baseline ventilation was 1 (50%), and 1.5 (IQR 1 to 2) versus 2 (56%) and 2 (IQR 1 to 3) for intranasal naloxone with a median difference of 1 (0.5 to 1.5, p = 0.0002). In one subject, reversal with 2 IN dose of naloxone was insufficient and 0.4 mg intravenous naloxone was administered due to a significant increased pCO_2 level in the absence of adequate breathing activity. In none of the subjects rescue IV naloxone was indicated following administration of IM naloxone. Arithmetically adding the IV dose as a regular IN dose resulted in change of the mode and median dose to 2 (50%) and 2.5 (IQR 1.5-3.5, IN vs IM: p = 0.0001).

Time to full reversal is presented in the Kaplan-Meier curve in Figure 3. It shows that 2 min after the first naloxone dose full reversal of OIRD was achieved in seven subjects (44%) after IM, but only in one subject (6%) following IN naloxone administration. After 4 min, full reversal was observed in 100% (n = 16) of IM and 63% (n = 10) of IN treated subjects. Complete reversal with IN naloxone was observed in all subjects after 9.2 min (n = 16), with significant differences between the two administration forms (IM: 3.9 min; log-rank test: p = 0.0002). On average the time to reversal was 2.2 ± 0.8 min (mean ± SD; n = 16) after IM naloxone and 4.0 ± 2.0 min after IN naloxone, with a mean difference of 2.0 min (95% CI 1.1 to 2.9 min, p = 0.002). Median reversal times were 2.3 (IQR 0.9) min after IM and 3.4 (1.6) min after IN naloxone. The percentage coefficient of variation of the time to reversal was smaller for IM than IN naloxone: 35% vs 52%, indicating more consistent reversal with IM naloxone. No renarcotization was observed after treatment with either IM or IN naloxone.

Following fentanyl, muscle rigidity was observed in one of the opioid-naïve volunteers following IN naloxone (supplemental document), and no withdrawal symptoms were observed following IM or IN naloxone administration.

Plasma concentrations of fentanyl and naloxone following IM and IN naloxone dosing are given in Figure 4A-E. For IM naloxone, the data of individuals that received 1 dose (n = 8) and those that received 2 doses (n = 8) are given in panels B and C, showing the marked increase (91%) in plasma concentration with the doubling of the IM dose. For IN naloxone, the data of individuals that received 2 doses (n = 9) and those that received 3 doses (n = 5) are given in panels D and E, showing a 25% increase in peak plasma concentration with the increasing dose (from 8 to 12 mg). Mean naloxone peak plasma concentrations were 37 ± 10 ng/mL at t = 6.5 min (1 IM dose; n = 8), 71 ± 20 ng/mL at t = 6.5 min (2 IM doses; n = 8), 20 ± 4 ng/mL at t = 14.5 min (2 IN doses; n = 9) and 25 ± 9 ng/mL at t = 16.5 min (3 IN doses; n = 6). The estimated peak fentanyl concentration was 38 ng/mL occurring at t = 90 s following the start of the fentanyl

administration (Fig. 4A).

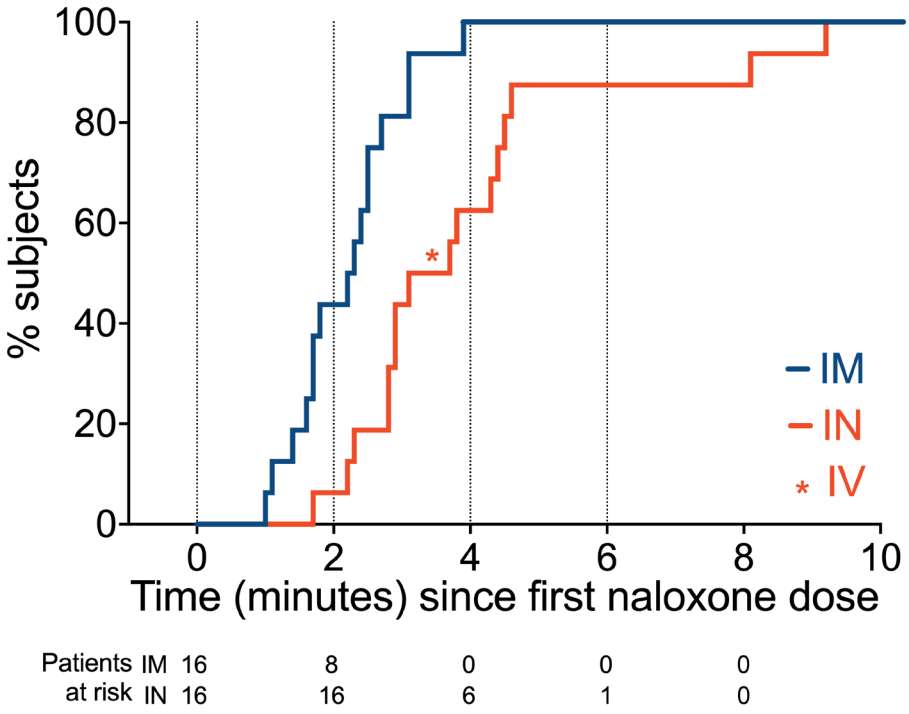

Figure 3. Kaplan-Meier survival analysis depicting the number of healthy volunteers (n = 16) that reached 80 to 100% of adequate ventilation versus time.
Below the graph, the number of subjects at risk at time points 0, 2, 4 or 6 min (vertical lines) is given. * administration of rescue intravenous naloxone (0.4 mg) in one subject; this subject received a 4 min penalty because of the need for intravenous (IV) rescue. Earlier reversal was observed following intramuscular (IM) naloxone compared to intranasal (IN) naloxone (log-rank test: p = 0.0002) at less dose requirements. Source data are provided as a Source Data file.

Chronic opioid users

We included a small sample of Caucasian chronic opioid users as an exploratory part of our study, to determine feasibility of administering high dose naloxone in this population, determine adverse effects and get an indication of the direction of naloxone effect in IN versus IM arms in this population. A total of nine individuals who used opioids on a daily basis were assessed for participation in the study. Three subjects were excluded (Fig. 1): one because of not meeting the inclusion criteria, two others for logistic reasons. One subject was randomized to the intervention but did not develop respiratory depression and therefore no naloxone was administered. Since this subject did not receive the intervention, his data were not included in the data analysis. Table 2 gives the demographics including opioid use of the

participants. Their mean daily morphine milligram equivalents (MME) ± SD were 201 ± 139 mg (range 90 to 450 mg). Excluding the subject who did not receive the intervention gives a daily MME of 151 ± 75 mg (range 90 – 270 mg).

The number of IM doses needed for return of adequate breathing to baseline levels was 1 in three subjects and 2 in two subjects; no rescue IV naloxone was needed after IM naloxone. For IN naloxone, 2 doses were given to two subjects, 3 to two subjects and 4 to one subject (Table 2); rescue IV naloxone was needed in 2 subjects. One subject received 0.4 mg intravenous naloxone after 3 IN doses due to a pCO_2 > 12 kPa (90 mmHg), the other subject after 2 IN doses because of desaturation below 70%.

The mode (frequency) and median (IQR) number of naloxone doses was 1 (60%) and 1 IM dose (IQR 1-2) versus 2 (60%) and 2 IN doses (IQR 2-3.5); median difference in dosing was 1 (IQR 1-1; IM vs IN). Time to return to baseline ventilation ranged from 1.4 to 3.2 min in the IM treated arm and 2.3 to 10.8 min in the IN treated arm. Median reversal times were 2.4 (IQR 1.1; n = 5) min for IM and 7.3 (3.1; n = 5) min for IN naloxone (Table 2).

Plasma naloxone concentrations after IM and IN doses are given in Figure 4, panels f and g. The naloxone concentrations following a single IM doses (n = 3) were similar to that observed in opioid-naïve individuals (compare panels b and f). The concentrations after 2 IN doses were in the same range as those observed in opioid-naïve individuals (compare panels d and g). We obtained pharmacokinetic data from a single participant after 4 IN doses (red line in panel g) that showed absence of dose effect. We relate this to saturation in nasal naloxone uptake.

Two subjects developed muscle rigidity upon administration of fentanyl, as objectified by the myotonometer (see the Supplemental Information File). Muscle rigidity developed slowly and became apparent after fentanyl and intranasal naloxone were administered. We did not observe muscle rigidity when fentanyl was followed by intramuscular naloxone.

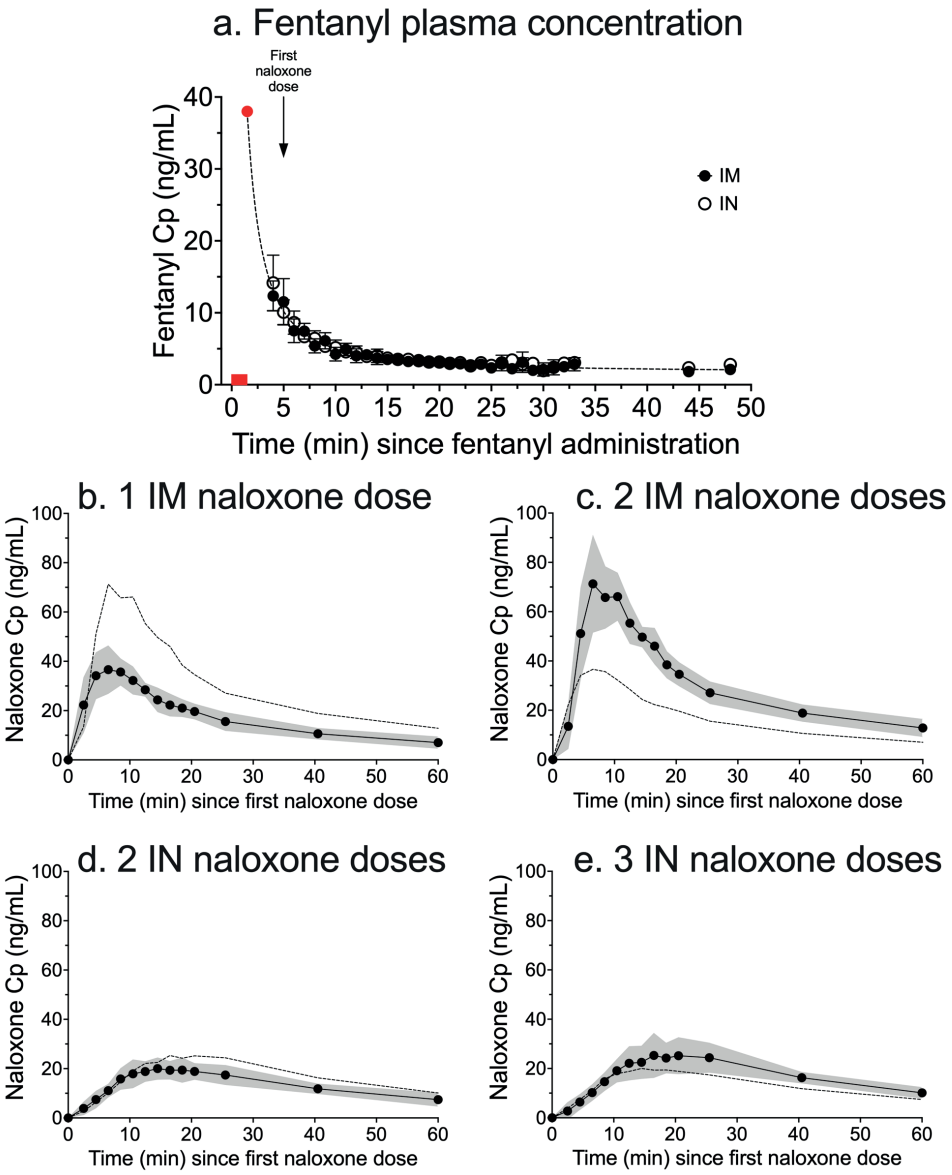

Figure 4. Plasma concentrations of fentanyl and naloxone following intramuscular (IM) and intranasal (IN) naloxone dosing.
a. Mean fentanyl concentrations over time. The red symbol is the estimated peak fentanyl concentration occurring at t = 90 s. The red bar depicts the 90 s 10 µg/kg fentanyl infusion. The dotted line through the data is the estimated exponential decay. b and c: Mean plasma naloxone concentrations observed in opioid-naïve subjects that received 1 dose (a; n = 8) and those that received 2 IM doses (c; n = 8). d and e. Mean plasma naloxone concentrations observed in opioid-naïve subjects that received 2 doses (d; n = 9) and those that received 3 IN doses (c; n = 6). f and g: Mean plasma naloxone concentrations observed in chronic opioid users that received 1 IM dose (f; n = 3) and those that received 2 and 4 IN doses (f; n = 2 received 2 doses, n = 1 received 4 doses (red line). In panels b, c, d and e, the dotted lines represent the plasma concentration of the alternate dosing group for either IM or IN dosing in opioid naïve participants. Data are mean ± SD (grey areas). ON are opioid-naïve participants; OU are chronic opioid users. Source data are provided as a Source Data file.

In four subjects, withdrawal symptoms occurred following treatment with naloxone. The severity of symptoms in two subjects was similar and considered mild by the investigators and participants and required no treatment (in 1 subject after IM naloxone, in the other after IV naloxone). In 2 other subjects (T and U, Table 2) symptoms were moderate to severe after both IM and IN naloxone (subject U), and IM naloxone and IV (after IN) naloxone (subject T).

These subjects were treated with intravenous clonidine (range 75 to 150 µg) after which they both received low-dose propofol for 1 h to sedate them during the withdrawal episode (after IN and IM naloxone treatment). Most prevalent symptoms included agitation, perspiration, nausea, shivering/shaking, hypertension, tachycardia and a burning sensation in the throat. All symptoms were absent upon discharge from the clinical research unit.

In the Supplemental Information File, the following secondary endpoints are presented: rigidity, pupillometry and end-tidal pCO_2 data.

Discussion

In this randomized, open-label trial, we compared the number of required IM versus IN naloxone doses in their ability to effectively reverse fentanyl-induced apnea. We studied healthy individuals and a small exploratory sample of individuals that use a daily average of 200 MMEs. In both populations, IM naloxone was more effective in reversing fentanyl-induced apnea compared to IN naloxone, without a need for intravenous rescue naloxone. The median times to restoration of ventilation to baseline levels was similar (2.3 min in opioid naïve individuals and 2.4 min in chronic opioid users) following IM naloxone. In contrast, IN naloxone administration was not only less effective (i.e., 1 extra dose was needed) but IV rescue naloxone was needed to restore adequate breathing activity in 1 healthy participant (6.3%) and 2 opioid users (40%) with median recovery times 3.4 min (healthy volunteers) and 7.3 min (chronic opioid users).

Opioid-induced apnea followed by cardiac arrest results from hypoxia and possibly direct opioid effects on the heart through an opioid effect on potassium channels.[1, 2, 13] This leads to significant mortality and morbidity given the large number of individuals that abuse potent opioids, particularly in the US and Canada, but certainly also in some other countries.[3] In the US alone, 80,000 people died from an opioid overdose in 2023.[14] Dealing with potent opioid-induced respiratory depression, apnea and cardiac arrest poses a significant challenge due to the recent substantial increase in the strength of illegally consumed opioids, especially of the potent opioid fentanyl (100 times more potent than morphine) or its congener

carfentanil (100 times more potent than fentanyl).[4] The increase in high affinity and potent opioids requires higher and more concentrated naloxone doses to counteract the opioid effect in the brainstem,[4] the site where opioids cause the loss of adequate breathing activity and apnea.[15]

The opioid receptor antagonist naloxone remains the primary treatment of an opioid overdose. While originally available as an injectable for IV or IM use in the clinical setting, new formulations were recently approved for use outside the hospital.[2, 4] IN and IM naloxone are easily administered by first responders and bystanders.[5] However, the efficacy and optimal dosing regimen has not been investigated in a controlled setting, particularly not in individuals that are apneic following the rapid IV administration of high-dose fentanyl. To the best of our knowledge, this is the first respiratory study that quantifies the effect of IM and IN naloxone following a 2-min period of fentanyl-induced apnea. This experimental study was designed to as best as possible reproduce an opioid overdose in the community setting, albeit in a safe and monitored setting. During the study, personnel with airway management and resuscitation skills were continuously present. The use of supplemental oxygen prevented serious hypoxic adverse events. This is certainly different from the real-world setting where overdose victims breathe air with 21% oxygen; this should be considered when extrapolating our findings to community overdose settings. In real-world settings, oxygen desaturations will occur rapidly and negatively impact cardiac output complicating effective treatment with any naloxone formulation due to suboptimal drug distribution (see also below). As discussed before,[2, 16] rescue of opioid overdose victims should include naloxone administration combined with resuscitation efforts, i.e. ventilatory support ensuring rapid uptake of oxygen and chest compression ensuring restoration or maintenance of circulation.

Our results align with previous findings. Yousefifard et al.[9] conducted a meta-analysis of studies on prehospital overdose rescue with naloxone. They concluded that the onset of action of IN naloxone was slower compared to IM naloxone, with a 2.7 times greater need for naloxone IV rescue medication following IN than IM dosing. In patients managed by ambulance personnel for an opioid overdose, Skulberg et al.[10] found that 80% of individuals returned to adequate ventilation after 1.4 mg (in 0.1 mL) IN naloxone versus 97% after 0.8 mg (in 2 mL) IM naloxone. Finally, Dietze et al.[8] conducted a double-blind, randomized trial in a medically supervised injection facility. They found that 0.8 mg IM naloxone (in 1 mL) was more efficient and required less rescue doses compared to 0.8 mg IN naloxone (in 1 mL). All of these studies performed in actual overdose victims are difficult to compare to the findings from our study. Apart from the different setting, heroin was suspected to be the cause in the majority

of opioid overdoses in these earlier studies. Considering respiratory depression, heroin is an opioid that is considerably less potent than fentanyl and about equipotent compared to morphine.[17] Moreover, we remain uninformed on the cardio-respiratory state of these overdose victims at the time of reversal. We argue that the use of heroin and lower equivalent fentanyl doses may have resulted in the high success rescue rates and do not necessarily translate to the current situation in the United States and Canada. We studied a fixed high-dose of fentanyl with ensuing apnea in almost all subjects. Given the delay in reversal that we have observed, particularly with IN naloxone, we argue that lower naloxone doses would have failed to restore adequate respiratory activity within acceptable safety limits in our current study, i.e. within acceptable time frames (< 10 min) and without the occurrence of hypoxia.

We attribute the difference in the number of IM and IN administrations and rescue times to the differences in naloxone plasma concentrations after IM and IN naloxone (Fig. 4), which we relate to differences in dose, administration route, absorption rate, bioavailability and concentration. The package inserts of the IM and IN formulations report that peak naloxone concentrations are higher after 5 mg/0.5 mL IM naloxone than after 4 mg/0.1 mL IN naloxone: 17.2 versus 4.8 ng/mL, occurring 15 and 30 min after administration, respectively.[11, 12] Note that these data were obtained from healthy individuals who were not exposed to opioids. We observed higher naloxone plasma concentrations (Fig. 4) than expected from the IM and IN naloxone package inserts. We relate this to the fact that naloxone was administered at a fixed dose regardless of the participants' weight (our subjects had a mean body weight of 72 kg), possible differences in sample scheme (we applied frequent sampling), arterial rather than venous blood sampling in our study (due to local metabolism and tissue uptake naloxone concentrations derived from arterial samples will be higher than from venous samples), high pCO_2 due to 2 to 6 min of apnea (this results in an increase in cardiac output and high tissue perfusion, particularly of the muscles, with more rapid uptake and enhanced distribution of naloxone), and opioid-naloxone pharmacokinetic interactions. The later was previously observed for the interaction between remifentanil and intranasal naloxone.[18] Irrespective, we conclude that compared to intranasal naloxone, the amount of naloxone that reaches the brain after IM naloxone will be higher and will reach the brain faster due to the higher and more concentrated dose and greater bioavailability.[19] This indicates that our results are attributable to difference in IM versus IN naloxone dose and bioavailability. Our data further suggest that earlier published venous naloxone pharmacokinetic data from individuals not exposed to high-dose opioid-induced prolonged periods of apnea might underestimate the arterial naloxone concentrations. This deserves further study, particularly of the possible pharmacokinetic interaction between fentanyl and naloxone. Finally, the absence of dose-dependency

for nasal naloxone is concerning and suggests that multiple doses in the same nostril in short periods of time have little effect in rapidly increasing the naloxone plasma concentration.

We observed muscle rigidity in just a few subjects. Since rigidity may be related to the dose and speed of administration, the lack of rigidity in the majority of participants may be related to the relative slow infusion rate (fentanyl was infused over 90 s). Given the small sample size, additional studies are required to examine the occurrence, implications and treatment of rigidity.

Our study has some limitations. Unlike real-world conditions, subjects were not subjected to physical or verbal stimulation (as recommended in the guidelines of the American Heart Association) before, during, or after naloxone administration.[20] Such stimulation could potentially increase breathing and lower the required naloxone dose. We opted to compare the pharmacodynamic effect of the two naloxone formulations on chemical control of breathing rather than behavioral control that occurs when external stimuli are applied.[21] Additionally, such external stimuli are difficult to standardize across study subjects and hence may have unpredictable effects on breathing that may have impacted the two arms of the study differently.

We used a small sample of individuals that use opioids on a daily basis. An important reason for this was to determine feasibility and assess whether the induced withdrawal symptoms would be manageable and treatable. We conclude that this indeed is possible with clonidine and sedation with propofol. Opioid users were allowed to use their habitual opioids on the morning of the visit to the laboratory. Whether this affected the study outcome remains unknown, but we argue that such conditions were similarly present on randomized IM and IN study days. Still, it may be the cause for the lesser efficacy of naloxone in this population compared to the healthy individuals, particularly after IN naloxone.

As stated previously,[22] in the real word, many overdose victims are found hours after the overdose occurred in a state of severe hypoventilation, hypoxia, hypercapnia and/or hypothermia, with low cardiac output. Particularly, the lack of brain hypoxia and low cardiac output in our current study may have affected outcome. For example, brain hypoxia diminishes the efficacy of naloxone.[23] Moreover, in case of a low cardiac output the distribution of naloxone may be altered. Whether this is more severe for IM naloxone, that is dependent on muscle perfusion, seems plausible, but requires more study.

We studied fentanyl as the opioid of choice. In the abuse setting fentanyl is often used together with other illicit substances, such as tranquilizers, that negatively affect respiration. This will likely decrease naloxone efficacy, although it remains unknown to what extent. Future studies should address this issue.

Finally, the high doses of naloxone in both formulations may trigger acute precipitated withdrawal in individuals with an opioid use disorder. While this could lead to reluctance in administering an opioid antagonist, we believe that saving a life outweighs the concern of inducing withdrawal. Nevertheless, the high-dose naloxone formulation remains a valuable medical countermeasure in public health emergencies, such as large-scale deployment of weaponized synthetic opioids.[24, 25]

In conclusion, in this small and descriptive study, we compared the efficacy of IM versus IN naloxone following high-dose fentanyl-induced apnea. The study was performed in 16 healthy volunteers. Additionally, we conducted an exploratory adjunct study in 6 daily opioid users. In healthy volunteers, we observed greater efficacy in reversal of apnea after IM compared to IN naloxone with the need for lesser IM doses and no need for rescue naloxone. The decreased IN naloxone efficacy was replicated in the small set of chronic opioid users. While our results are relevant, further larger studies are required, particularly in chronic daily opioid users, focusing not only on respiration but also on muscle rigidity and withdrawal symptoms.

Methods

Ethics and Subjects

This single center, randomized, crossover, open label study on the comparison of the number of doses intramuscular (IM) and intranasal (IN) naloxone required to reverse fentanyl-induced apnea was approved under the European Clinical Trial Regulation (ECTR) by the local Medical Review Ethics Committee (METC LDD) on August 25, 2023, after submission of the protocol at the EU Clinical Trials Information System (CTIS, identifier 2023-505338-93-00). The protocol was registered and is available at the ISRCTN registry under identifier 21068708 (https://doi.org/10.1186/ISRCTN21068708) on September 6, 2023. All subjects were studied on two separate occasions, once receiving IM naloxone, and once receiving IN naloxone; the sequence of administration was random. Before enrollment, all participants gave written informed consent. Study procedures were performed according to good clinical practice guidelines and the Declaration of Helsinki. The study was conducted from September 21, 2023 (study initiation with start recruitment) with first subject in on November 2, 2023, to September 6, 2024 (last subject out) at the Anesthesia & Pain Research Unit of Leiden University. All subjects

were recruited via folders displayed on the University campus and through web-based advertisements of our study. The subjects received a financial compensation for their participation.

The study was conducted in healthy volunteers and in self-reported chronic daily opioid users. Eligibility criteria were age 18 to 65 years (inclusive) and body mass index 19 to 40 kg/m^2 (inclusive). Chronic opioid users were required to use at least 60 mg morphine equivalents per day. All participants were required to be in a good health condition based on a medical evaluation conducted by an independent physician, based on the subject's medical and surgical history, physical examination and vital signs. For opioid users, a 12-leads electrocardiogram, and hematology and blood chemistry safety checks were performed. Exclusion criteria included presence or history of relevant medical or psychiatric diseases, pregnancy or lactation, a history of allergic response to study medication. Additionally, for healthy volunteers, a positive breath alcohol test or a positive drug urine dipstick on screening and study days were exclusion criteria. Chronic opioid users that met the criteria for diagnosis of a substance use disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 other than opioids, caffeine or nicotine, were excluded, as well as those individuals that received medication-assisted treatment for their opioid use, including treatment with mixed agonists-antagonists or benzodiazepines.

Study design and apparatus

The study was performed in our clinical research unit, an area with similar monitoring and access to airway management equipment that is available in an operating room. Upon arrival at the unit, all subjects received two intravenous access lines, one for fentanyl and one for possible intravenous naloxone administration. The intravenous naloxone served as escape medication in case the study drug intervention was unsuccessful (i.e. absence of adequate breathing within 8 min after the first naloxone dose) or in case safety rules were met (see below). An arterial line was inserted in the radial artery of the non-dominant hand for continuous monitoring of hemodynamic parameters and blood sampling. Additionally, arterial oxygen saturation via a finger clip and the electrocardiogram were monitored continuously throughout the study day.

Prior to any drug administration, breath-to-breath minute ventilation was measured using a mask fitted over nose and face, that was connected to a pneumotachograph and pressure transducer system (Hans Rudolph Inc., USA). Inspired and expired gas concentrations were measured at the mouth using the Masimo Root ISA OR plus capnograph (Masimo, USA). Subjects inhaled supplemental oxygen (flow set at 4-6 L/min) to prevent desaturation during

episodes of apnea and respiratory depression. Data were presented onscreen allowing real-time assessment of the hemodynamic and ventilatory state of the subject. All respiratory parameters were collected breath-to-breath for analysis.

Additional measurements muscle rigidity that was measured at the brachial muscle of the dominant upper arm using a myotonometer (Myoton, Tallin, Estonia), and the pupil size that was measured with the PLR-3000 pupilometer (Neuroptics, USA).

Measurements, safety rules and drug administration

Ventilation

After a 10-15 min period of steady-state oxygen breathing through a facemask, a 10 μg/kg intravenous fentanyl dose was administered over 90 s. The dose was based on an earlier study and aimed at producing an episode of apnea within 2-4 min.[26] This dose is within the range of fentanyl doses consumed in the community setting by individuals that use fentanyl in a range of administration forms (including oral, nasal or intravenous use and smoking; dose range reported 0.25 to 1.0 g).[27] The relatively slow infusion rate will diminish the likelihood of muscle rigidity. When apnea occurred, defined as inadequate ventilation below 2 L/min, a timer was set and one of either naloxone formulations was administered at 2-min intervals until adequate reversal of respiratory depression was observed. IM naloxone was injected in the rectus femoris muscle, IN naloxone was given in alternate nostrils for repetitive doses, if required. The aim of naloxone administration was the return of adequate and sustained ventilation to at least 80% of pre-fentanyl baseline levels (henceforth referred to as “baseline ventilation”). Per protocol, a maximum of 4 doses of the IM and IN formulations could be given. The timing of naloxone dosing was based on the 2-3 min administration interval as outlined by the United States Prescribing Information.[4] Assuming a bioavailability of 50% for intranasal naloxone and >75% for intramuscular naloxone,[20] we hypothesized that reversal would be achieved after 2 to 3 doses of intranasal naloxone and 1 to 2 doses after intramuscular naloxone. If ventilation remained below this target after all doses were given, a naloxone dose of 0.4 mg intravenous naloxone was to be administered. Respiratory measurements continued for 2 hours from the administration of the first naloxone dose (t = 0). In contrast to the guidelines of the American Heart Association,[20] we refrained from verbal and/or tactical stimulation of our subjects during naloxone administration. We did so to enable the study of the naloxone pharmacodynamics driven by its pharmacokinetics without the confounding effects of activation of behavioral control of breathing. We agree that such a design differs from the guidelines. Stimulation may have an effect, but this is not certain as stimulation may have limited effect in case of loss of consciousness of the overdose victim (opioid narcosis).

Drugs

All drugs were prepared by the local pharmacy and dispensed on the morning of the study: fentanyl citrate 0.05 mg/mL (Hameln Pharma GmbH, Hameln, Germany), Zimhi (naloxone hydrochloride) 5 mg in 0.5 mL (DMK Pharmaceuticals Co., San Diego, CA, USA, and since May 2024 Zmi Pharma Inc, Carlsbad, CA, USA),[11] Narcan Nasal Spray (naloxone hydrochloride) 4 mg in 0.1 mL (Adapt Pharma Inc. Radnor, PA, USA)[12] and intravenous naloxone hydrochloride 0.4 mg/mL (Hameln Pharma GmbH, Hamel, Germany). IM or IN naloxone were administered on separate days in a random fashion (1:1) according to a randomization code generated by a study-independent statistician at the study center. The study had a crossover design with a 7-10 days washout period in-between study days.

Blood sampling and analyses.

Five mL arterial blood samples were obtained in K2EDTA tubes at regular intervals for measurement of fentanyl and naloxone concentrations in plasma. Plasma was separated from blood and stored at -80 °C until analysis. The samples were analyzed by Ardena Bioanalysis BV (Assen, the Netherlands) using validated liquid chromatography tandem mass spectrometry assays (LC-MS/MS) with limits of quantitation of 0.05 ng/mL (lower limit) to 500 ng/mL (upper limit) for both naloxone and fentanyl. Quality control revealed values for precision (coefficient of variation) for naloxone 1.1% (within-run precision) and 2.0% (between-run precision) and equivalent values for fentanyl 2.2% and 1.7%, respectively, while values for accuracy (bias) were for naloxone 2.2% (within-run bias) and 3.4% (between-run bias) and equivalent values for fentanyl -0.6% and 2.4%, respectively.

Safety

In case of the following circumstances, 0.4 mg naloxone was to be administered intravenously to ensure safety of the participants: end-tidal partial pressures of CO_2 greater than 90 mmHg (12 kPa) for more than 3 min, oxygen saturation of 70-80% for more than 1 min, oxygen saturation < 70%, or any other situation of condition that could affect the health of the subject, as judged by the attending anesthesiologist.

Sample size and statistical analysis

No experimental data are available from the current literature that compare 4 mg IN and 5 mg IM naloxone dose requirements for complete (i.e. 80 to 100% of baseline ventilation) reversal of minute ventilation following fentanyl-induced apnea. We therefore performed a (crossover) trial simulation. We simulated a median difference of 1 dose between IM (1 dose) and IN (2 doses) naloxone with a variability of 1 (IQR) for IM and 2 (IQR) for IN naloxone. We

simulated up to 20 subjects and obtained a power > 90% with $p < 0.05$ to detect a 1 dose difference with 12 subjects. To consider any discontinuations or deviations from our assumptions, we added 4 subjects to attain a test population of 16 healthy volunteers. The chronic opioid user population was added for comparative reasons and that part of the study should be considered exploratory. We chose a convenience sample of 6 individuals that were chronic, daily opioid users.

The primary endpoint of the study was the number of IM versus IN administrations to reach full reversal of minute ventilation (± 20%) following induction of apnea with an intravenous dose of 10 µg/kg fentanyl. These data were analyzed with the Wilcoxon signed rank test with continuity correction. A secondary endpoint was the time to 80 to 100% reversal of adequate minute ventilation (± 20%) from the moment of the first naloxone administration. For the subjects that were treated with intravenous naloxone (after IN naloxone), a penalty of 4 min was added, to reflect the time required to fetch and draw the naloxone from its container. These data were analyzed using the Wilcoxon signed rank test, and Kaplan-Meier analysis with log-rank (Mantel-Cox) testing in GraphPad Prism, version 10 for Windows (GraphPad Software, Boston, MA, USA). P-values < 0.05 were considered significant. Data analyses were further performed in R (R Foundation for Statistical Computing, Austria, http://www.R-project.org/). The data from the healthy volunteers are presented with statistical testing, the data from the chronic opioid users are presented descriptively.

Additional secondary endpoints were (i) the number of IM and IN administrations to restore stable breathing in chronic opioid users; (ii) breath-to-breath minute ventilation and end-tidal pCO_2; (ii) plasma concentrations of fentanyl and naloxone measured at specific time points in opioid-naïve individuals; the fentanyl measurements and analysis are exploratory as they were not prespecified in the protocol; (iii) pupil diameter and (iv) muscle tone. These secondary endpoints are presented without formal statistical analyses.

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