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CLINICAL TRIAL

Pharmacokinetics and -dynamics of intramuscular and intranasal naloxone: an explorative study in healthy volunteers

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Abstract

Purpose This study aimed to develop a model for pharmacodynamic and pharmacokinetic studies of naloxone antagonism under steady-state opioid agonism and to compare a high-concentration/low-volume intranasal naloxone formulation 8 mg/ml to intramuscular 0.8 mg.

Methods Two-way crossover in 12 healthy volunteers receiving naloxone while receiving remifentanil by a target-controlled infusion for 102 min. The group were subdivided into three different doses of remifentanil. Blood samples for serum naloxone concentrations, pupillometry and heat pain threshold were measured.

Results The relative bioavailability of intranasal to intramuscular naloxone was 0.75. Pupillometry showed difference in antagonism; the effect was significant in the data set as a whole ($p < 0.001$) and in all three subgroups ($p < 0.02$–$p < 0.001$). Heat pain threshold showed no statistical difference.

Conclusions A target-controlled infusion of remifentanil provides good conditions for studying the pharmacodynamics of naloxone, and pupillometry was a better modality than heat pain threshold. Intranasal naloxone 0.8 mg is inferior for a similar dose intramuscular. Our design may help to bridge the gap between studies in healthy volunteers and the patient population in need of naloxone for opioid overdose.

Trial registration clinicaltrials.gov: NCT02307721

Keywords Naloxone · Intranasal · Pharmacodynamics · Pharmacokinetics · Drug overdose · Remifentanil

Introduction

Worldwide, approximately 100,000 people die annually from opioid overdoses, and this figure is increasing, particularly in the USA [1, 2]. Opioid intoxication is recognised by miosis, respiratory depression and reduced consciousness. Naloxone has a key role in emergency treatment of respiratory arrest caused by opioid intoxication. It is a drug with an excellent

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safety profile, and it has little pharmacological effects in the absence of opioids. However, in the opioid-dependent patient it may precipitate acute withdrawal symptoms [3].

The provision of naloxone to people likely to witness an opioid overdose is a recommendation from the WHO [4]. Naloxone has been available since 1971, and many stakeholders have advocated the development of novel and user-friendly naloxone formulations. Nasal naloxone has long been used in various off-label formulations, but the use has been criticised on scientific, regulatory and legal basis [5, 6].

Despite the widespread use of nasal naloxone, there has been an absence of pharmacologic studies. Bioavailability as low as 0.04 has been reported [7]. Regardless, clinical studies comparing intranasal (IN) to intramuscular (IM) naloxone have been promising [8]. IN drugs require high concentrations and low volumes to allow systemic uptake with a maximum volume of < 0.15 ml/nostril [9, 10]. This is particularly important for naloxone, which has a high first-pass liver metabolism [11]. The IN formulation approved in 2015 by the US Food and Drug Administration has a relative bioavailability compared to IM of 0.47 (4-mg dose) and 0.44 (2 × 4-mg dose) [12]. An absolute bioavailability of 0.54 was recently reported for the IN formulation used in the present study [13].

Various models to study pharmacodynamic effects of opioids exist. Commonly, opioid agonism such as pain relief, pupil size changes or drug-liking has been reported [14–16]. The study of the reversal, antagonism, of these effects is rarer. Alfentanil, tramadol and hydromorphone per oral administration combined with naloxone [17, 18] have been suggested, but neither of these models creates a steady and reproducible state of opioid agonism. Shram et al. used remifentanil bolus and pupillometry to demonstrate the effects of the μ-opioid receptor antagonist samidorphan [19]. Pupillometry is an easy and non-invasive measurement and is often used to study the pharmacodynamic effects of opioids [20–23]. Pupil size is also validated as diagnostic criterion in pre-hospital overdoses [24]. Heat pain threshold (HPT) has also been shown to increase with remifentanil infusions in healthy subjects [25, 26].

In the present study, remifentanil was administered as a target-controlled infusion (TCI) [27, 28] to achieve steady-state opioid agonism. The computerised infusion system delivered remifentanil to rapidly achieve a set plasma concentration using a multi-compartment pharmacokinetic model. To measure the effects of the drugs administered, pupillary size and heat pain threshold were assessed before, during and after naloxone administration. The aim of the study was to establish a model for studying the pharmacodynamics of naloxone and to compare intramuscular and intranasal administration of naloxone under steady-state opioid agonism in human volunteers. It also aimed to investigate whether pupillometry or HPT were best suited to describe the pharmacodynamics of opioid reversal by naloxone.

Methods

Ethics

This study was conducted according to the Declaration of Helsinki and The International Conference on Harmonisation and Good Clinical Practice. It was approved by The Regional Committees of Medical and Health Research Ethics (2014/740) and the Norwegian Medicines Agency (EudraCT 2014-001465-27). Informed written consent was obtained from all prior to inclusion. Participants were insured through the Drug Liability Association, Norway, and compensated for each treatment visit with 1500 NOK (160 Euro/175 USD).

Subjects

Healthy men and women aged 18–40 years were eligible to participate. “Healthy” was defined as American Society of Anesthesiologists class I [29]. A full medical history and targeted examination including 12-lead electrocardiogram without pathologic abnormalities and blood samples within normal reference values for haemoglobin, creatinine, ASAT, ALAT and gamma GT were required. Women had to use safe contraception throughout the study period and have a serum HCG below 3 IU/l at inclusion. Breast-feeding women were excluded. Participants taking any medications including herbal products, with any known drug allergies, having any local nasal disease or nasal surgery for the last 2 months or a cold for the last week were excluded. Participants with a history of contact with police or authorities in relation to alcohol or drug offences, a history of prolonged use of opioid analgesics, who had access to remifentanil or other potent opioids in their daily workplace or who had a history of drug and/or alcohol abuse were excluded. Potential participants had to answer the CAGE-AID questionnaire [30]; anyone answering yes to two or more questions was not allowed to participate.

Nineteen subjects were screened for inclusion; five did not meet the criteria. Fourteen subjects were included. One subject withdrew consent and one started medication that lead to exclusion, both prior to randomisation. Twelve participants were randomised and completed the study: six men and six women, with mean age of 23.8 (22.6–25) years, mean height of 175.3 cm (168.6–182.0), mean weight of 68.9 kg (61.3–76.5) and mean BMI of 22.3 kg/m^2.

Design

This was a phase 1, open, randomised, two-way, crossover, pharmacokinetic and pharmacodynamic study in human volunteers. Participants were exposed to remifentanil and naloxone twice. Each study session lasted 7 h; the sessions were separated by at least a 72-h wash-out period. The order of treatments was decided by concealed randomisation by an

Internet-based service that conducted block randomisation without stratification. A formal sample size calculation was not performed. Twelve subjects are commonly used in phase 1 studies, as it usually provides adequate data for estimates of inter-individual variations of the pharmacokinetics of the study drug. The study was conducted at the Clinical Research Facility, St. Olavs Hospital, Trondheim, Norway, from December 2014 to April 2015.

The primary endpoint in this study was comparison of the pharmacodynamic profile of IN and IM naloxone by pupillometry and heat pain threshold. Secondary endpoints included the pharmacokinetic profile of IN and IM under opioid influence (bioavailability, Cmax and Tmax) and safety of formulation.

Naloxone were administered as 0.8 mg IM or 0.8 mg IN. Naloxone B. Braun 0.4 mg/ml (Braun, Melsungen, Germany) IM was supplied by the St. Olavs Hospital Pharmacy and administered as 2.0 ml in the deltoid muscle. The nasal formulation contained naloxone hydrochloride 8 mg/ml and was produced by the Department of Biopharmaceutical Production, Norwegian Institute of Public Health (FHI), Oslo, Norway. The formulation is previously published in detail [13]. IN naloxone was administered in a Unitdose disposable nasal spray device from Aptar Pharma (Louveciennes, France). IN naloxone was administered as 0.1-ml puff in one nostril with the participant supine. The IN doses were chosen on the basis of previous studies of the same naloxone formulation [13] as it corresponds to the lowest recommended starting dose for opioid overdose (0.4 mg). The IM dose of 0.8 mg naloxone is the most commonly used dose for reversal in the Oslo Ambulance Service, and it falls within the recommended starting dose for titration in pre-hospital opioid overdoses, which is between 0.4 and 2.0 mg in both Europe and the USA [3, 31, 32]. Thus, dose-response correlation of the model could also be observed.

During the course of the study, concerns regarding the nasal spray production and possible leakage from spray containers were raised. The study was halted for 2 weeks and all sprays where weighed at delivery to the Clinical Research Facility, during storage, at and after dose administration. The sprays with a change in weight of more than 0.0001 g where excluded.

Remifentanil hydrochloride (Ultiva, GlaxoSmithKline, Brentford, UK) was administered by TCI plasma control Minto model, using Alaris PK Guardrail syringe pumps (CareFusion Cooperation, UK). This computer-based dosing system delivers the drug as an initial bolus and frequently changes the speed of the infusion to rapidly achieve steady state. Remifentanil is ideally suited to create a state of stable opioid influence during the time of infusion. It has a half-life of only 3–10 min and no active metabolites [33]. Participants received remifentanil for a total of 102 min each visit (Fig. 1); the initial target was 2.5 ng/ml ($n = 4$), followed by 1.3 ng/ml ($n = 5$) or 1.0 ng/ml ($n = 3$). A similar model is previously used [27, 28]. The infusion was started at a dose of 1.0 ng/ml for 1 min, then increased to target for 11 min. The combination of a drug with an ultra-short half-life [33] and the bolus dose given by the TCI pump [34] 12 min should ensure steady state. Remifentanil infusion was continued for a further 90 min at the target concentration set. Naloxone was administered 12 min after the remifentanil was started.

Safety

Participants were required to fast before a study session [35]. They were monitored by continuous oxygen saturation and three-lead ECG and intermittent non-invasive blood pressure throughout. An anaesthetist was present during and minimum 1 h after the administration of remifentanil. For safety and to avoid adverse events from remifentanil metoclopramide 10 mg intravenous (IV) once, ondansetron 4 mg IV once, ephedrine 10 mg IV once and oxygen on nasal prongs (max 2 l/min) were allowed as concomitant medications in our study. Additional IV naloxone was available as rescue medicine.

Pharmacodynamic measurements

Pupil size was measured using a Neuroptics VIP 200 Pupillometer (Neuroptics, Irvine, CA, USA). To ensure similar light conditions, the research facility had low and uniform ambient lighting at all study visits. The light was controlled using the application Light Meter version 2.1 by Vlad Polyanskiy for iPhone 5 at the start and end of each session reading mean 39.51 (38.18–40.84) lux. The pupils were given time to adapt prior to start of study. Pupillometry was measured at times − 23, − 18, − 14, 0, 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90 and 120 min, with 95, 100, 105, 110 and 115 min added after four participants for higher resolution.

HPTs were measured using the Somedic MSA Thermotest (Somedic AB, Hørby, Sweden). This apparatus measures the relationship between the intensity of controlled thermal stimuli and the associated perception. The stimulus (1 °C per second rise time from the 32 °C start temperature) was applied to intact skin by a hand-held thermode (area 25 × 50 mm = 12.5 cm^2) placed over the non-dominant thenar eminence while monitoring the temperature. Participants were instructed to stop the increase in temperature once the sensation changed from warm to painful. The HPT was measured in °C and we calculated the average of three repeated single HPTs. The HPT was measured at times − 21 min as a test to familiarise subjects with the procedure and then at − 17, − 13, 0, 3, 7, 12, 17, 20, 30, 45, 60, 90 and 120 min. The individual HPT baseline was defined as the 0 thresholds, and the HPT baseline response difference was calculated for the following measurements. Maximum temperature was set to 52 °C. If participants did

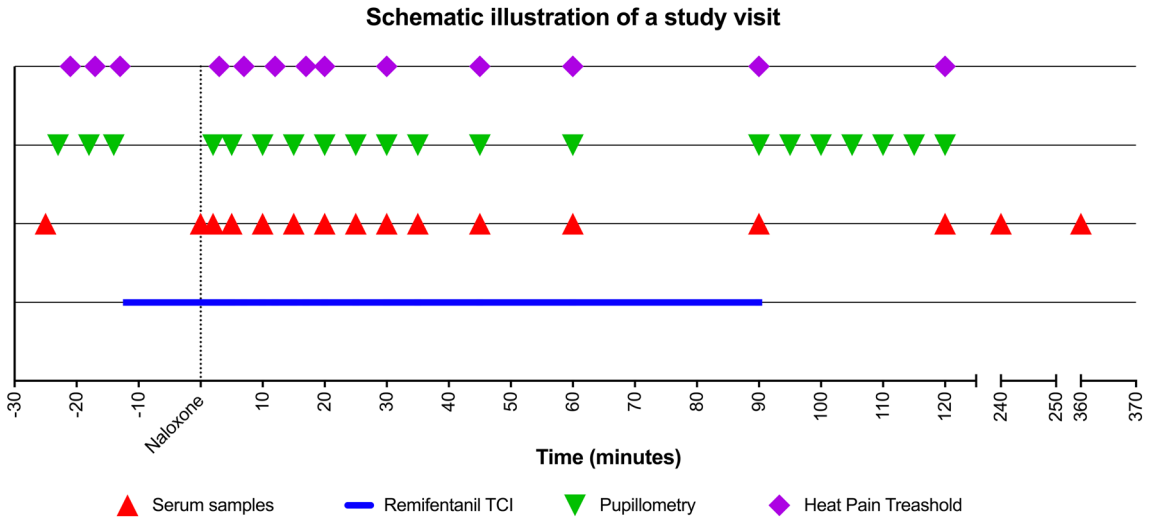

Fig. 1 Schematic illustration of samples and measurements and sequence of events in the protocol for each session. Remifentanil infusion lasts 102 min and naloxone is administered at $t = 0$

not stop, the stimulus prior to the maximum temperature 52 °C was set as the result of that individual test.

Blood samples and analysis

Blood samples were drawn from an IV cannula placed in the antecubital fossa in the opposite arm of naloxone and remifentanil administration. Blood for naloxone analysis was collected in Vacuette tubes without gel and left to coagulate for 30 min, centrifuged for 10 min at 2200*g*. Serum was transferred to cryotubes and immediately frozen at − 20 °C, and stored in an − 80 °C freezer before the end of the day. Naloxone samples were drawn at − 25, 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240 and 360 min. Naloxone was analysed by a validated liquid chromatography tandem mass spectrometry method and deuterated naloxone-d5 was used as an internal standard. The calibration range was from 0.02 to 10 ng/ml and the limit of quantification (LOQ) was 0.02 ng/ml with a coefficient of variation (CV) < 6.8% and inaccuracy < 4.0% ($n = 17$). CV and inaccuracy of the quality controls QC1, 2 and 3 (0.05, 5.0 and 8.0 ng/ml) were < 7.4 and 0.6% (QC1), < 3.3 and 0.6% (QC2) and < 2.2 and 0.9% (QC3) respectively in the pre-run validation ($n = 18$). During in-run validation ($n = 18$), the CV and inaccuracy were < 5.1 and 5.1% (QC1), < 3.0 and 0.4% (QC2) and < 3.6 and 0.2% (QC3). The method is published in full [13].

Statistics

Pharmacodynamic measurements were analysed in the whole group and in the different remifentanil dose subgroups using the statistics software R, version 2.13.1 (open source). A mixed linear model analysis with the combination of time and treatment as the fixed effects was employed for all comparisons reported; exceptions are clearly stated. To account for repeated measurements on each participant, participant ID was included as a random effect. Using a likelihood ratio test, the time course for the two treatments between $t = 2$ and $t = 90$ was compared against the lowest point, and the time course from $t > 0$ was compared between the treatments. When the time course for the two treatments was significantly different, the treatments were compared at each time point using a Wald test.

Serum concentration data was analysed by non-compartmental techniques using WinNonlin Standard version 6.4 (Pharsight Corporation, NJ, USA). Area under the curve (AUClast (linear trapezoidal rule), terminal elimination half-life, Cmax and Tmax were calculated by computerised curve fitting. Dose-corrected AUCs were used to calculate the relative bioavailability. Comparing the present data with historic PK data was performed in accordance with the bioequivalence criteria [36] using independent sample *T* test on logarithmically transformed PK data. Descriptive statistics were performed in SPSS version 21 (IBM, NY, USA).

Concentration measurements below LOQ were not used in the analysis. Outlier points of the serum concentration profile that deviated more than twice, or less than half, of the expected value were taken out of the analysis. Missing data were not imputed. There were three missing samples and four outliers out of a total of 336 samples.

Results

The primary endpoint of this study was to describe the pharmacodynamic (PD) profile of IN versus IM naloxone. Data is reported as mean and 95% confidence intervals (CI) unless clearly stated.

Changes in pupillary size

All data pooled show mean pupil diameter before remifentanil of 6.6 mm (6.2–7.0) in the IN group and 6.8 mm (6.6–7.1) in the IM group. After the start of remifentanil administration, the nadir ($t = 0$) was 2.9 mm (2.6–3.2) in both groups. After naloxone administration ($t = 0$), the reversal of miosis was seen in both treatment groups, but more prominent in the IM group. This effect was apparent in the whole dataset ($n = 12$) and in each remifentanil subgroup. After remifentanil infusion was terminated ($t = 90$), the pupils returned to initial size.

Difference in pupil size from nadir

Analysis of changes in pupillary size (pooled data, $n = 12$) from a horizontal line drawn from the nadir ($t = 0$) showed (Fig. 2) that the time course is different from this low point for both treatments ($p = 0.002$ for IN and $p < 0.001$ for IM). A subgroup analysis showed a time course different from nadir for IM ($p < 0.01$) but not for IN ($p = 0.68$) in the 2.5-ng/ml remifentanil TCI group ($n = 4$). In the 1.3-ng/ml TCI group ($n = 5$), both IN and IM showed time courses different from nadir ($p < 0.01$), and in the 1.0-ng/ml TCI group ($n = 3$), neither of the treatments produced a time course different from nadir (IN $p = 0.38$, IM $p = 0.14$).

Difference in pupil size between IN and IM

Figure 2 shows the time course of pupillary size, how remifentanil induces miosis and how naloxone reverses this. The IM and IN curve separated after naloxone is administered ($t = 0$) and joined up at $t = 45$ until the end of the study session. This effect was apparent in the data set as a whole ($p < 0.001$) and in all the three subgroups ($p < 0.02$–$p < 0.001$).

When comparing each time point (pooled data), the difference in miosis reversal was significantly different between IM and IN from 5 to 35 min after naloxone administration (Supplementary material 1). The difference was not significant for the rest of the study session. The apex of miosis reversal was 15 min for IM and 30 min for IN. Miosis became more apparent from 60 min and until remifentanil was stopped at 90 min, and pupillary size returned to initial size. The TCI subgroup analysis showed a difference between the two routes of administration at all time points from 5 to 25 min for the

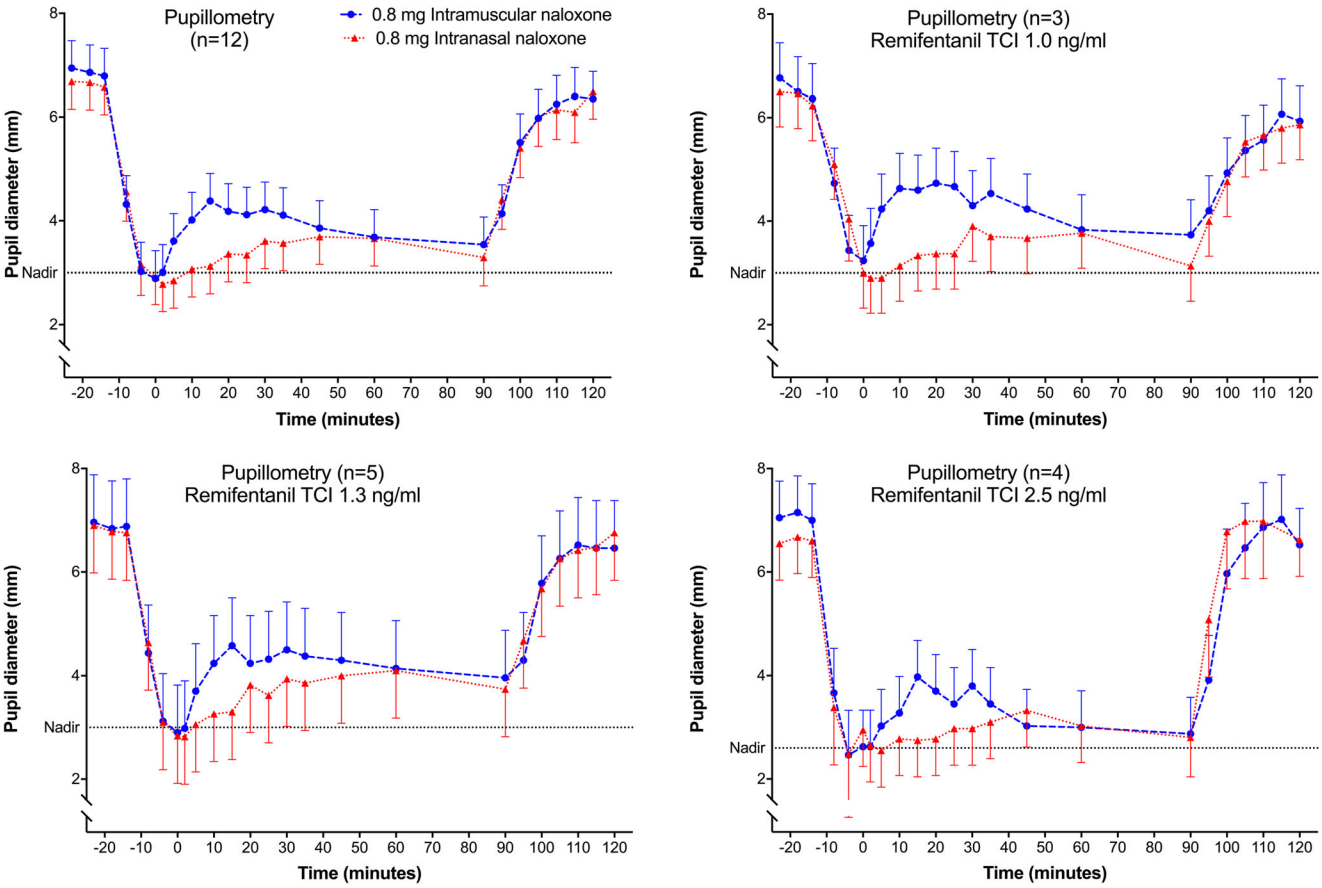

Fig. 2 Time course of variation of pupil size (mean, 95% confidence interval). Pupils are adapted to low ambient light and remifentanil TCI is administered between $t = -12$ and $t = 90$ min; 0.8 mg naloxone is administered at $t = 0$ as either nasal spray (triangles) or intramuscular injection (circles)

remifentanil TCI 1.0-ng/ml group. For the remaining two groups, only two time points showed significant difference between the two routes of administration, at 10–15 min for the 1.3-ng/ml remifentanil group and at 15–20 min for the 2.5-ng/ml group (Supplementary material 1).

Heat pain threshold

Figure 3 shows the results from the HPT measurements. The between-subjects variability, shown as 95% confidence intervals of means in the figure, was large as expected (average SD = 2.67 °C) while the within-subjects variability was small (average SD = 0.96 °C). HPT means increased from the pre-remifentanil recording (− 13- and − 17-min means) to $t = 0$ in both groups (by 1.1 °C in IN and 0.5 °C in IM). A consistent HPT decrease from the peak at $t = 0$ seemed to occur for both treatments, most consistently for about 30–60 min, but the effect size was moderate (about − 0.8 °C at 30 min in both the treatment groups). Neither with time, nor between IM and IN, statistically significant different time courses appeared in the material as a whole ($p = 0.89$). In the analysis of the TCI subgroups, only the 1.0-ng/ml group displayed a significant different time course ($p = 0.004$) between the routes of administration. A comparison of the two routes showed only three significant time points ($t = 12$, 90 and 120) and no apparent pattern or systematic difference.

Pharmacokinetic variables

The secondary endpoint in this study was the pharmacokinetic (PK) profile of IN and IM naloxone under opioid influence.

Both IN sprays and IM syringes were accurately weighed before and after administration, and the actual dose naloxone administered was calculated to form the base of the PK analysis. Mean IN dose was 0.75 mg and mean IM dose was 0.82 mg.

The main variables are presented in Table 1 and Fig. 4. The relative bioavailability (F) of IN compared to that of IM naloxone was 0.75 (95% CI 0.63–0.87) ($n = 11$). One individual missed serum naloxone samples at $t = 240$ and 360 min, so the elimination rate constant could not be calculated. This

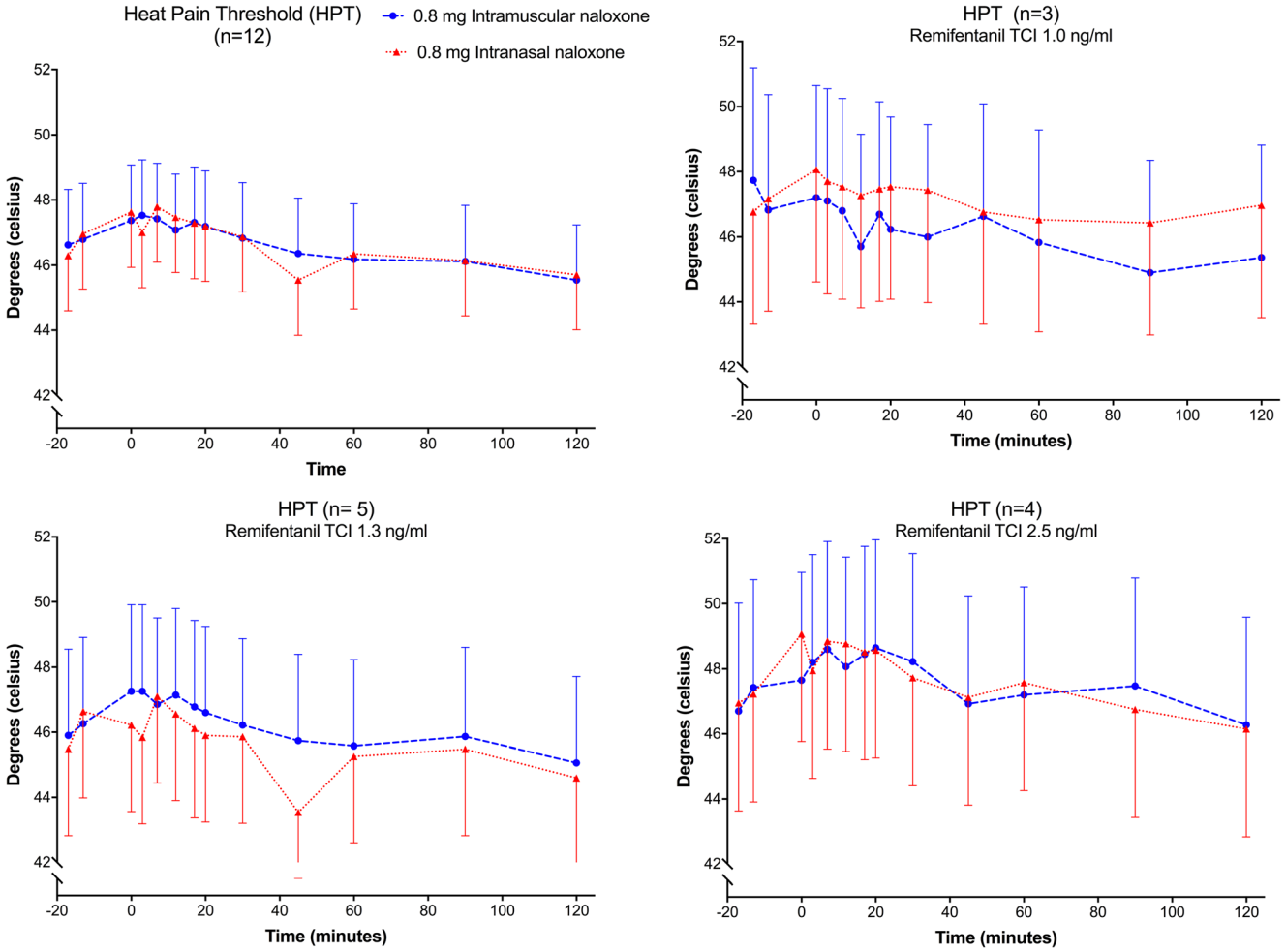

Fig. 3 Time course of variation of heat pain threshold (mean, 95% confidence interval). Remifentanil TCI is administered between $t = -12$ and $t = 90$ min and naloxone administered at $t = 0$; 0.8 mg naloxone is administered at $t = 0$ as either nasal spray (triangles) or intramuscular injection (circles)

Table 1 Pharmacokinetic calculations for intranasal and intramuscular naloxone. Data are presented as mean (95% confidence intervals). *Cmax* maximum concentration, *Tmax* time to maximum concentration, *AUClast* area under the curve until last measurement

	Cmax (ng/ml)	Tmax (min)	AUClast (min × ng/ml)	Half-life (min)	Clearance /F^a (ml/min)	Volume of distribution/F^a (l)	Bioavailability (F)
IM 0.8 mg	3.62 (2.64–4.60)	7.75 (5.01–10.5)	244 (197–292)	69.7 (59.5–79.8)	3150 (2600–3719)	325 (232–419)	–
IN 0.8 mg	1.63 (1.25–2.02)	28.0 (22.0–34.0)	160 (125–195)	63.7 (59.2–68.2)	3420 (2745–4095)	317 (245–390)	0.75

[a] For extravascular models in WinNonlin, the fraction of dose absorbed cannot be estimated; therefore, volume and clearance for these models are actually volume/F or clearance/F where F is the fraction of dose absorbed. We have estimated this to be 1 for IM and 0.75 for IN

participant was therefore excluded from bioavailability, clearance and distribution volume analysis. Extrapolation of area under the curve (AUC) last to AUC∞ was 2.5% for IN and 3.0% for IM, indicating that our sampling schedule covers above 97% of the serum concentration curve.

Cmax and AUClast for IM were about twice those of IN, IN Tmax was three times faster (7.75 versus 28 min), while $t1/2$, clearance and volume of distribution were similar.

We calculated the time to 50% and 80% of maximum concentration (Tmax50 and Tmax80). Mean Tmax50 for IN was 11.4 min and that for IM 4.25 min. Tmax80 was 19.8 min for IN and 6.42 min for IM.

PK/PD comparison

The hysteresis plots show a counter-clockwise direction for both IN and IM naloxone (Fig. 5). Visual inspection of the curve indicates a maximum reversal of miosis at around 2.5 ng/ml naloxone and 15 min for IM 0.8 mg. IN naloxone never reached this serum concentration level. The hysteresis loop for the TCI 2.5-ng/ml group shows a very small degree of reversal by IN naloxone at that remifentanil dose.

Safety

Adverse events were reported using the Common Terminology Criteria for Adverse Events version 4.0. No serious adverse events were reported. Four cases of intercurrent illness and three cases of adverse events were reported in seven individual participants. All cases resolved spontaneously with no sequelae. The adverse events were all headaches and were defined as having a possible relationship to the IN naloxone formulation. No participants required the administration of the concomitant medications allowed in the protocol.

Mean (min–max) total remifentanil doses for TCI 1.0 ng/ml were 307 (239–375) μg, those for TCI 1.3 ng/ml were 426 (393–460) μg and those for 2.5 ng/ml were 771 (654–888) μg.

Discussion

The major findings of this study were that a target-controlled infusion of remifentanil provided satisfactory conditions for studying the pharmacodynamics of naloxone and that pupillometry was a better modality than heat pain threshold.

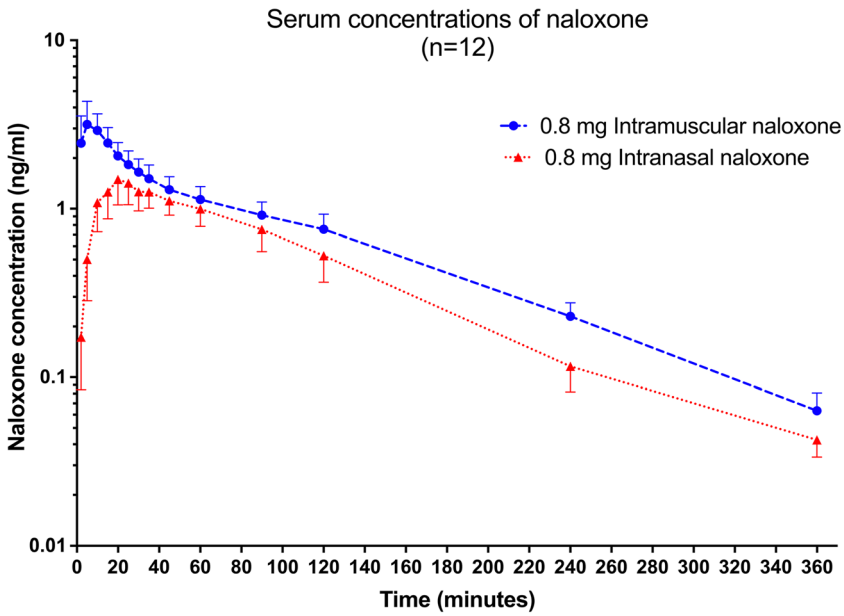

Fig. 4 Time course of serum concentrations of naloxone (ng/ml) mean and 95% confidence interval after intranasal or intramuscular administration of 0.8 mg naloxone in healthy human volunteers receiving a remifentanil infusion ($n = 12$). Triangles represent the nasal spray and circles the intramuscular naloxone

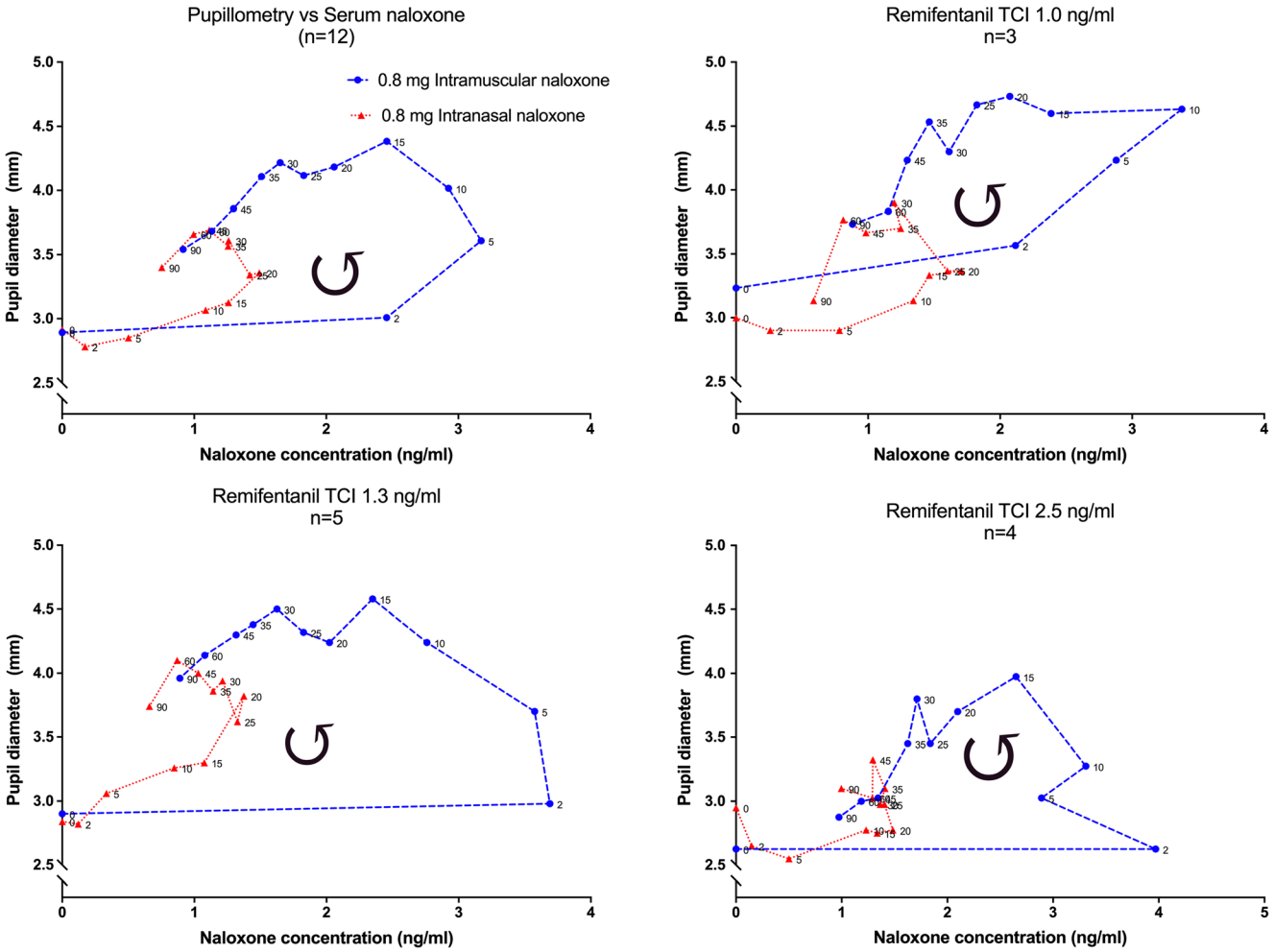

Fig. 5 Hysteresis plot of pupil diameter and naloxone concentration during a stable remifentanil infusion. Each point is numbered corresponding to time it was measured. Error bars are removed for clarity. The arrow indicates the direction of time. Triangles represent the nasal spray and circles the intramuscular naloxone

There was a significant delay in the transfer of naloxone from blood to the site of action. In this model, the time course of the naloxone antagonism was clearly displayed, and the effect of 0.8 mg naloxone IM was both more rapid and profound than that of 0.8 mg IN, the latter with a bioavailability of 0.75 relative to IM. These observations were compatible with the differences in the respective serum concentration time course curves.

Several models for studying the pharmacodynamics of naloxone have been published [17, 20, 37, 38]. They have all in common that they lacked the potential of obtaining reproducible conditions for the agonist that is necessary for studying the time course of naloxone action only. In this study, target control infusion was used for its potential to rapidly obtain and maintain steady-state conditions. TCI administers remifentanil based on a complex model that renders reproducible conditions across individuals and occasions to a higher degree than ordinary, arbitrary infusion regimens. All the remifentanil doses used in this model expose participants to levels of remifentanil below the threshold of < 0.1 μg/kg/min expected to produce opioid tolerance or hyperalgesia [39]. Such effects would confound the pharmacodynamic measurements.

It was expected in this explorative study that both IN and IM doses would provide a significant antagonism of the remifentanil 2.5 ng/ml target infusion-induced miosis, as a similar dose of naloxone reversed miosis with similar remifentanil doses in an earlier PD study [40] and 2.5 ng/ml were used in a similar research protocol measuring HPT earlier [28]. This assumption turned out to be wrong as the pupillary response to naloxone doses were poor under the 2.5 ng/ml TCI of remifentanil. The division into three dosing subgroups reducing from the initially planned target of 2.5 ng/ml was done to improve resolution of the pharmacodynamic measurements. Regardless, the pupillometry model gave good resolution as it could both demonstrate time course effects of naloxone and separate the effects of two different administration forms/doses. This was in contrast to the HPT

model which was insensitive to the experimental conditions in this study in all the groups.

The counter-clockwise hysteresis plots show a time delay between the serum naloxone concentration and the effect in pupil size regardless of administration form. This is similar to the plots seen for the opioid antagonist samidorphan and for the opioid fentanyl [19, 41]. Although similar to these related drugs, we cannot answer whether this is a distribution delay to the effect site, slow receptor kinetics or other mechanisms.

In this crossover study, it was clearly shown that the 0.8-mg naloxone dose given IM performed better than the 0.8 mg IN, both with respect to speed of onset and extent of reversal. This was expected as the absolute bioavailability of IN for this formulation is 0.54 [13], and that the relative bioavailability of IN to IM naloxone was found to be 0.75. Certainly, this should be taken into account when deciding a clinical useful concentration of nasal naloxone.

There may be an interaction between remifentanil and naloxone. A higher AUC of naloxone was found in this study compared with data from previous trials using the same formulation [13] and other studies of high-concentration/low-volume naloxone formulations [42, 43]. Applying independent sample *T* test and the bioequivalence criteria on the present and using the historic data as reference, mean difference and 90% CIs were 0.62 (0.48–0.81) for AUC0–∞ ratio and 0.87 (0.63–1.20) for maximum concentration (Cmax) ratio, respectively. The difference was statistically significant for AUC; this likely indicates a clinically relevant interaction. If true, this may be relevant for overdose victims but needs further investigation.

The time to maximum concentration (Tmax) of 8 min after IM administration indicates an extremely rapid uptake of naloxone. Previously reported Tmax for IM naloxone has been in the mean range of 10 to 25 min [44, 45]. Again, we may speculate whether this is a result of the remifentanil infusion. Otherwise, the PK parameters were within previously reported ranges.

Any naloxone formulation intended to treat opioid overdoses must weigh the dose and onset of action between rapid and sufficient reversal of respiration against the precipitation of acute withdrawal. IN formulations have slower uptake as shown by higher Tmax than injection [44], resulting in a slower onset of action as shown in this study. However, as nasal sprays can be administered to overdose patients prior to the arrival of emergency medical staff, it can still shorten time to treatment effect. The somewhat slower onset may also reduce the symptoms of withdrawal. IN naloxone is becoming more available and is increasingly forming the basis of public health intervention to combat death from opioid overdoses. PD studies and our model may help to bridge the gap between PK studies in healthy volunteers and the patient population where the drug is meant to serve.

Besides exploring a PK/PD model for opioid reversal, the objective of this study was to explore an IN dose of 0.8 mg naloxone to the clinically relevant dose of 0.8 mg IM naloxone. The overall conclusion is that an IN dose of 0.8 mg, as expected, is inferior to the same nominal dose IM. Further development of IN naloxone for emergency reversal of opioid intoxication requires higher doses.

Limitations

The dataset is limited with a low number of participants in each subgroup of remifentanil, especially in the 1.0-ng/ml group with $n = 3$. Negative observations may be caused by low power and the results therefore have to be interpreted with caution. A higher dose of IN naloxone administered, more equivalent to the IM dose, would have yielded more significant change in pupillometry in the IN group. Remifentanil is a potent opioid with a unique elimination by blood esterases and may have different physiological effect to those of opioids more commonly associated with overdose. Pupillometry is a pharmacodynamic measurement with no direct clinical significance, although it is one of the cardinal symptoms in opioid overdose. In an overdose, the respiratory depression is the main symptom to treat, and caution is required to translate the PD effects on miosis directly to the desired effects needed to reverse an opioid overdose. Adequate PK/PD modelling cannot be conducted as we do only have venous blood concentrations.

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Author contributions OD was principal investigator and contributed to all aspects of this study. AKS and IT has written the manuscript, designed the research protocol, conducted the research and analysed data. ØS has performed the mixed model statistical analysis. TN and SS has analysed serum samples and prepared data for PK analysis. TS has designed the HPT measurement program. TL has been pivotal in the development of the IN naloxone formulation, the fundament of this study. All authors have reviewed the final draft of the text.

Compliance with ethical standards

Conflict of interest Norwegian University of Science and Technology (NTNU) and its subsidiary Technology Transfer Office (TTO) have a licencing agreement with Den norske Eterfabrikk (DnE) regarding the naloxone formulation studied. DnE has sent an application for marketing authorization for a drug for human consumption. NTNU, TTO and Ola

Dale (OD) have financial benefit from these contracts. OD has been engaged by DnE as Principle Investigator in a pharmacokinetic study of naloxone for which OD receives no personal honorarium. DnE has compensated OD for two travels from Trondheim to Oslo.

Arne Kristian Skulberg (AKS) has signed a non-compete contract with DnE lasting the duration of his PhD program (estimated 2018). This does not limit AKS right to publish results and he receives no royalties or other financial benefits from DnE/NTNU. Other authors declare they have no conflicts of interest.

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