Eur J Clin Pharmacol
DOI 10.1007/s00228-016-2191-1

PHARMACOKINETICS AND DISPOSITION

Pharmacokinetics of a new, nasal formulation of naloxone

Ida Tylleskar[1] · Arne Kristian Skulberg[1,2] · Turid Nilsen[1] · Sissel Skarra[1] · Phatsawee Jansook[3] · Ola Dale[1,4]

Received: 28 September 2016 / Accepted: 28 December 2016

Abstract
Purpose Nasal naloxone is wanted for bystander administration in opioid overdose and as a needle-free alternative for emergency medical personnel. Epidemiologic studies have indicated a therapeutic effect of bystander administration of low-concentration/high-volume formulations. The objective for this study was to describe the nasal pharmacokinetics of a new high-concentration/low-volume nasal formulation of naloxone.
Methods This was an open, randomized triple crossover trial in healthy, human volunteers (n = 12) where two doses of nasal naloxone (0.8 and 1.6 mg) and one intravenous dose (1.0 mg) were compared. Fifteen serum samples were collected before and until 6 h after naloxone administration. Quantification of naloxone was performed by a validated liquid chromatography-tandem mass spectrometry method.
Results Bioavailability was 0.54 (0.45–0.63) for the 0.8 mg and 0.52 (0.37–0.67) for the 1.6 mg nasal naloxone formulation. Maximum concentration levels (C_{max}) were 1.45 ng/ml (1.07–1.84) for 0.8 mg and 2.57 ng/ml (1.49–3.66) for the 1.6 mg. Time to maximum concentrations (T_{max}) were reached at 17.9 min (11.4–24.5) and 18.6 min (14.4–22.9) for the 0.8 mg and the 1.6 mg doses, respectively.
Conclusion This nasal naloxone formulation had a rapid, systemic uptake and higher bioavailability than naloxone formulations not designed for IN use. This indicates that an optimized high-concentration/low-volume nasal spray formulation may deliver a therapeutic dose. The 1.6 mg nasal dose provided serum concentrations that surpassed those of 1.0 mg IV after 15–20 min and stayed above for the rest of the study period.

Keywords Nasal · Intranasal · Naloxone · Pharmacokinetics · Bioavailability · Overdose

Introduction

Opioid overdoses are a worldwide epidemic, affecting both users of illicit drugs and patients taking prescribed opioids. About 12–21 million use opioids worldwide, the annual death toll is 69,000 [1] and the number of non-fatal overdoses are many times higher [2]. Opioids cause respiratory depression, which may progress to cardiac arrest and death. Although ventilatory support is the primary intervention, administration of an antidote such as naloxone is of vital importance.

Naloxone competitively displaces opioids from the μ-opioid receptor and antagonizes the effects. It is usually administered intravenously (IV) or intramuscularly (IM) with a starting dose of 0.4–2.0 mg naloxone hydrochloride and titrated to desired response. Some advocate a lower starting dose of 0.04 mg naloxone when treating overdoses in the emergency room or iatrogenic overdoses [3, 4]. IV used to be the preferred treatment because of the faster action; however, there is now a widespread clinical use of IM [4]. Reversal of

Electronic supplementary material The online version of this article (doi:10.1007/s00228-016-2191-1) contains supplementary material, which is available to authorized users.

✉ Ola Dale
ola.dale@ntnu.no

[1] Department of Circulation and Medical Imaging, NTNU, Norwegian University of Science and Technology, Trondheim, Norway

[2] Division of Emergencies and Critical Care, Department of Anaesthesiology, Oslo University Hospital, Oslo, Norway

[3] Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

[4] Department of Research and Development, St. Olav's University Hospital, Trondheim, Norway

Published online: 31 January 2017

symptoms is rapid, but acute withdrawal symptoms can be precipitated, particularly in opioid-dependent subjects. Precipitation of withdrawal symptoms is associated with intravenous administration and higher doses [5]. Otherwise, naloxone is considered a safe drug with few side effects [6–8].

As a response to the overdose epidemic, there has been a growing interest for take home naloxone (THN) among politicians, medical staff, and caretakers. Since most opioid overdoses are witnessed [9, 10], the World Health Organization has recommended that people likely to witness an opioid overdose should have access to naloxone [10]. A needle-free naloxone alternative would be favorable. Nasal administration (IN) is quick and easy to use compared to IV injection [11]; it protects against accidental blood exposure and allows bystanders to intervene in an overdose situation. In the USA, a particularly sharp rise in deaths from opioids has taken place the last few years [12]. This has led the US Food and Drug Administration (FDA) to grant fast track applications to speed up development of adequate nasal naloxone formulations.

Nasal administration of naloxone has a great potential to change treatment guidelines. The nasal cavity has a thin mucosa and a rich blood supply, which allows for quick absorption of xenobiotics; also, nasally absorbed drugs bypass the liver first-pass metabolism, which is extensive for naloxone [13–15]. Other nasal drugs such as methadone, fentanyl, or midazolam are known to have bioavailabilities of more than 0.60 [16–18]. These formulations have in common is that they are delivered in volumes of 0.1 ml, respecting the volume limitation for the nostril of about 0.1 to 0.15 ml [19, 20].

Today, there is a widespread use of off-label intranasal naloxone kits in THN-programs, often using dilute formulations intended for injection, connecting the syringe to an atomizer. Spray volumes of up to 2.5 ml per nostril have been used with a resulting bioavailability of 0.04, making these formulations unsuitable for the delivery of systemic, therapeutic doses [21]. FDA requires that a nasal naloxone should at least generate serum concentration comparable with those of IV, IM, or subcutaneous naloxone administration [22]. Consequently, the concentration of naloxone must be much higher than that commonly found in formulations for injection. This principle was adopted recently for an FDA-fast-track-approved naloxone nasal spray (Narcan® (naloxone hydrochloride) nasal spray, Adapt Pharma, PA, USA) having a concentration of 40 mg/ml delivered in 0.1 ml. The relative bioavailability of the nasal formulation relative to IM was 0.47 [15, 23, 24]. Unfortunately, its absolute bioavailability was not reported.

Several clinical studies have evaluated the potential of nasal naloxone. The common issue in these studies was the same as that for the THN-programs; in the use of formulations not optimized for nasal administration [25–27], and in the few randomized controlled trials conducted, the need for naloxone rescue was 13% higher in the IN group compared to that in IM administrations [7, 8] confirming that the dose delivered systemically may have been too small, as expected from the biological considerations above.

All over, the reviews of the evidence of intranasal naloxone have concluded that IN naloxone could be useful, but there is currently not sufficient evidence to fully support IN naloxone as the first line treatment by paramedics or for community management of opioid overdose. Even though an FDA-approved formulation is now available, there is still a need for research on the disposition of nasal naloxone formulations, optimal dosing, and the clinical efficacy of these sprays [10, 25, 28].

The aim of the present study was to investigate the pharmacokinetic profile with emphasis on absolute bioavailability of a novel naloxone formulation designed for nasal use, with a high-concentration/low-volume and excipients to enhance uptake.

Material and methods

Formulation and production

The solution was formulated for intranasal delivery using naloxone hydrochloride dihydrate (C_{19} $H_{21}NO_4$ ·HCl ·$2H_2O$, CAS number: 51481-60-8). The naloxone concentration was 8 mg/ml and contained well-known excipients such as glycerine (12 mg/ml), polyvinyl pyrrolidone (1.0 mg/ml), and sodium edetate (0.5 mg/ml) as absorption enhancers and benzalkonium chloride (0.2 mg/ml) as preservatives. Citric acid-sodium citrate buffer (2.0 and 2.8 mg/ml, respectively) was used to maintain the formulation's pH of 4.3.

A bidose disposable nasal spray device from Aptar Pharma (Louveciennes, France) was used. The formulation was produced, and the device was assembled by the Department of Biopharmaceutical Production, Norwegian Institute of Public Health (FHI), Oslo, Norway. The production complied with Good Manufacturing Practice. The sprayers are constructed to deliver 2 × 0.1 ml. This requires that the container to be prefilled with 0.230 ml of naloxone solution as 0.030 ml remained in the container after actuation for correct delivery of 0.1 ml × 2.

Participants

Healthy men and women aged 18–45 with hemoglobin, creatinine, ASAT, ALAT, and gamma-GT within reference values and a normal ECG were eligible for inclusion. Regular use of medications, including herbal medicines, was not allowed. Female participants required a negative pregnancy test, high efficacy contraception, and could not be breastfeeding during the study period. Subjects with a history of previous nasal surgery, a history of drug allergies, or drug addiction were also

excluded. Fifteen subjects were screened for inclusion (12 men and 3 women). Two participants did not meet the inclusion criteria. Thus, 11 men and two women, aged 21–32, all Caucasians, were included. One subject was excluded during the study. Average BMI was 23.5 (24.2 for men and 21.2 for women). All over, BMI ranged from 20.7 to 27.8.

Setting and design

The study was conducted at the Clinical Research Facility, St. Olav's University Hospital, Trondheim, Norway, during February–May 2014. This was a phase 1, open-label, randomized, three-way crossover study. The subjects were exposed to naloxone three times, twice intranasally (IN) and once intravenously (IV). Each study session lasted for 6–7 h and the sessions were separated by at least 72 h wash-out period. The order of treatments was decided by randomization. This was performed in a concealed fashion by an internet-based service that conducted block randomization without stratification. A formal sample size calculation was not performed. Twelve subjects are commonly used in such phase 1 studies, as it usually provides adequate data for inter individual variations of the pharmacokinetics of the study drug.

Drug doses and administration

The subjects received intranasal naloxone 0.1 ml 8 mg/ml once (0.8 mg) and twice (1.6 mg) one in each nostril, and 1.0 mg of IV naloxone at three separate visits. The administration was performed by trained study nurses while subjects were seated in a reclined position. The protocol did not specify the duration of the reclining period, but subjects maintained the sitting the first hour. Spray devices were precisely weighed (ME235P, Sartorius, NY, USA), before and after actuation. When only one puff was delivered to a study subject, a second actuation was performed, weighing was done before and after each of the actuations. The hospital pharmacy at St. Olav's University Hospital delivered Naloxon B. Braun 0.4 mg/ml (Melsungen, Germany) for intravenous administration.

Procedures

Subjects had to abstain from all medications for 7 days before treatment. No fasting or other meal restrictions were required. IV cannulas for sampling were placed in the antecubital fossa, and participants were monitored with oxygen saturation and non-invasive blood pressure for safety. Venous blood samples were taken prior to naloxone administration and at 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240, and 360 min after dose delivery. Six milliliter blood were drawn each time and collected in serum separator clot activator tubes (Vacuette®, Greiner Bio-One, Austria). Samples were centrifuged, and 2 ml serum was frozen in cryotubes at −80 °C until analyzed. Within 4 weeks after the last pharmacokinetic session, there were short follow-up visits.

Naloxone analysis

Naloxone was analyzed by a validated high-performance liquid chromatography-tandem mass spectrometry method with a lower limit of quantitation (LOQ) of 0.02 ng/ml at the Proteomics and Metabolomics Core Facility (PROMEC), NTNU, Norway. The analytical method was validated according to Dadgar et al. [29] and Shah et al. [30]. For details, see supplementary file 1, available online.

Primary and secondary outcome measurements

The primary outcome was the absolute bioavailability of the nasal formulation of naloxone. Secondary aims were to compare time to maximum concentrations (T_{max}), the maximum concentration levels (C_{max}) and safety of the nasal formulation. Time to 50 and 80% of maximum concentration (T_{max50}, T_{max80}) were later calculated.

Statistics

Serum concentration data was analyzed by non-compartmental techniques using Win-Nonlin Standard version 6.4 (Pharsight Corporation, NJ, USA). Area under the curve (AUClast (linear trapezoidal rule)), terminal elimination half-life, C_{max} (maximum serum concentration), and T_{max} (time to maximum serum concentration) were calculated by computerized curve fitting. Dose-corrected AUCs were employed to calculate the absolute bioavailability. Dose-corrected values for AUClast and C_{max} for 0.8 and 1.6 mg IN doses were compared with paired t test. A p value < 0.05 was considered significant. Within- and between-subject variability of bioavailability and C_{max} were examined using mixed models with subject specific random intercepts. Data was described as mean and 95% confidence intervals (95% CI) if not specified otherwise. SPSS version 21 (IBM, NY, USA) was employed for descriptive statistics, while Stata version 14.1 (StataCorp, TX, USA) was used for the mixed models. 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.

Results

Twelve subjects were included in the final analysis. For one of the participants, a complete study session was removed from the analysis due to potential spray device failure (see below).

Two sampling points of a total of 540 samples were excluded, as they deviated more than twice their expected value as described above. Results below the limit of quantitation were also excluded.

Pharmacokinetics

The bioavailability of the present nasal naloxone formulation was 0.54 (0.45–0.63) for the 0.8 mg and 0.52 (0.37–0.67) for the 1.6 mg IN (Table 1 and Fig. 1). The dose-corrected AUClast values were 137 (105–169) and 135 (90.0–180) for 0.8 mg and 1.6 mg IN ($p = 0.892$), respectively.

The mean maximum serum concentrations were 1.45 ng/ml (1.07–1.84) for 0.8 mg and 2.57 ng/ml (1.49–3.66) for 1.6 mg, Table 1. The respective dose-corrected values were 1.72 (1.24–2.19) and 1.61 (0.93–2.29) ($p = 0.674$). Time to maximum concentration was reached at 17.9 min (11.4–24.5) and 18.6 min (14.4–22.9) for the 0.8 mg and the 1.6 mg doses, respectively.

Naloxone was quantified above the LOQ in all but two samples at 2 min, both from the IN 0.8 mg arm. The T_{max50} was 8.34 min (7.62–9.07), and the T_{max80} was 12.1 min (10.9–13.3) for 0.8 mg. For 1.6 mg, T_{max50} was 10.5 min (9.74–11.2); T_{max80} was 16.8 min (15.7–17.9). The mean terminal half-lives varied from 70 to 90 min. The extrapolation from AUClast to AUCinfinity was about 5% of total AUC0-infinity.

The 1.6 mg IN serum concentrations surpassed the IV serum concentrations at 15–20 min (Fig. 2) and stayed above for the rest of the examined period. The 0.8 mg IN serum concentrations never reached the IV serum concentration levels.

Variance

For bioavailability, total variance in the model was 0.047. The within-subject variability component of the variance was 0.012, and the between-subject variability component of the variance was 0.035 (Fig. 3).

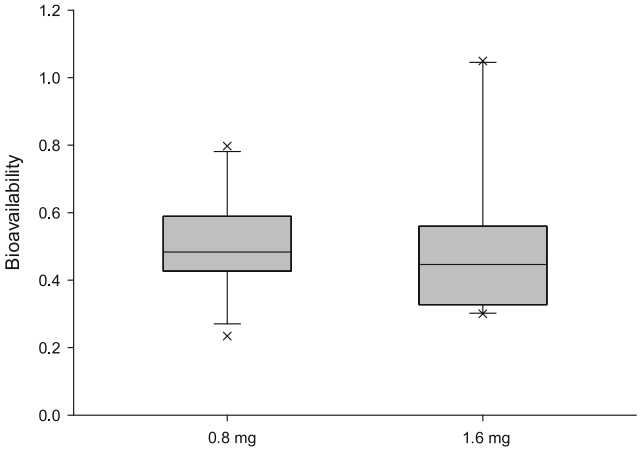

Fig. 1 Absolute bioavailability of two doses nasal naloxone (0.8 and 1.6 mg) compared to 1.0 mg IV. *Horizontal lines* depict median values, *boxes* the 25 and 75 percentiles, *whiskers* the 95% percentiles, and *crosses* the outliers. $n = 12$ (0.8 mg) and $n = 11$ (1.6 mg)

Total variance in the C_{max} model was 0.994. The within-subject variability component of the variance was 0.387, and the between-subject variability component of the variance was 0.607 (Fig.3).

Safety and adverse events

Thirteen subjects were exposed to the test product. All subjects who received at least one dose of the test drug were included in the safety analysis. Subjective complaints or abnormal physical findings were recorded using the NCI Common Terminology Criteria for Adverse Events (CTCAE v 4.0).

There were no serious adverse events. Fifty percent experienced a short-lived mild, although a bitter taste in the pharynx after 1–20 min of nasal administration. Two other incidents are reported, one feeling of numbness in the nose which was resolved spontaneously and one nosebleed. This subject had suffered spontaneous nosebleeds prior to inclusion that he did not divulge to the research team. During the study, he was excluded as he no longer fulfilled the inclusion criteria due to a

Table 1 Pharmacokinetic variables in healthy volunteers after intranasal and intravenous administration of naloxone in an open, randomized three-way crossover trial

Treatment	C_{max} (ng/ml)	T_{max} (min)	T_{max50} (min)	T_{max80} (min)	Terminal half-life (min)	AUClast (min × ng/ml)
0.8 mg intranasal naloxone ($n = 12$)	1.45 (1.07–1.84)	17.9 (11.4–24.5)	8.34 (7.62–9.07)	12.1 (10.9–13.3)	89.7 (76.8–103)	99.0 (76.7–121)
1.6 mg intranasal naloxone ($n = 11$)	2.57 (1.49–3.66)	18.6 (14.4–22.9)	10.5 (9.74–11.2)	16.8 (15.7–17.9)	79.0 (65.3–92.7)	185 (123–248)
1.0 mg intravenous naloxone ($n = 12$)	14.2 (9.13–19.2)[a]	2.25 (1.70–2.80)[a]			70.1 (60.1–78.7)	240 (207–273)

Data are presented as mean values (95% confidence intervals). C_{max} maximum concentration, T_{max} time to maximum concentration, T_{max50} time to 50% of maximum concentration, T_{max80} time to 80% of maximum concentration, AUClast area under the curve until last measurement

[a] For comparison, "C_{max}" and "T_{max}" (concentration at the first sample drawn) are reported for IV

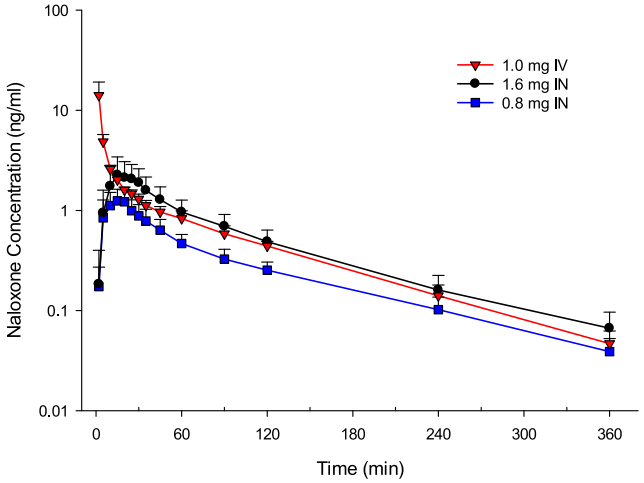

Fig. 2 Time course of serum concentrations (mean (error bars 95% CI)) of naloxone after intravenous (1.0 mg) and intranasal (0.8 and 1.6 mg) administration in healthy human volunteers (n = 12 for IV and IN 0.8 mg, n = 11 for IN 1.6 mg). *Squares* are 0.8 mg IN, *dots* are the 1.6 mg IN and *triangles* are the 1.0 mg IV

nasal cauterization after a spontaneous nosebleed of the contralateral nostril during the trial.

Spray device performance and actual dosing

After the study was completed, concerns regarding the production and performance of the spray devices were raised. There were two different issues: (1) questions regarding the amount of drug filled and (2) questions regarding the assembly of the spray device and possible leakage.

The sprays gave on average (95% CI) 0.0933 ml (0.0889–0.0977) and 0.187 ml (0.182–0.192) for the 0.8 and 1.6 mg dosing, respectively. As almost all devices delivered a lower volume than predicted, the producer FHI controlled the filling volume in additional tests by weighing the containers before and after filling. This test showed that average filling weight volumes were 0.217 ml (range 0.2148–0.2217 ml), 0.013 ml less than specified from the device producer Aptar. Thus, the lower than required filling volume may explain the all over lower than expected spray delivery.

However, one of the sprays deviated significantly from the other 23 sprays by delivering a volume of 0.162 ml, far less than the anticipated 0.200 ml. The low performance of this spray may indicate a leakage problem, and the session in which it was involved was excluded from the pharmacokinetic

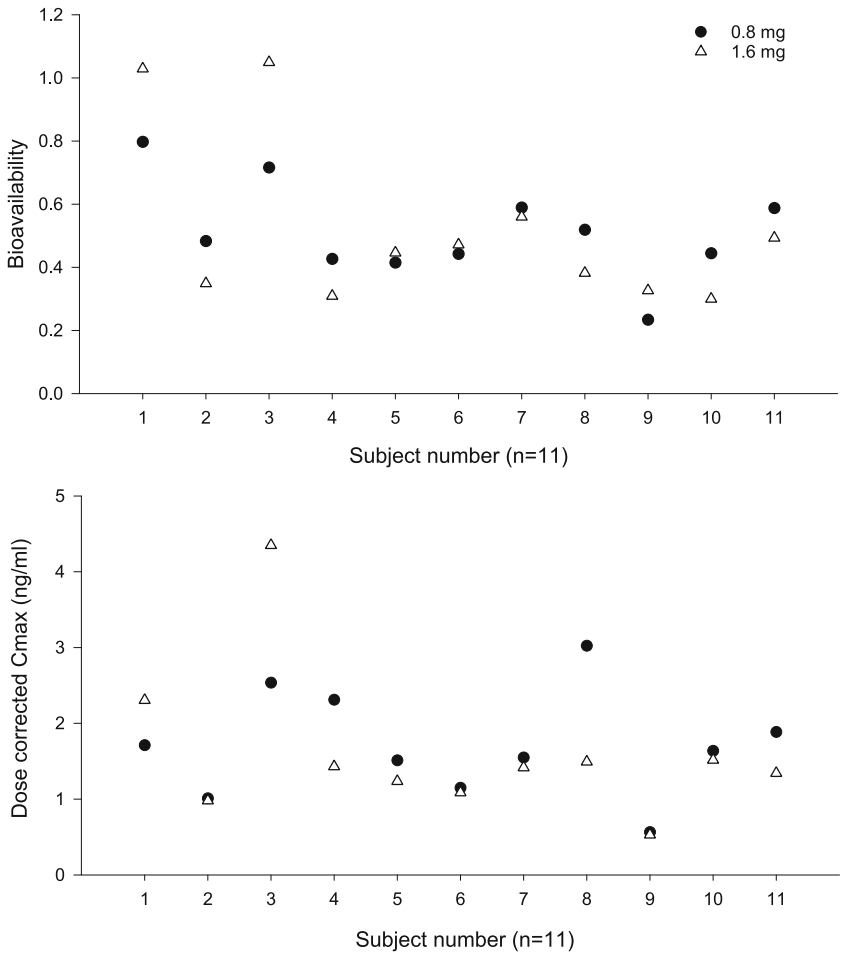

Fig. 3 Between- and within-subject variability in healthy human volunteers (n = 11) for bioavailability and dose-corrected C_{max} of nasal naloxone. *Dots* are measurements for 0.8 mg, *triangles* for 1.6 mg

analysis. It had no significant impact on mean bioavailabilities (0.54 and 0.51 for 0.8 mg with and without subject 2, compared to 0.52 for 1.6 mg).

Discussion

The 8-mg/ml nasal formulation was made to provide systemic exposure that falls within a recommended dosing range of 0.4–2.0 mg for intravenous naloxone. The major findings were that the nasal administration of the formulation resulted in a rapid systemic uptake and a higher absolute bioavailability than previously shown [15, 21, 31], thereby providing a systemic, therapeutic dose of naloxone. Serum concentrations after the nasal 1.6-mg dose surpassed those of 1 mg IV after 15–20 min and stayed above for the rest of the study period. The 0.8-mg nasal dose never reached IV concentrations.

The doses were chosen as we from the unpublished data expected the nasal spray to have a bioavailability of about 0.50, and the recommended initial dose is 0.4–2.0 mg naloxone, aiming at the lower end of this scale as 0.4 and 0.8 mg is the common initial doses in the Norwegian prehospital care. The intravenous dose was chosen arbitrarily within this dose range. Others have chosen to justify their choice of nasal dosing with 2 mg IM as comparison, aiming at the higher end of the initial dosing recommendation [24].

The primary outcome measure was absolute bioavailability of a high-concentration/low-volume naloxone nasal spray that has not been reported previously in peer-reviewed literature. The observed bioavailabilities of 0.52 and 0.54 are somewhat lower than for other nasal formulations such as fentanyl, methadone, and midazolam [16–18]. Regardless, the bioavailability of the present formulation is far higher than that of only 0.04 previously reported by Dowling et al. [21] using 0.4 mg/ml naloxone concentration. The major shortcoming of this study [21] was that high volumes were given, up to 2.5 ml in each nostril, which is more than 15 times the recommended maximum amount for nasal administrations. Although not directly comparable, our absolute bioavailability compares well with the relative bioavailability to IM of 0.47 in the Patient Information Leaflet of the recently FDA-approved spray in 4 mg/0.1 ml [15]. However, the exact bioavailability is not important as long as the nasal spray can deliver a systemic, therapeutic dose in one actuation as shown in the present study.

An important aspect of the knowledgebase for all drugs for human use, particularly in emergency medicine, is variability between individuals and variability between different doses in the same individual. Differences in the characteristics of the individual nose such as blood flow, mucociliary clearance, and anatomy [32] probably contributes to the between-subject variability. For nasal bioavailability, 74.5% of the variance may be explained by between-subject variability. For C_{max}, the same figure was 61% (Fig. 3). Thus, it seems that the variability mainly comes from differences between the individuals rather than from factors within the same individual. A low within-subject variability may indicate that the results are reliable, as subjects have acted as their own controls.

When treating opioid overdose, one wish to avoid the precipitation of withdrawal symptoms and at the same time be sure to have a sufficient duration to prevent recurrence of the overdose as the naloxone concentration is declining [5]. As the type and amount of opioids often are unknown in the emergency setting, this is difficult. Many places in the world see increasing overdoses from very potent or long lasting opioids, such as fentanyl or methadone [12, 33]. Knowledge of the patterns of local opioid use must be reflected in local naloxone-dosing guidelines. Intravenous administration of drugs is characterized by immediate high serum concentrations compared to all other administration forms. Precipitation of acute withdrawal symptoms and agitation after overdose reversal is related to rapidly rising, high naloxone concentrations [5], as seen for IV in Fig.2. This is one of the reasons why current clinical practice has moved from IV to IM naloxone—to prevent withdrawal symptoms [5, 34]. The C_{max} found after 0.8 mg IN in this study was higher than the C_{max} (1.1–1.2 ng/ml) reported after 0.4 mg naloxone IM in the study of Evzio®(naloxone hydrochloride) IM auto injector (Kaléo Pharma, VA, USA) [35]. Narcan® nasal spray, achieves a C_{max} of 4.8 ng/ml after a single 4 mg naloxone IN dose and 9.7 ng/ml after 2 × 4 mg [15]. Our formulation reaches C_{max} values between these naloxone products. Our 1.6-mg dosing reaches twice as that of Evzio® C_{max}, and about half that of the 4-mg dose of Narcan® nasal spray. The application of two doses of Narcan® nasal spray (2 × 4 mg) has a C_{max} close to our IV 1 mg at 2-min sampling point, which is a higher dose than commonly applied as initial dose in clinical practice, at least in Norway. In relation to IV naloxone, our 1.6-mg dose provided lower C_{max}, than the IV 2-min sample, but maintained higher concentration after 15–20 min (Fig.2). This may indicate a lower risk for precipitation of withdrawal symptoms combined with a possibly longer duration of action for IN naloxone compared to IV naloxone. Compared to the Narcan® nasal spray, the doses are substantially lower. This allows for the titration to clinical response that is highly recommended. This could maximize the effect and minimize the occurrence of withdrawal reactions, and still being within the FDA requirement as it produces serum concentration comparable with those after 0.4 mg IM which for most patients will give a sufficient reversal [5].

In an overdose situation, the time for naloxone to reach and build up in the blood is important to reverse the respiratory depression. Our solution has a T_{max} of 18 min for both 0.8 and 1.6 mg (Table 1). This is similar to the T_{max} of 15–20 min reported for IM naloxone and to the T_{max} of 0.50 h (30 min) and 0.33 h (20 min) reported for the Narcan® nasal spray [15,

35]. Furthermore, naloxone was quantifiable in all but two samples taken 2 min after drug administration and T_{max50} and T_{max80} were about 8 and 12 min after the 0.8-mg single dose administration, respectively. The clinical effect is therefore expected to precede the T_{max}.

The secondary outcome in this study, the terminal half-life of naloxone, compares well with previous reports [4, 35], indicating external validity of our study. The extrapolation from AUClast to AUCinfinity (fig not shown) was only about 5% indicating that our sampling schedule covers about 95% of the serum concentration curve.

Instead of including an IM arm, two doses of nasal naloxone were studied. Due to the crossover design, information regarding the relationship between within- and between-subject variability could be presented as discussed above. Moreover, it provided a reasonable indication of a dose-concentration relationship, as both AUClast and C_{max} were almost twice as high for 1.6 mg versus 0.8 mg doses as the dose-corrected figures were similar for both.

No clinically significant adverse event was observed; however, the taste of the nasal spray was commonly reported. No definitive conclusions regarding the safety of the spray can be drawn from the 12 subjects, but it seems to be well tolerated.

Our study is limited by its relatively small sample size. The volunteers included were healthy and with no concomitant medications or drugs, thus not representative of the patient population who usually receives naloxone, reducing external validity in this regard. Intravenous naloxone 1.0 mg is higher than the usual first dose for overdose reversal in Norway, but in between the two doses studied. A comparison with intramuscular naloxone in clinically relevant doses would have been of significant interest.

Conclusion

Our spray formulation resulted in a rapid systemic uptake of naloxone, with higher bioavailability than previously reported [21]. This indicates that an optimized high-concentration/low-volume nasal spray formulation of naloxone can deliver a therapeutic dose. The 1.6-mg nasal dose provided serum naloxone concentrations that surpassed those of 1.0 mg IV after 15–20 min and stayed above for the rest of the study period. This serum concentration time course may indicate a lower risk for precipitation of withdrawal symptoms combined with a possibly longer duration of action for IN naloxone compared to that for IV naloxone. The study did not elicit data of worrying side effects in the exposed subjects. All over, the results are promising and further development of the product is warranted. The relative bioavailability of nasal to intramuscular naloxone for this formulation needs to be determined. Moreover, pharmacodynamic outcomes such as pupillary size, analgesia or respiratory rate of nasal, and injected naloxone should be compared. Finally, a clinical trial comparing low-volume/high-concentration nasal formulations and standard treatment is needed.

Acknowledgements Thanks to the Unit for Applied Clinical Research, NTNU, for the assistance with GCP monitoring and providing the internet-based randomization, to the Clinical Research Facility, St. Olav's University Hospital, for conducting the study, and to the Proteomics and Metabolomics Core Facility, PROMEC, NTNU, for conducting the naloxone analysis. These infrastructures are all funded by the Faculty of Medicine, NTNU and the Central Norway Regional Health Authority. We also wish to acknowledge professor Thorsteinn Loftsson, University of Iceland, Reykjavík, Iceland for his valuable advises regarding the formulation. Thanks also to Richard Poulsson, Azanta, Denmark for valuable guidance and genuine interest in the project, and to Inge Christoffer Olsen at ICOStatistics for his aid with the statistical modeling. This study was funded by grants from the Laerdal Foundation for Acute Medicine, Unimed Innovation/St. Olav's University Hospital and Felles Forskningsutvalg, NTNU/St. Olav's University Hospital, Norway.

Compliance with ethical standards

Ethical approval and informed consent All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Helsinki declaration, the principles of the International Conference on Harmonisation, and Good Clinical Practice guidelines. It was approved by the Regional Committee of Medical and Health Research Ethics (2013/1519/REK sør-øst A) and the Norwegian Medicines Agency (EudraCT number: 2013–000050-22) and registered in clinicaltrials.gov (NCT02158117). Informed written consent was obtained from all subjects prior to inclusion. Participants were insured trough the Drug Liability Association, Norway, and compensated for each treatment visit with 1500 NOK (160 Euro/ 175 USD).

Declarations of interest Ola Dale's (OD) employer Norwegian University of Science and Technology (NTNU) have recently signed cooperation and licensing contracts with Den norske Eterfabrikk (DnE) to seek commercialization of the nasal naloxone formulation developed by OD. The latter regulates potential royalties for OD through NTNU. OD is engaged by DnE as Principle Investigator in a pharmacokinetic study of naloxone for which OD receives no personal honorarium. DnE has compensated OD for one travel from Trondheim to Oslo.

Arne Kristian Skulberg (AKS) has signed a non-compete contract with DnE lasting the duration of his PhD program at NTNU (estimated 2018). This does not limit AKS right to publish results. AKS will receive no financial benefit from the license agreement between DnE and NTNU. Other authors declare that they have no conflicts of interest.

References

1. Degenhardt L, Hall W (2012) Extent of illicit drug use and dependence, and their contribution to the global burden of disease. Lancet 379(9810):55–70. doi:10.1016/s0140-6736(11)61138-0
2. Darke S, Mattick RP, Degenhardt L (2003) The ratio of non-fatal to fatal heroin overdose. Addiction 98(8):1169–1171
3. Boyer EW (2012) Drug therapy: management of opioid analgesic overdose. N Engl J Med 367(2):146–155

4. Health Products Regulatory Authority Naloxone 400 micrograms/ml solution for injection/infusion - Summary of Product Characteristics. http://www.webcitation.org/6g4edopWl Accessed 17/03/2016 2016
5. Clarke SF, Dargan PI, Jones AL (2005) Naloxone in opioid poisoning: walking the tightrope. Emerg Med J 22(9):612–616. doi:10.1136/emj.2003.009613
6. Buajordet I, Naess AC, Jacobsen D, Brors O (2004) Adverse events after naloxone treatment of episodes of suspected acute opioid overdose. Eur J Emerg Med 11(1):19–23
7. Kerr D, Kelly AM, Dietze P, Jolley D, Barger B (2009) Randomized controlled trial comparing the effectiveness and safety of intranasal and intramuscular naloxone for the treatment of suspected heroin overdose. Addiction 104(12):2067–2074. doi:10.1111/j.1360-0443.2009.02724.x
8. Kelly AM, Kerr D, Dietze P, Patrick I, Walker T, Koutsogiannis Z (2005) Randomised trial of intranasal versus intramuscular naloxone in prehospital treatment for suspected opioid overdose. Med J Aust 182(1):24–27
9. McGregor C, Darke S, Ali R, Christie P (1998) Experience of non-fatal overdose among heroin users in Adelaide, Australia: circumstances and risk perceptions. Addiction 93(5):701–711
10. World Health Organization (2014) Community management of opioid overdose, WHO guidelines approved by the guidelines review committee. World Health Organization, Geneva
11. McDermott C, Collins NC (2012) Prehospital medication administration: a randomised study comparing intranasal and intravenous routes. Emerg Med Int 2012:476161. doi:10.1155/2012/476161
12. Rudd RA, Aleshire N, Zibbell JE, Gladden M (2016) Increases in drug and opioid overdose deaths—United States, 2000–2014. Morbidity and Mortality Weekly Report (MMWR) 64(50–51):1378–1382
13. Hussain A, Kimura R, Huang CH, Kashihara T (1984) Nasal absorption of naloxone and buprenorphine in rats. Int J Pharm 21(2):233–237
14. Ngai SH, Berkowitz BA, Yang JC, Hempstead J, Spector S (1976) Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action. Anesthesiology 44(5):398–401
15. Adapt Pharma (2015) NARCAN® (naloxone hydrochloride) nasal spray - Patient Information Leaflet. http://www.webcitation.org/6g1Upt7eu. Accessed 15 March 2016
16. Dale O, Hoffer C, Sheffels P, Kharasch ED (2002) Disposition of nasal, intravenous, and oral methadone in healthy volunteers. Clin Pharmacol Ther 72(5):536–545. doi:10.1067/mcp.2002.128386
17. Foster D, Upton R, Christrup L, Popper L (2008) Pharmacokinetics and pharmacodynamics of intranasal versus intravenous fentanyl in patients with pain after oral surgery. Ann Pharmacother 42(10):1380–1387. doi:10.1345/aph.1L168
18. Dale O, Nilsen T, Loftsson T, Hjorth Tonnesen H, Klepstad P, Kaasa S, Holand T, Djupesland PG (2006) Intranasal midazolam: a comparison of two delivery devices in human volunteers. J Pharm Pharmacol 58(10):1311–1318. doi:10.1211/jpp.58.10.0003
19. Dale O, Hjortkjaer R, Kharasch ED (2002) Nasal administration of opioids for pain management in adults. Acta Anaesthesiol Scand 46(7):759–770
20. Costantino HR, Illum L, Brandt G, Johnson PH, Quay SC (2007) Intranasal delivery: physicochemical and therapeutic aspects. Int J Pharm 337(1–2):1–24. doi:10.1016/j.ijpharm.2007.03.025
21. Dowling J, Isbister GK, Kirkpatrick CM, Naidoo D, Graudins A (2008) Population pharmacokinetics of intravenous, intramuscular, and intranasal naloxone in human volunteers. Ther Drug Monit 30(4):490–496. doi:10.1097/FTD.0b013e3181816214
22. Hertz S (2012) Naloxone for outpatient use: data required to support an NDA. U.S. Food and Drug Administration. http://www.webcitation.org/6kK5i0JH1 Accessed September 6th 2016
23. U.S. Food and Drug Administration (2015) FDA moves quickly to approve easy-to-use nasal spray to treat opioid overdose. http://www.webcitation.org/6g1W28jYq Accessed 15/03/2016 2016
24. Krieter P, Chiang N, Gyaw S, Skolnick P, Crystal R, Keegan F, Aker J, Beck M, Harris J (2016) Pharmacokinetic properties and human use characteristics of an FDA approved intranasal naloxone product for the treatment of opioid overdose. J Clin Pharmacol. doi:10.1002/jcph.759
25. Kerr D, Dietze P, Kelly AM (2008) Intranasal naloxone for the treatment of suspected heroin overdose. Addiction 103(3):379–386. doi:10.1111/j.1360-0443.2007.02097.x
26. Doe-Simkins M, Walley AY, Epstein A, Moyer P (2009) Saved by the nose: bystander-administered intranasal naloxone hydrochloride for opioid overdose. Am J Public Health 99(5):788–791. doi:10.2105/ajph.2008.146647
27. Barton ED, Colwell CB, Wolfe T, Fosnocht D, Gravitz C, Bryan T, Dunn W, Benson J, Bailey J (2005) Efficacy of intranasal naloxone as a needleless alternative for treatment of opioid overdose in the prehospital setting. J Emerg Med 29(3):265–271. doi:10.1016/j.jemermed.2005.03.007
28. Strang J, McDonald R, Tas B, Day E (2016) Clinical provision of improvised nasal naloxone without experimental testing and without regulatory approval: imaginative shortcut or dangerous bypass of essential safety procedures? Addiction. doi:10.1111/add.13209
29. Dadgar D, Burnett PE, Choc MG, Gallicano K, Hooper JW (1995) Application issues in bioanalytical method validation, sample analysis and data reporting. J Pharm Biomed Anal 13(2):89–97
30. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layloff T, Viswanathan CT, Cook CE, McDowall RD et al (1991) Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet 16(4):249–255
31. Middleton LS, Nuzzo PA, Lofwall MR, Moody DE, Walsh SL (2011) The pharmacodynamic and pharmacokinetic profile of intranasal crushed buprenorphine and buprenorphine/naloxone tablets in opioid abusers. Addiction 106(8):1460–1473. doi:10.1111/j.1360-0443.2011.03424.x
32. Pires A, Fortuna A, Alves G, Falcao A (2009) Intranasal drug delivery: how, why and what for? J Pharm Pharm Sci 12(3):288–311
33. Zuckerman M, Weisberg SN, Boyer EW (2014) Pitfalls of intranasal naloxone. Prehosp Emerg Care 18(4):550–554. doi:10.3109/10903127.2014.896961
34. Robertson TM, Hendey GW, Stroh G, Shalit M (2009) Intranasal naloxone is a viable alternative to intravenous naloxone for prehospital narcotic overdose. Prehosp Emerg Care 13(4):512–515. doi:10.1080/10903120903144866
35. Kaléo Pharma (2014) Evzio prescribing information. http://www.webcitation.org/6g1VdDwWl. Accessed 15 March 2016