Ida Tylleskär

Nasal naloxone

A pilot study of the pharmacokinetics of a concentrated formulation

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Last but not least, I would like to thank the participants in the study, without their willingness to participate, the study could never have been conducted.

DECLARATION OF INTERESTS

Norwegian University of Science and Technology (NTNU) have signed a cooperation and licensing contracts with Den norske Eterfabrikk (DnE) to seek commercialisation of the nasal naloxone formulation. These agreements do not limit NTNUs right to publish results.

The nasal naloxone formulation was developed by my supervisor, Ola Dale (OD). The licensing contracts between DnE and NTNU regulates potential royalties for OD through NTNU. OD have also been engaged by DnE as Principle Investigator in a pharmacokinetic study of naloxone for which he received no personal honorarium. DnE has compensated OD for two travels from Trondheim to Oslo for meetings regarding the project.

I have no interests to declare. I am involved in this project as a medical student at NTNU, and will receive no financial benefit from the license agreement.

SUMMARY

Introduction: Naloxone is the antidote against heroin and other opioids. As a measure to combat overdose deaths, nasal naloxone is wanted for bystander administration by lay people. The objective of this study was to investigate the pharmacokinetic profile of nasal naloxone delivered as in a high-concentration/low-volume formulation. The primary objective was to get a preliminary estimation of bioavailability of intranasal naloxone in human, healthy volunteers. Secondary objectives were a preliminary estimation of maximum serum concentration ($C_{max)}$ and time to maximum serum concentration ($T_{max)}$, and also to investigate the safety of the formulation.

Materials and methods: This was a phase 1, single centre, open-label, randomised, two-way crossover trial in healthy male volunteers, n=5, age 18-45 years. 1.0 mg intravenous naloxone was compared to 2 mg intranasal naloxone given as 0.1 ml of 20 mg/ml nasal spray. Blood samples were drawn at predetermined intervals, and serum concentrations of naloxone was determined by a validated liquid chromatography-mass spectrometry method, and analysed by non-compartmental techniques. A 72-hour washout period was enforced between treatments. A post-study interview was performed.

Results: Bioavailability (mean (95% confidence interval) were 47.1% (38.4-55.8) for the intranasal naloxone. C_{max} were 4.24 (1.48-7.00). T_{max} was reached after 16.0 min (5.80-26.2). The mean half-lives varied from 80-90 min. No clinically significant adverse event was observed. Moreover, the spray provoked no unexpected adverse events. The only reported adverse drug reaction was taste of the nasal spray.

Conclusions: The nasal sprayer resulted in a rapid systemic uptake, and a higher bioavailability than previously reported for low-concentration/high-volume formulations. The nasal spray provided serum concentration that surpassed the intravenous after 10 min, and stayed above until 240 min. The spray did not elicit worrying side effects in the exposed subjects. The results are promising and further development of the product is warranted. Based on these results we have chosen to study 8 and 16 mg/ml in the next study. Further trials comparing the nasal spray with clinically relevant doses of intramuscular naloxone, and studies investigating the pharmacodynamic properties of the product, is also needed.

LIST OF ABBREVIATIONS

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
AUC/ AUClast	Area under the curve/AUC until last sample
AUC∞ /AUCinfinity	Area under the curve extrapolated to infinity
BMI	Body mass index
CI	95% confidence interval
Cmax	Maximum serum concentration
CRF	Case Report Form
CV	Coefficient of variation
DnE	Den norske Eterfabrikk
ECG	Electrocardiography
FDA	US Food and Drug Administration
FHI	Norwegian Institute of Public Health (Folkehelseinstituttet)
GCP	Good Clinical Practice
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
IM	Intramuscular
IN	Intranasal
IMP	Investigational Medicinal Product
IV	Intravenous
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
LOQ	Limit of quantitation
NTNU	Norwegian University of Science and Technology
PK	Pharmacokinetic
QC	Quality control
SPC	Summary of Product Characteristics
THN	Take Home Naloxone

Tmax	Time to maximum serum concentration
Tmax50	Time to 50% of maximum serum concentration
Tmax80	Time to 80% of maximum serum concentration
WHO	World Health Organisation

TABLE OF CONTENTS

INTRODUCTION

BACKGROUND AND RATIONALE

Overdose from illicit opioids with a potential terminal outcome is a serious problem among opioid abusers worldwide. Those who inject heroin or other opioids are considered to have the highest risk for death from overdose.

Opioids cause respiratory depression, which may progress to cardiac arrest and death. To save lives, immediate treatment with a μ-opioid antidote such as naloxone is required. The antidote reverses the life threatening respiratory depression within minutes. Usually intravenous (IV) or intramuscular (IM) administration is employed, the former requires considerable skill, and the latter have a slower onset of action. The usual procedure in Norwegian emergency medicine is to administer 0.4-0.8 mg IM, and thereafter 0.4 mg IV, the IV dose for rapid onset and the IM for longer duration (1, 2). This is important, as the duration of action of the intoxicating agent is usually longer than for the antidote.

Nasal (IN) naloxone has been suggested as an alternative for emergency teams and possibly also by bystanders (3-5). There has been a growing interest for take home naloxone (THN) among politicians, medical staff, and caretakers around the world, and a needle-free naloxone alternative would be favourable. It has been shown that bystanders administering IN naloxone to overdose victims may save lives (5). However, the place IN naloxone in this setting is not established (4, 6). For medical personnel this eliminates the risk of needle stick injuries and blood exposure from a risk population. Moreover, cannulation of the needle abusers may be very challenging (7) and nasal administration might shorten time to treatment. As a response to the overdose epidemic in the United States, the National Institute of Drug Abuse initiated development of adequate naloxone nasal sprayers through American pharmaceutical companies and the US Food and Drug Administration (FDA) granted fast track applications to speed up this development.

At the time when this study was conducted, all previous published studies of IN naloxone for treatment of opioid overdose had used inappropriate formulations with a low concentration and a high volume (4, 5). In short, they could not deliver a therapeutic dose in a recommended volume. Naloxone had to be given in volumes up five to 25 times larger than the recommended maximum volume of 0.1-0.2 ml for IN administrations (8, 9). Therefore,

important information regarding the pharmacokinetics of IN administered naloxone was scarce; one study indicated a bioavailability as low as of 4% (10). This in contrast to a nasal bioavailability of more than 65 % for proper formulations of other drugs (11-13). However, in a study of 2 mg naloxone administered nasally as powder, the bioavailability was 30 % with a maximum serum concentration (C_{max}) and a time to maximum serum concentration (T_{max}) of 1.6 ng/ml and 20 min, respectively (14). This showed that when the issue of volume was circumvented by delivering naloxone as a powder, a higher bioavailability was achieved. Thus a well formulated naloxone high-concentration/low-volume spray, might be able to deliver a therapeutic dose of naloxone through the nose.

There were several publications on prehospital use of nasal naloxone. However, the systematic review of Ashton et al (7) concluded that the evidence was weak and that there are conflicting results regarding the efficacy of IN naloxone. Kerr et al (4) in her systematic reviews agreed, but called for comparative studies evaluating alternative doses, drug formulations and delivery devices. Kerr et al followed up on this and published an open randomised controlled study comparing 1mg IN and 1 mg IM naloxone for suspected heroin overdose in which 172 patients were included (15). They concluded that the IN compared well with the IM route. In that trial 18 % of the patients receiving intranasal naloxone received rescue naloxone compared to 4.5% after intramuscular administration. It was speculated if this could be due to the non-blinded design of the study, but there is also a possibility that the nasal naloxone had less effect due to the high volume it was administrated in. Despite the lack of evidence 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 atomiser.

After the completion of this study, several pertinent research reports have been published on this issue. In 2014 World Health Organisation (WHO) published a report on community treatment of opioid overdose concluded that there were few well conducted studies. WHO gave a conditional recommendation to the use of nasal naloxone in THN-programs, but pointed out that these did not use licenced products. Moreover, they stated that questions remain about the optimal dosing and formulation for the intranasal route of administration (16). There has been reports of improvised nasal sprays failing to for instance adequately reverse a case of fentanyl overdose (17). It was speculated whether this could be due to the potency and long duration of action of fentanyl (8), but a recent article suggest that the

bioavailability of the nasal spray used was only 11% (18), and the amount of drug reaching the systemic circulation may therefore be far below he recommended minimum dose of naloxone in opioid overdose.

In 2014 the naloxone auto-injector Evzio® (Kaléo Pharma, VA, USA) for THN application was approved for the US market (19). FDA requires that a nasal naloxone should at least generate serum concentration comparable with those of IV, IM, or subcutaneous naloxone administration (20). 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 47% (21-23). Unfortunately, its absolute bioavailability was not reported. The concept of high concentration/low volume nasal naloxone sprayer has also been proven in another study, which showed that nasal naloxone had an absolute bioavailability of 25-28%, and they concluded that it had an absorption time-course that made it suitable for emergency treatment (24).

At the time when the present study was conducted the reviews of the evidence of intranasal naloxone concluded that IN naloxone could be useful, but there was currently insufficient evidence to fully support IN naloxone as the first line treatment by paramedics or for community management of opioid overdose (7, 16). At the present, even though an FDA-approved nasal spray is now available on the US market, there is still a need for research on the disposition of nasal naloxone formulations, optimal dosing, and the clinical efficacy of these sprays (4, 16, 25).

In 2013 we conducted this pilot study of a new, nasal naloxone spray to explore the bioavailability in a high-concentration/low-volume formulation. The present Investigational Medicinal Product (IMP) had a concentration of 20 mg/ml. This was a much higher concentration than studied before, and allowed for delivery of adequate dosing in a small volume (0.1 ml). The Aptar bidose disposable nasal sprayers were used to administer the IMP. The formulation contained well-known excipients, among them absorption enhancers and antimicrobial agents. To identify the bioavailability is paramount to continue the development of the drug, since accurate dosing relies on knowledge of such information.

AIMS AND STUDY OBJECTIVES

The primary objective of this study was to give a preliminary estimation of absolute bioavailability of this nasal formulation of naloxone in healthy human volunteers. The data from this study were later used to select the doses to be tested in a subsequent study where the pharmacokinetic parameters of this formulation were finally estimated.

Secondary aims were to compare time to maximum serum concentration (T_{max}) and the maximum serum concentration (C_{max}). Additional objectives were to evaluate if the subjects experienced discomfort from the nasal spray, and other observations regarding the safety of the nasal formulation.

MATERIALS AND METHODS

ETHICS

The study was conducted according to the principles of the Declaration of Helsinki, and was consistent with International Conference on Harmonisation-Good Clinical Practise (ICH-GCP) guidelines. This study was approved by the Regional Committee of Medical and Health Research Ethics, region South East-C (ref no. 2012/1970) and by the Norwegian Medicines Agency (EudraCT number: 2012-004989-18) before inclusion of participants. It was also recorded in clinicaltrials.gov with the identifier NCT01939444.

The Regional Committee of Medical and Health Research Ethics approved the participant information and the informed consent form along with the approval of the study. Participants were given both written and verbal information about the nature, purpose, possible risk and benefit of the study before they consented to participation. They were informed about the strict confidentiality of their participant data, and that we did not access their medical records at the hospital. It was emphasised that the participation was voluntary and that they without consequence might terminate their study participation at any time.

Documented informed consent was obtained for all participants included in the study before they were screened for inclusion. Potential participants received a copy of the written information. Those who were interested were called in to a meeting with me and my supervisor where I explained each section of the letter and the participant could ask questions freely. The process took about 20 min. Those wanting to participate then signed the consent form. A copy of the study information and the consent were also given to the participants.

Registration and storage of participant data were carried out in accordance with national legislation and regulations on medical research and privacy issues. Participant's medical records was not accessed. The subjects were identified by participant number and initials used in the Case Report Forms (CRF) and all other documents. The identifier was kept in a safe, and only study personnel have access. The identifier list included full names, social security numbers, and last known phone numbers and addresses. The participant compensation were 1500 Norwegian kroner (170 EUR /180 USD) for each visit. The participants were insured through the Drug Liability Association, Norway, during the trial.

STUDY DESIGN AND SETTING

This was a phase 1, open label, randomised, two-way crossover study of a new formulation of nasal naloxone was compared to intravenous naloxone administration in 5 healthy volunteers. The study was open label (no blinding) and treatment orders were decided by concealed randomisation. The nasal dose was 2.0 mg and the IV comparator dose was 1.0 mg naloxone. All subjects received both treatments. Subjects therefore acted as their own control. Each study session lasted for 6-7 hours, and the sessions were separated by at least 72 hours wash-out period. The study was conducted at the Clinical Research Facility, St. Olavs Hospital, Trondheim University Hospital, Norway, during August - October 2013.

SAMPLE SIZE

The purpose of this study was to provide estimations of the bioavailability of the present formulation of nasal naloxone. A formal sample size estimation was not conducted as there was no previous knowledge about the pharmacokinetics of a 20mg/ml naloxone formulation given in 0.1 ml. Five subjects were chosen as this would provide the data required for this preliminary estimation of bioavailability aiming to determine which concentrations (4, 8, 16 or 20 mg/ml) to proceed with. If this nasal spray showed any potential for delivering a therapeutic dose, it would be followed up by a more extensive study for a final determination the bioavailability of the formulation.

PARTICIPANTS

Initial phase 1 pharmacokinetic studies are usually conducted in a healthy population for obvious reasons such as normal physiology and absence of potential interacting medications.

INCLUSION CRITERIA

Healthy male subjects aged 18-45 years were eligible for inclusion. All of the following conditions must apply to the prospective study subject at:

- Healthy
- Normal ECG (electrocardiography)
- Laboratory values within the reference values for the following*:
  - Haemoglobin: (male: 13.4 – 17.0 g/dl)
  - Creatinine: (male: 60 – 105 micromol/l)

- Aspartate aminotransferase (ASAT): (male: 15 – 45 U/l)
- Alanine aminotransferase (ALAT): (male: 10 – 70 U/l)
- Gamma GT: (male: 10 – 80 U/l)

*Laboratory reference values at St. Olavs Hospital, for the relevant hematological and biochemical tests for inclusion 18- 40 years of age. If any subject between the ages 40 and 45 were included the laboratory at St. Olavs Hospital, would be consulted for any differences in reference values for our tests.

- Signed informed consent and expected cooperation of the subjects for the treatment and follow-up had to be obtained and documented according to ICH-GCP, and national/local regulations.

No information regarding gender aspects can be expected in this study with a small study sample (n=5), and there is no evidence suggesting that there is a sex difference in the pharmacokinetics of naloxone. For these reasons, and because of the risk of pregnancy in fertile females, it was decided to have males only in this first trial.

EXCLUSION CRITERIA

Participants were excluded from the study if they met any of the following criteria:

- Taking any medications including herbal medicines the last week prior to first treatment visit
- Having any local nasal disease or nasal surgery or recent cold for the last week, (if applicable)
- History of drug abuse
- History of prior drug allergy
- Any reason why, in the opinion of the investigator, the patient should not participate.

Individuals who had undergone nasal surgery or had a nasal disease might have a different nasal absorption and was therefore excluded in this initial study. Concerning any medications taken last week prior to study days, this was not allowed as they may interact with naloxone absorption, distribution and elimination, or the analysis of naloxone. This is standard procedure in phase I trials. Safety concerns were the reason for the exclusion criteria applying to subjects having a history or either drug abuse or drug allergy.

FORMULATION AND PRODUCTION

The solution was formulated for intranasal delivery using naloxone hydrochloride dihydrate ($C_{19}H_{21}NO_4 \cdot HCl \cdot 2H_2O$, CAS number: 51481-60-8). The naloxone concentration was 20 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. They deliver 0.1 ml of liquid per actuation. CEO Richard Poulsson, Azanta, Denmark provided valuable guidance and aided us with establishing contact with Aptar. The formulation was produced and the device assembled by Department of Biopharmaceutical Production, Norwegian Institute of Public Health (FHI), Oslo, Norway. The production complied with Good Manufacturing Practice. All the participants received nasal naloxone from the same batch.

Picture 1. The Aptar bidose nasal spray

The Aptar Bidose was used for delivering the nasal spray.

The formulation was developed as contractual work by Phatsawee Jansook, Pharm D, PhD, University of Bangkok under guidance of professor of formulation pharmacy Thorsteinn Loftsson, Pharm D, PhD, University of Iceland, under whom Jansook previously had served as a post doc.

DRUG DOSES AND ADMINISTRATION

All subjects received both treatments with a minimum three-day washout period between treatments. The order of treatments was randomised, and the subjects received 2.0 mg IN naloxone, and 1.0 mg of IV naloxone at two separate visits. The nasal spray had a naloxone concentration of 20 mg/ml and was given in 0.1ml, a total dose of 2.0 mg.

The administration was performed by trained study nurses while subjects were seated in the recline position. The protocol did not specify the duration of the reclining period, but subjects maintained the sitting the first hour. Spray devices were weighed (ME235P, Sartorius, NY, USA), before and after actuation to determine the actual dose given. All treatment (single dosing at each session) of subjects was conducted in the Clinical Research Facility under supervision of trained study personnel. Compliance was therefore complete.

Comparator was produced by B. Braun Melsungen AG, Melsungen, Germany. All the participants received test drugs from the same batch. The ampoules were labelled and delivered by Sykehusapoteket (Hospital Pharmacy), St. Olavs Hospital, Trondheim University Hospital, Norway.

Table 1. Treatments studied

Treatment	Formulation	Administration route	Concentration	Volume	Dose
IV 1.0	Naloxon B. Braun	Intravenous	0.4 mg/ml	2.5 ml	1.0 mg
IN 2.0	IMP	Intranasal	20.0 mg/ml	0.1 ml	2.0 mg

SELECTION OF DOSES IN THE STUDY

The different ambulance services in Norway have different treatment guidelines for administration of naloxone in opioid overdoes, but the usual procedure is to administer 0.4-0.8 mg IM, and thereafter an IV dose of 0.4 mg. Dose may be repeated until satisfactory

effect up to a maximum dose of 2 mg naloxone (1, 2). The maximum dose allowed in the Summary of Product Characteristics (SPC) is 10 mg (26). The IV dose is administered to achieve rapid onset of action, the IM for possibly longer duration. This is important, as the duration of action of the intoxicating opioid is usually longer than for the antidote.

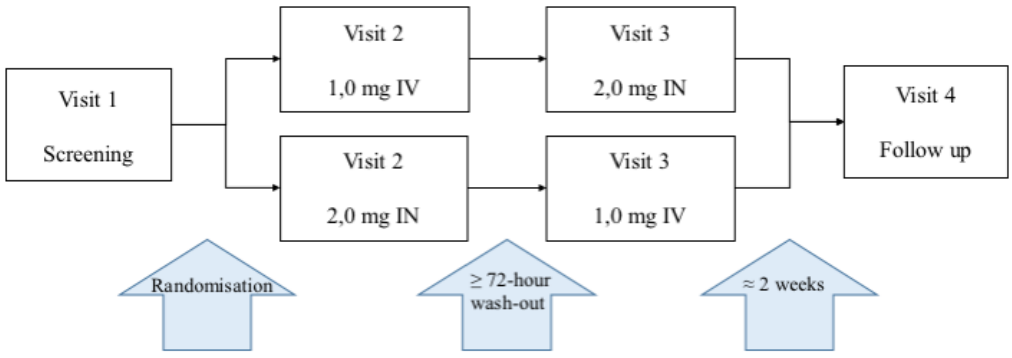
The formulation has been described for four different concentrations: 4 mg/ml, 8 mg/ml, 16 mg/ml and 20 mg/ml. If the nasal spray had a bioavailability of 20% - 50 %, the dose would be equivalent to the 0.4 – 1.0 mg given parenterally in clinical practice. As we had no experience with this nasal spray, and previous trials of nasal naloxone estimated 4% bioavailability, we chose to study the highest dose.

The intravenous dose was 1.0 mg. This is half the recommended upper dose for initial naloxone treatment. It was expected that the nasal and intravenous naloxone concentration would be in about the same range.

PROCEDURES

Potential subjects were screened for inclusion by interview, ECG, examination and clinical chemistry after giving informed, written consent as described above. When clinical chemistry results were available, the subjects were evaluated for inclusion. Consenting subjects fulfilling the inclusion/exclusion criteria were included and subsequently randomised to treatment order. The two pharmacokinetic (PK) sessions were then conducted according to randomised order. Finally, subjects met for follow-up about 2 weeks afterwards.

Figure 1. Overall study design



The subjects had 4 visits each. A screening visit, two intervention visits and a follow-up visit. Those who were included after screening were randomly allocated to one of two treatment orders. All participants received both treatments in a cross-over fashion.

Visit 1 (Screening): Assessment of clinical state included a general physical examination and a subject interview. Age, weight, height, sex, natural stimulants, previous diseases, use of medication and allergies were recorded during screening. Blood were sampled for clinical chemistry (haemoglobin, creatinine, ASAT, ALAT and gamma GTenzyme activity) was analysed by standard procedures at St. Olavs Hospital, Trondheim University Hospital. An ECG was also taken.

Randomisation: Participants who fulfilled the inclusion criteria were included and randomised. Treatment sequences were randomised in a concealed fashion by an internet based solution delivered by Unit for Applied Clinical Research, Faculty of Medicine, NTNU. The system does block randomisation. In this study there was no stratification. The blocks vary in size, but the first, smallest and biggest blocks were determined regarding the total size of the study.

Visit 2 and 3 (PK study days): 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 ml blood were drawn each time and collected in serum clot activator tubes (Vacuette®, Greiner Bio-One, Austria). Samples were centrifuged and 2 ml serum was frozen in cryotubes at -80 ºC until analysed.

A medical doctor was present for the administration of the medication, and for the next 30 minutes. Any adverse reactions, including local symptoms from the nose were recorded. The drug has a terminal half-life of 60-90 minutes and study subjects were followed for 360 minutes, this is equal to 4-5 half-lives of naloxone.

Visit 4 (Follow-up): Interview with the subject about any symptoms/health aspects about 2 weeks after visit 3. Clinical examination and laboratory tests were conducted/taken if indicated. Any necessary follow-up measures were taken in the best interest of the subject.

Table 2. Trial flow chart

	Screening Period		Treatment sessions		Follow-up
Time	Screening	Inclusion & randomisation	Session 1	Session 2	Two weeks
Informed consent	X				
Medical history	X				X
Physical Examination	X				
Inclusion evaluation		X			
Randomisation		X			
Vital signs	X[1]		X[1]	X[1]	
Blood samples	X[2]		X[3]	X[3]	
IMP administration			X	X	
Use of medication	X		X	X	X
Adverse event registration			X	X	X

The trial flow chart describes what were done at each visit. 1) Blood pressure, heart rate and respiration rate. 2) Haemoglobin, Creatinine, ASAT, ALAT and Gamma GT. 3) For quantitation of naloxone.

NALOXONE ANALYSIS

As described previously, subjects received the test drug once and reference treatment once. The treatments sessions were 6 hours each, with a three-day minimum washout period. On study days a baseline blood sample was drawn before the drug was administered. Study drug was administered while the subjects were sitting. After drug administration, blood samples were collected at 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240 and 360 minutes. There were no fasting regime or diet restrictions, as oral drugs were not administered.

Quantification of naloxone in serum was conducted using a validated high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) method at the Proteomics and Metabolomics Core Facility (PROMEC), Faculty of Medicine, NTNU, Norway. The method was fully validated by assessing linearity, accuracy, precision, sensitivity, specificity/selectivity, in process and storage stability, dilution integrity and assay ruggedness according to Dadgar et al (27) and Shah et al (28).

Naloxone hydrochloride dihydrate ($C_{19}H_{21}NO_4 \cdot HCl \cdot 2H_2O$, CAS number: 51481-60-8) and deuterated naloxone-d5 solution ($C_{19}H_{16}NO_4D_5$, CAS number: 1261079-38-2) were used as reference material (Sigma-Aldrich, St. Louis, MO, USA), and acetonitrile (HPLC-grade) was from Lab-Scan Analytical Sciences (Gliwice, Poland). The calibration standards and quality controls were prepared with plasma from blood donors (St. Olavs Hospital, Trondheim University Hospital).

The analytical preparation procedure was essentially as for the method described by Edwards et al (29). Standards, quality controls and samples (200 µl) were spiked with the internal standard deuterated naloxone-d5 (20 µl, 50 ng/ml). Plasma proteins were precipitated with acetonitrile (0.9 ml), vortexed, and after 30 minutes (4ºC) centrifuged for 10 minutes at 12000 x g (10ºC). Supernatants were evaporated to dryness in a MiVac concentrator and reconstituted in 50 µl mobile phase (mobile phase = 20% acetonitrile in 0.1% formic acid). The reconstituted samples were injected (3 µl) in the mobile phase (flow = 300 µl/min) by a Shimadzu auto injector (20AC) to a Zorbax SB-C18 column (5 µm, 2.1 x 150 mm) and further introduced to the Applied Biosystems API 5500 triple quadrupole by an Turbo VTM Ion Source operating in positive ion mode. Ion pairs were 328.2/268.2 and 333.2/273.2 for naloxone and the internal standard, respectively. Sample analysis was performed by multiple reaction mode. The turbo ion-spray probe temperature was set to 625°C, nebulizer and curtain

gas flow rates of 70 psi and 30 psi. The ion-spray voltage was 5500 V, while the declustering and entrance potentials were set to 126 V and 10 V. The collision cell energy was 37 V using a collision activated dissociation (CAD) set at 9, the collision cell exit potential was 22 V. Quantitative determinations was done by using AB Sciex Analyst ver 1.5.

Calibration range was 0.02 – 45 ng/ml (9 calibration standards). The correlation coefficient (r2) was > 0.9985 for all the calibration curves. The limit of quantitation (LOQ) was 0.02 ng/ml, with the coefficient of variation (CV) < 15.9 % and inaccuracy < 1.1 % (n = 16). The quality controls (QC 1, 2, 3) were in the lower (0.05 ng/ml), middle (15 ng/ml) and upper (30 ng/ml) calibration range. In the pre-run validation (n = 18) CV and inaccuracy were found to be < 10.7 %, 4.2 % (QC 1), < 3.9 %, 5.9 % (QC 2) and < 4.2 %, 2.8 % (QC 3) respectively. During in-run validation CV and inaccuracy for the quality controls (n = 35) were < 9.8%, 4.3% (QC 1), < 10.5%, 6.1% (QC 2) and < 4.5%, 2.6 % (QC 3).

Stability tests were performed prior to analyses: Auto sampler stability (24 hours), freeze/thaw stability (three times), long terms stability (12 months). Stability data was within limits given (27, 28) and all samples were analysed within three months.

Factors that can be important for evaluation of PK measurements are kidney- and liver function. This study was conducted in healthy volunteers, and the screening procedures required that the participants had creatinine and liver blood tests within reference values. Therefore, this is not a concern in this study, and it was not evaluated further.

PRIMARY AND SECONDARY OUTCOME MEASUREMENTS

The primary outcome was the absolute bioavailability of the nasal formulation of naloxone. Bioavailability was determined by calculating the ratio for dose-corrected area under the curve (until last sample) for IN and IV administrations of naloxone.

Secondary aims were to determine time to maximum serum concentration (T_{max}), the maximum serum concentration (C_{max}) and safety of the nasal formulation.

A third task taken on in this report was that data from this pilot study was compared with the data of the following study to evaluate if more was learned about central tendencies and variability by conducting a larger study.

SAFETY

Naloxone is a well-known, well-tolerated drug with an excellent safety profile over many decades of use, and has virtually no effects on healthy subjects. Since the bioavailability of this formulation is unknown, the theoretical maximum dose that could be delivered was 2 mg. This required a bioavailability of 100% which was unlikely. According to the SPC for IV naloxone standard initial doses for opioid overdose is 0.4 -2.0 mg, which can be repeated until a total dose of 10 mg is given (26). Thus the maximum theoretically possible nasal dose is within the range of the initial IV dose. In addition, the maximum serum concentration of a nasally delivered dose will be far lower than those of an equivalent IV dose. Side effects of nasal administration is possible, for example may unpleasant taste be experienced. The excipients in the present nasal formulation are all well known. The risks to our participating subjects were therefore considered minimal, and there are significant benefits in developing an adequately formulated naloxone nasal spray for pre hospital use. All adverse events were registered according to ICH-GCP and national laws.

STATISTICS

Serum concentration data was analysed by non-compartmental techniques. Area under the curve; (AUC (linear trapezoidal rule), terminal elimination half-life, C_{max} and T_{max} were calculated by computerised curve fitting using the Win-Nonlin Standard version 6.4 (Pharsight Corporation, USA) as described in previous publications (8, 11, 12, 30). Dose-corrected AUClasts were employed to calculate the absolute bioavailability. Data were described as mean, SD and 95% confidence intervals (CI). The descriptive statistics were conducted with SPSS version 23. Time course naloxone concentration curves are presented in log-linear plots.

Measurements below limit of quantification (LOQ) were not used in the analysis. The accepted CV for LOQ was 20% (27). All samples with a naloxone concentration lower than 0.016 ng/ml were excluded from the analysis, as these results were considered unreliable. 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. Out of 150 samples analysed, only

two data points were removed due to this criterion. Missing data were not imputed. There was no interim analysis. No major changes to study design were conducted.

The results from this pilot study were combined with results from the larger study that followed (30). Minimum-maximum values were calculated to evaluate variation. Pearson's correlation test and linear regression were used to investigate if there were a dose-concentration relationship for AUClast and C_{max}. Pearson's correlation test was also conducted for bioavailability and dose-corrected values of AUClast and C_{max}.

MY INVOLVEMENT

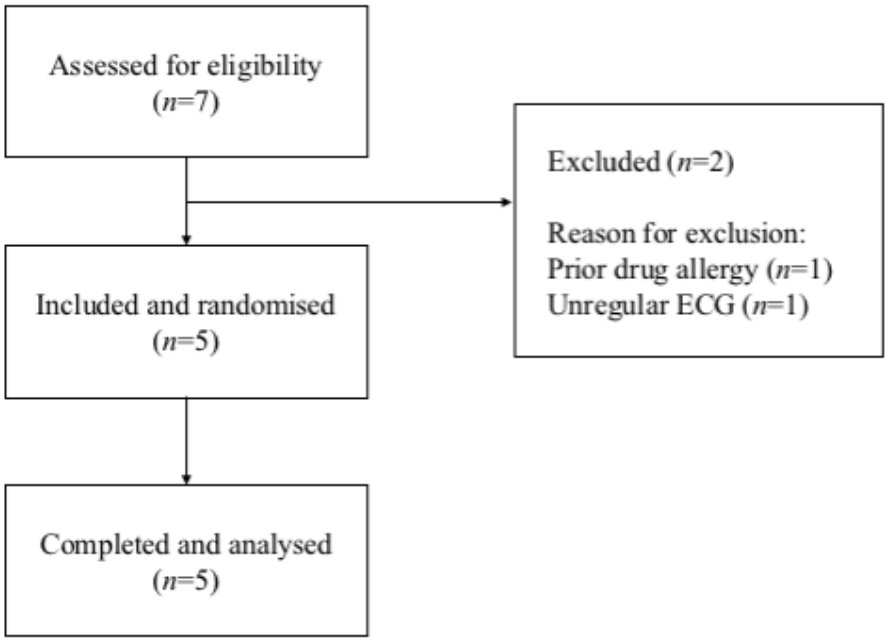
This was a clinical study of a new medication, and I was a third grade medical student with no previous research experience when it was conducted. I therefore had lots of help from my supervisor and the research group. However, they gave me gradually increased responsibilities. In the planning phase I was involved from the beginning and wrote the information letter about the study, and developed the case report form based on a template used previously.

When the study was ongoing, I was responsible for recruiting and held the information interviews and collected informed consents, as described above. I scheduled the visits with the Clinical Research Facility and the participants, and followed up on the CRFs making sure they were filled out correctly. Our senior engineers analysed the samples, but I analysed a set of samples parallel with the engineers to learn and understand more about the method. After the study was completed I got the responsibility for the study archive, conducted the statistical analyses of the data and made the graphical presentations of the results. I combined the two datasets, did all the statistics, results and discussion for the addendum of this thesis. I did most of the writing on the Final Study Report that was sent to the Norwegian Medicines Agency, and also wrote this thesis.

RESULTS

Seven participants were screened for inclusion. Two did not fulfil the inclusion criteria, five were included in the study. Five subjects completed the study, all Caucasians males with mean (min-max) age of 23.4 years (21-25), height of 179.6 cm (175-187) and weight of 73.9 kg (64.0-91.8). Average body mass index (BMI) was 22.8 and ranged from 20.9 to 26.2.

Figure 2. Flow chart of study participants



Seven participants were screened, and two were excluded as they did not fulfil the inclusion criteria. Five subjects completed the study.

PRIMARY ENDPOINT

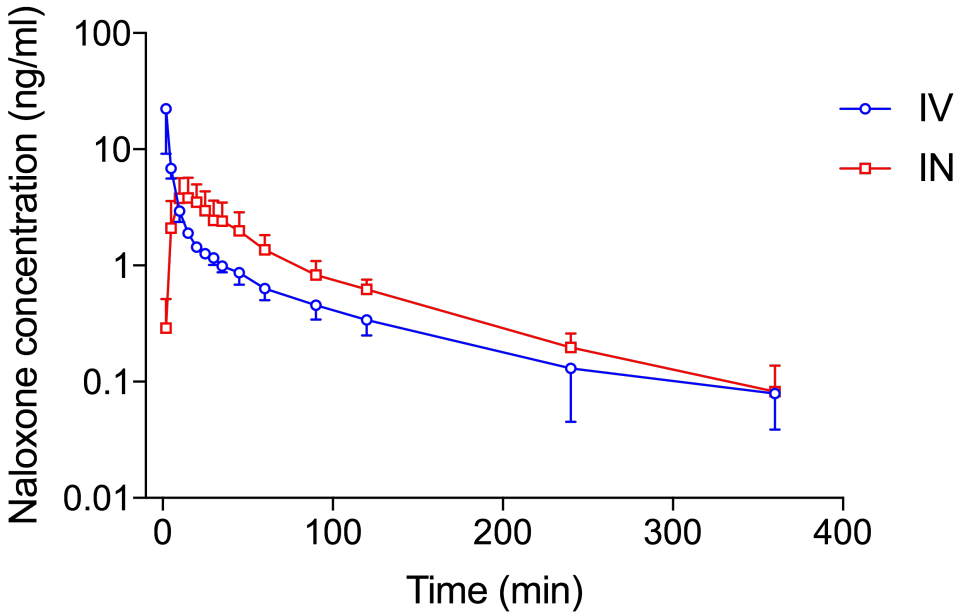
The absolute bioavailability was 47.1 (38.4-55.8) %, reported in mean (95% confidence interval). This was considerably higher than previously reported for nasal naloxone. For subjects 2 and 4 drops from the spray were discharged from the nose immediately after spraying. Their bioavailabilities were 24 and 53%, respectively.

SECONDARY ENDPOINTS

The secondary endpoints were C_{max} and T_{max}. C_{max} for the nasal spray was 4.24 (1.48-7.00) ng/ml, and T_{max} was 16.0 (5.80-26.2) minutes. Time to 50% and 80% of maximum

serum concentration, T_{max50} and T_{max80}, were also calculated. T_{max50} was 7.00 (3.60-10.4) min and T_{max80} was 13.0 (4.67-21.3) min. The mean half-lives were 80 and 90 min for the IN and IV, respectively (see table 3). The extrapolation of AUC from last measurement to infinity was 5% for the intranasal curve and 4% for intravenous curve.

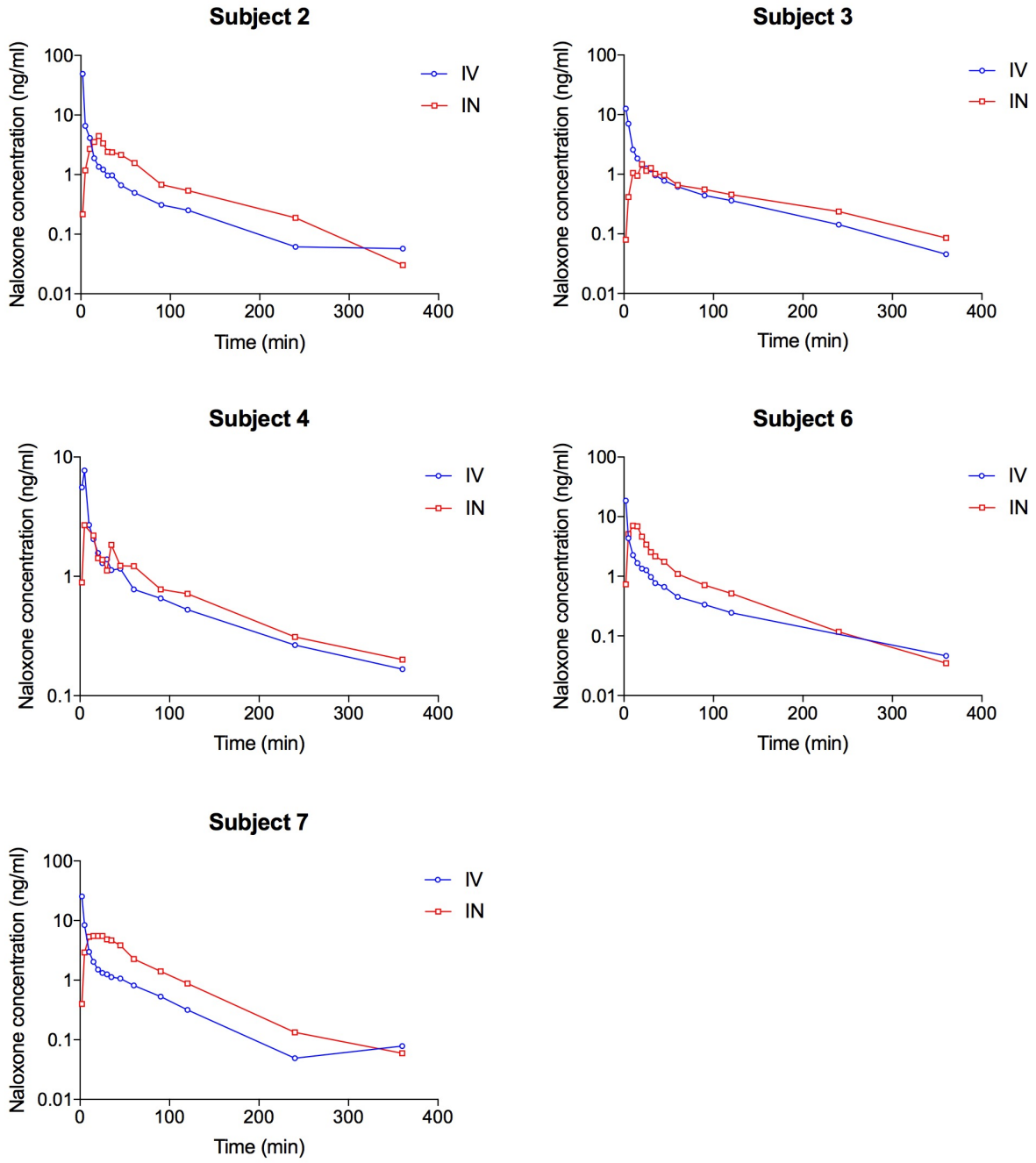
Figure 3. The time course for the mean serum concentration of naloxone in five subjects



Time course of mean serum concentrations of naloxone after intravenous (1.0 mg) and intranasal (2.0 mg) administration in healthy human volunteers (n = 5). Red line (squares) represents the 2.0 mg IN, and blue line (dots) the 1.0 mg IV. Error bars show 95% confidence interval.

The intravenous administration gave an instant high naloxone concentration, which thereafter fell rapidly. The concentrations following the intranasal treatment rose more slowly, and passed the IV after 10 minutes. IN administration continued to provide higher serum concentrations than IV until 240 minutes.

Figure 4. The time course for the mean serum concentrations of naloxone in each of the 5 subjects



Time course of serum concentrations of naloxone after intravenous (1.0 mg) and intranasal (2.0 mg) administration in five healthy human volunteers. Red line (squares) are the 2.0 mg IN, and blue line (dots) are the 1.0 mg IV. The main impression is the same as in figure 3, IV gives higher concentrations initially, but after a while IN produces higher concentrations.

Table 3. Pharmacokinetic variables in healthy volunteers after intranasal and intravenous administration of naloxone in an open, randomised two-way crossover trial (n=5)

Treatment	C_{max} (ng/ml)	T_{max} (min)	AUClast (min*ng/ml)	AUC∞ (min*ng/ml)	Distribution volume (ml)	Clearance (ml/min)	Half-life (min)
2.0 mg IN naloxone	4.24 (1.48-7.00)	16.0 (5.80-26.2)	264 (147-381)	276 (164-388)	429 900 (139 700-720 100)	3620 (2490-4740)	80.0 (35.3-125)
1.0 mg IV naloxone	22.7 (2.57-42.9)*	2.60 (0.93-4.27)*	282 (159-404)	293 (174-413)	482 300 (230 500-734 200)	3660 (2470-4840)	89.6 (56.1-123)

Data are presented as mean values ± 95% confidence intervals. Abbreviations: IN: intranasal, IV: intravenous, C_{max}: maximum concentration, T_{max}: time to maximum concentration, AUClast: area under the curve until last measurement at 360 min, AUC∞: area under the curve extrapolated to infinity.

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

SAFETY

The only adverse events reported were taste sensations of the nasal spray. It was recorded in 4 out of the 5 administrations. The time period of reporting was from 1-19 minutes after nasal spray administration. The participants described the nasal spray as "bitter", "tasting like medicine". There were only mild taste sensations, and none of the participants reported that the test product was unpleasant or distasteful. No one described itch, hurt or burning sensations. There was no cases of intercurrent illness.

ADDENDUM: COMBINING RESULTS OF THE PRESENT STUDY WITH THE RESULTS OF THE SUBSEQUENT, PUBLISHED STUDY

In the following I have used data from the present study as well as from the subsequent study already published (30). The aim was as said above to that data from this pilot study to evaluate if more was learned about central tendencies and variability by conducting a larger study.

CENTRAL TENDENCIES AND VARIATION

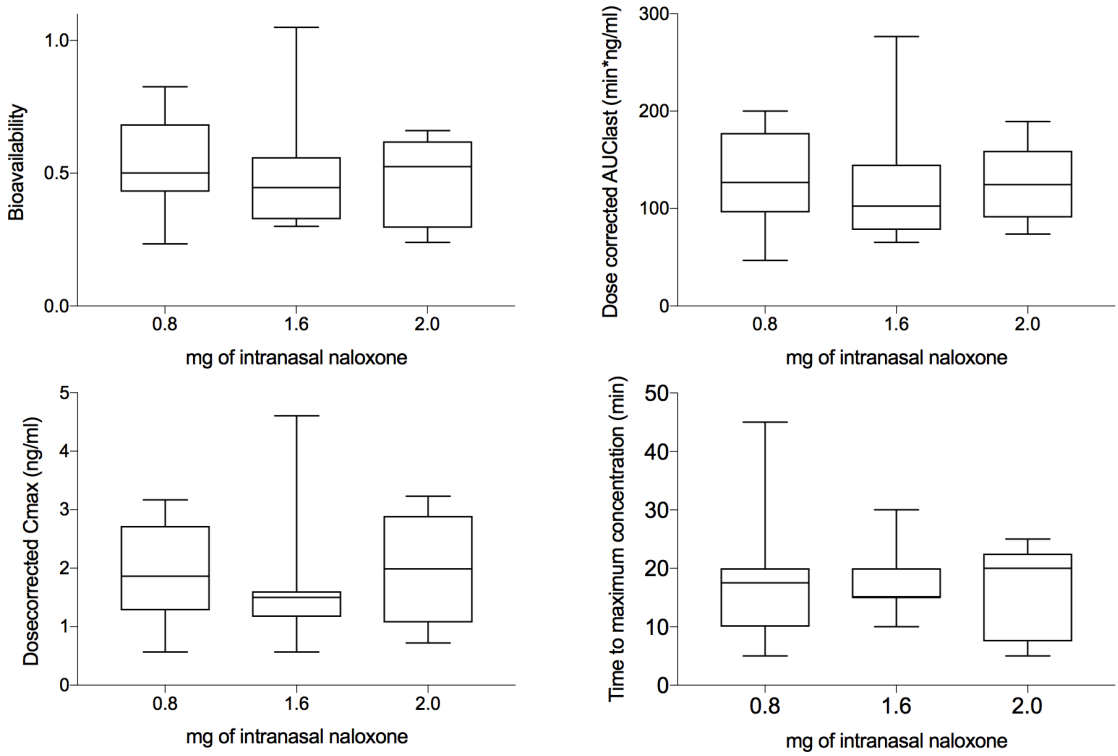
The data from both the studies are combined in table 4, and the central tendency is described as mean, and variation as 95% confidence interval and minimum – maximum values. The data is also for clarity illustrated in boxplots with median and percentiles in figure 5.

Table 4. Pharmacokinetic variables in healthy volunteers after intranasal and intravenous administration of naloxone. The results are combined from two open, randomised crossover studies. In the present study the participants received 2.0 mg naloxone (n=5), while they got 0.8 mg and 1.6 mg naloxone (n = 12/11) in the follow–up study (30).

Treatment	Bioavailability (%)	C_{max} (ng/ml)	Dose-corrected Cmax (ng/ml)	T_{max} (min)	AUClast (min*ng/ml)	Dose-corrected AUClast (min*ng/ml)
0.8 mg IN	54.0 (44.7-63.4) ((23.4-82.5))	1.45 (1.07–1.84) ((0.45-2.42))	1.93 (1.49-2.36) ((0.57-3.17))	17.9 (11.4–24.5) ((5-45))	99.0 (76.7–121) ((37.2-154))	131 (106-156) ((46.8-200))
1.6 mg IN	52.0 (36.8-67.2) ((30.0-105))	2.57 (1.49–3.66) ((0.85-6.96))	1.70 (1.10-2.30) ((0.57-4.6))	18.6 (14.4–22.9) ((10-30))	185 (123–248) ((97.6-418))	123 (88.2-157) ((65.3-277))
2.0 mg IN	47.1 (38.4-55.8) ((23.9-66.0))	4.24 (1.48-7.00) ((1.47-7.71))	1.98 (1.22-2.75) ((0.72-3.23))	16.0 (5.80-26.2) ((5-25))	264 (147-381) ((150-408))	125 (92.1-159) ((73.9-189))

Data are presented as mean (95% confidence interval) ((min-max)). Abbreviations: IN: intranasal, C_{max}*: maximum concentration,* T_{max}*: time to maximum concentration, AUClast: Area under the curve until last measurement at 360 min.*

Figure 5. Absolute bioavailability, dose-corrected AUClast, dose-corrected C_{max} and T_{max} for three doses of nasal naloxone (0.8, 1.6 and 2.0 mg). The results are combined from two different studies to illustrate central tendencies and variation.



Horizontal lines depict median values, boxes the 25 and 75 percentiles, whiskers the 95% percentiles, and crosses the outliers. n = 12 (0.8 mg), n = 11 (1.6 mg) and n=5 (2.0 mg).

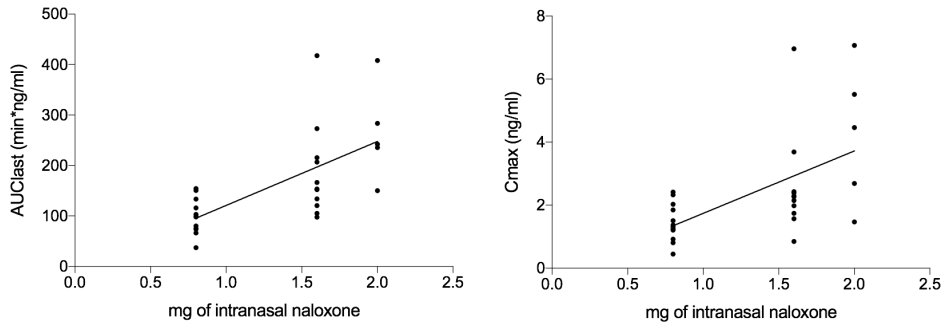
DOSE-CONCENTRATION RELATIONSHIP

The information on AUClast and C_{max} from our two studies was combined to investigate if there was a dose-concentration relationship. Pearson's correlation test showed a strong correlation with $r = 0.65$ for AUClast and $r = 0.57$ for C_{max}. However, not all variables were normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$). There were significant outliers, if they were taken out, the r stayed the same for C_{max} , but caused a small change for AUClast to $r = 0.71$. However, as the change was not that large it was decided to keep all data points in the analysis.

To assess linearity a scatterplot of AUClast and C_{max} against naloxone dose with superimposed regression line was plotted (figure 6). Visual inspection of these two plots

indicated a linear relationship between the variables. A linear regression analysis established that dose naloxone statistically significantly predicted AUClast, F (1, 26) = 19.35, $p < 0.0005$ and dose naloxone given accounted for 42.7% of the explained variability in AUClast. The regression equation was: predicted AUClast = -5.727 + 126.68 x (naloxone dose). For C_{max} we found the same relationship, F (1, 26) = 12.43, $p = 0.002$, dose naloxone given explained 32.3% of the variability in C_{max}. The regression equation was: predicted C_{max} = -0.237 + 1.979 x (naloxone dose).

Figure 6. Scatterplot with regression line for AUClast and C_{max}. The results are combined from two studies.



Three doses of nasal naloxone have been tested, 0.8 mg (n = 12), 1.6 mg (n=11) and 2.0 mg (n=5).

Bioavailability is a ratio, and is therefore directly comparable between studies. Pearson's correlation test showed that there was no apparent correlation between dose and bioavailability, with r = -0.106 (p=0.59). The dose given to the participant explained 1% of the variation in bioavailability. As above, not all variables were normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$). There were no outliers outside 3 SD. AUClast and C_{max} are dose dependent, and the numbers must therefore be dose-corrected to be comparable for comparison of central tendencies and variation. Pearson's correlation test showed no correlation between dose and the dose-corrected data for AUClast and C_{max}, r = -0.065 (p=0.74) and r = -0.040 (p=0.84) respectively. This indicated dose linearity.

DISCUSSION

OVERALL

In the following, each finding of this study is first discussed in relation to the knowledge available at the time of the study, followed by their relations to updated knowledge. At the end of the Discussion, data from this pilot study is compared with the data of the following study to evaluate if more was learned by conducting a larger study.

STUDY DESIGN

This was a phase I trial to explore pharmacokinetic characteristics and safety of a new, nasal formulation of naloxone. The aim of the study was to estimate the bioavailability of this new formulation intended for nasal use. Healthy individuals were included to secure all treatments in an equal setting. This was possible as naloxone is considered a safe medication.

Crossover studies are the preferred design in pharmacokinetic trials if possible, because the subjects' acts as their own control in a crossover study. The crossover design usually reduces the number of subjects required compared to a parallel group design. The study gains precision as treatment was compared within, rather than between, participants. This eliminates the variation between participants for each intervention. This study design is in accordance with best practice within this field.

The crossover study design is susceptible to carry-over effects between treatments. Effects of one treatment can affect the outcome in the following period. To avoid carry-over effects a 72-hour washout period after naloxone administration was enforced. The half-life of naloxone is 60-90 minutes (26), and five half-lives corresponds to about 5- 7.5 hours. A three days wash-out period secured that naloxone was eliminated before the next administration. Most participants had more than three days between treatments. If naloxone remained in the body from last study session this would also be discovered by naloxone being present in the “zero”-sample.

A potential adaption effect was that the uptake, distribution or elimination could be altered to the second or third time the subjects received treatment. In this study it was unlikely to occur as this was one-administration treatments, they were administrated both intravenously and intranasal, and the order was randomised. The possibility that an adaption effect could influence the results was therefore small.

To provide a reliable sampling schedule, it is recommended to have frequent sampling in the beginning, to avoid the risk C_{max} being the first sampling point. If C_{max} is the first sampling point, we cannot describe the uptake and distribution sufficiently. In this study we drew about 4 samples before C_{max} was observed, indication that the basis for determining T_{max} was sound. The sampling schedule (AUClast) should cover at least 80% of AUCinfinity to give a reliable estimate of the extent of exposure (31). The extrapolation from AUClast to AUCinfinity was only about 5%. This indicates that our sampling schedule covers about 95% of the serum concentration curve. We also find that the terminal half-life of naloxone compares well with previous reports (26), indicating external validity of our study.

RESULTS

The present study was the first trial of a new, nasal naloxone spray. The study indicated an absolute bioavailability of this nasal naloxone formulation of 47 % on average, which was higher than previously shown for nasal naloxone. It demonstrated a fairly rapid systemic uptake with a T_{max} of 16 min, and a considerable C_{max} of about 4 ng/ml. The nasal spray provided serum concentration that surpassed those of IV after 10 min, and stayed above until 240 minutes.

PRIMARY OUTCOME

The primary outcome measure was an estimation of absolute bioavailability of this a high-concentration/low-volume nasal naloxone formulation. The observed bioavailability of 47% was somewhat lower than for some other nasal formulations such as fentanyl (13), midazolam (12) and methadone (11) bioavailabilities of 89%, 68-71% and 85%, respectively. Regardless, the bioavailability of this formulation was far higher than the nasal bioavailability for naloxone of only 4% previously reported by Dowling et al (10). It is recommended that the volume of nasal administrations should not exceed 0.1-0.2 ml (8, 9). The subjects in Dowling et al's study (10) were given up to 5 ml of the 0.4 mg/ml solution in the nose, 25 times the recommended maximum amount for nasal administrations. As the authors pointed out the subjects, despite best efforts, swallowed a considerable amount of the drug. Naloxone has a low oral bioavailability due to almost complete first pass metabolism (32), thus it is likely that an unknown portion of the dose given never reached the site of action.

Take-home naloxone programs often use a 1mg/ml formulation for their improvised nasal sprayers. However, this formulation has also showed a poor absolute bioavailability of only 11% (18), which is far lower than that of the present formulation.

Except our own follow-up study of this pilot, only one other study on high-concentration/low-volume formulations have reported the absolute bioavailability. They found a bioavailability of 25-28% (24) compared to our 47%. There are three explanations for this difference. They studied doses of 8 and 16 mg, 10 times our dose, which might suggest an uptake saturation at high doses. The other difference was that their sampling time were only 30 minutes. In our material we see a higher concentration over time that might "cancel" the initially high concentrations from IV, but as their sampling ends at 30 minutes they will not have these effect. It might also be that our nasal spray has a better uptake and bioavailability, due to the absorption enhancers added in the formulation.

For the newly approved Narcan® nasal (dose: 4 mg/0.1 ml) they only report the relative bioavailability to intramuscular naloxone which was 47% (21). If the absolute bioavailability of intramuscular administration is 100%, the bioavailability of the two products is similar, however, it is likely somewhat smaller. Nevertheless, the bioavailability of high-concentration/low-volume formulations are much higher than previously reported for nasal naloxone, and allows for administration of therapeutic doses in one spray.

Our nasal spray had a mean absolute bioavailability of 47 %. A prominent finding was the variability of the bioavailability with min-max of 24-66% (range 42%), indicating a considerable inter individual differences in nasal uptake of naloxone. This has been shown for other nasal formulations with adequate volume of nasal spray. For instance for nasal methadone, the bioavailability was 85% (CI 95%: 70-110%) (11), and for the nasal midazolam, with a median bioavailability of 71%; the range was 59 % (12). The inter individual variability may be related to many factors; such as blood flow, mucociliary clearance and anatomy (33). As this is a small study with only five subjects, the range of bioavailabilities can be even larger than what have been showed here. The variation probably reflects both the biological variation as well spraying technicalities. For two of the subjects, drops were discharged from the nasal cavity almost immediately after spraying. Their bioavailabilities were 24% and 53%. Thus their true biological bioavailability might have been be higher if the drug has stayed in the nose, and is probably more correctly reflected in the three other subjects with 35, 58 and 66 %, respectively. However, when it happens in a

controlled study setting, it might as well happen in real life situations with the use of the spray under more complex circumstances. In the clinical setting however, nasal naloxone will always be individually titrated with respect to its effect on the respiratory rate. Thus the inter individual variability may not pose a significant clinical problem.

SECONDARY OUTCOMES

We found a C_{max} of 4.2 ng/ml after 2 mg naloxone delivered in 0.1 ml to the nose. This was much higher than the 0.53 ng/ml reported after the 2 mg nasal spray delivered in 2 ml (18) which is the concentration of naloxone most often used in the THN-programs outside America today. The C_{max} found after IN in this study was also 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) intramuscular auto injector (Kaléo Pharma, VA, USA) (19). Our 2 mg dose provided a C_{max} close to that of the recently approved Narcan® nasal spray of 4.8 ng/ml after a single 4 mg naloxone IN dose (twice that of ours) (21). It should be emphasized that the Narcan® nasal spray aims at an equivalence of about 2 mg IM, the upper part of the recommended range of 0.4 to 2.0 mg for initial dosing of naloxone for opioid overdose. Regardless, this indicates that our nasal spray achieved clinically relevant concentrations and subsequently that a therapeutic dose was provided in one single shot.

On the other hand, these IN concentrations were far lower than the initial concentrations measured after IV administration (on average 22.7 ng/ml 2 minutes after administration). The much higher initial serum concentrations after IV than nasal administration may in some deeply intoxicated subjects be beneficial, but for those suffering a less severe intoxication, this may cause withdrawal symptoms and agitation that is related to the rapidly rising and high naloxone concentrations (34). This might trigger aggression, refusal of follow-up health care services and active drug seeking (35).

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 16 min (Table 3). This is similar to the T_{max} of 15–20 min reported for IM naloxone (19) and to the T_{max} of the 20-30 min reported for other naloxone nasal sprays (18, 21, 24). Furthermore, naloxone was quantifiable in all samples taken 2 min after drug administration and T_{max50} and T_{max80} were about 7 and 13 min after the nasal administration. These findings are consistent with the report of a T_{max50} of 7-8 minutes in another study (24). It should be kept in mind that a

clinical effect of naloxone precedes the T_{max}.

As showed in figure 3, the nasal concentration surpassed those of IV after 10 min. The uptake delay imposed by an extravascular administration was carried all the way to at least 240 min. This means that the opioid antagonist action will be stronger for a longer time after administration of 2 mg IN than for 1 mg IV, and probably reduce the risk for relapse of the respiratory depression induced by the overdose. Due to a lower and somewhat slower uptake one also avoids the initial risk for provoking withdrawal symptoms compared to IV as discussed above. This is also the reason for the change in clinical practice from intravenous to intramuscular administration. However, it should be noted that the use of intramuscular naloxone as the first treatment is off label according to the SPCs, unless it is not possible to obtain IV access (26).

It seems like the present IN formulation have the capacity to provide serum concentration corresponding to those of established clinical practice and consequently be able to provide the necessary therapeutic safety. But the pharmacokinetic relationship between commonly applied intravenous, intramuscular and appropriate nasal formulations need to be studied further.

In this study a bidose sprayer was used, and it contained two doses of 2.0 mg each. This should make it possible to give a second dose if the first dose should fail. One may only speculate whether failures such as the fentanyl overdose case described previously would have been avoided with this formulation (17). As the nasal spray show promising pharmacokinetic results, it was followed up by a new study where the bioavailability was finally determined in 12 subjects. With an anticipated bioavailability of about 50%, we chose to study 0.8 mg and 1.6 mg in this follow-up study as this corresponds to the initial naloxone of 0.4 or 0.8 mg dosing used in Norway in suspected opioid overdose (1, 2). This study was recently published and will be discussed below (30).

ADDENDUM: COMBINING RESULTS OF THE PRESENT STUDY WITH THE RESULTS OF THE SUBSEQUENT, PUBLISHED STUDY

In the final part of this discussion, the results from the present and the subsequent studies are combined to evaluate what could be learned regarding central tendencies and variation from increasing sample size and studying two other intranasal doses with the same IV dose. The follow-up study was a three-way crossover trial in 12 healthy volunteers receiving 0.8 mg IN, 1.6 mg IN and 1.0 mg IV naloxone (30).

CENTRAL TENDENCIES AND VARIATION

The observations for the follow-up study are paired, while they are independent from the observations of the current study. As statistical tests are usually for either paired observations or independent observations, this makes ordinary statistics analysis difficult. However, we can study the central tendencies and variations and visually inspect their plots, but these findings have to be interpreted with caution.

Bioavailability is a ratio, and is therefore directly comparable between studies. T_{max} is a time point, and also directly comparable. AUClast and C_{max} are dose dependent, and the numbers must therefore be dose-corrected to be comparable for central tendencies and variation. Studying the results described in table 4, we start looking at bioavailability. The studies all show a mean bioavailability of around 50%. The dose-corrected AUClast show numbers between 123 and 131 min*ng/ml, T_{max} is between 16 and 19 min and dose-corrected C_{max} is between 1.7 and 2.0 ng/ml. Overall the impression is that the means are almost the same across the two studies and within the doses of the following study, and that also a small study was usable to get an estimation of the central tendencies.

On the other hand, by illustrating the difference in inter quartile ranges (figure 5) we see that the variability differs between the doses and studies. It seems to maybe be smaller in the current study (n=5), this is also seen in the data presented in table 4. The min-max values of bioavailability are for example 24-66% in the current study, while the same values are 23-83% and 30-105% in the 0.8 and 1.6 mg arm of the follow-up study. This illustrates that the dataset from the current study probably is too small to disclose the real variability of the whole population. On the other hand, there are outliers that have a significant impact on the results on variability, especially in the 1.6 mg arm. However, the same pattern is seen for

T_{max}. The min-max values were 5-25 minutes, whereas in the follow-up study the same number were 5-45 and 10-30 minutes, respectively.

DOSE-CONCENTRATION RELATIONSHIP

Having results from three different doses of the same nasal spray raises the interesting question on whether there is a dose-concentration relationship, and whether this relationship is linear. Pearson's correlation test showed a strong correlation between higher dose and both higher AUClast and C_{max}. However, some of the assumptions of the test are not met as not all variables were normally distributed, but the test is somewhat robust in this respect. There were outliers that can affect the analysis, but taking them out did not change the results much. The linear regression showed that dose predicted 42.7% and 32.3% of the variability in AUClast and C_{max} respectively. One of the assumptions of the regression is that the observations should be independent, however in this sample some of the observations are paired. The findings therefore have to be interpreted with caution.

The results suggest that there is a strong correlation between dose and AUClast and C_{max}. The relationship seems to be linear and positive, such that a higher dose giver higher AUClast and C_{max}. This is consistent with the findings of a relationship between dose and AUClast and C_{max} in an overview of patent applications on nasal naloxone formulations (18).

Pearson's correlation test was conducted for bioavailability, dose-corrected AUClast and dose-corrected C_{max}. They showed no correlation at all. If the uptake was saturated with high naloxone doses, it might had showed a lower bioavailability or dose-corrected AUClast and C_{max} at higher doses, but here we cannot identify a relationship related to dose, indicating linearity.

To conclude, the follow-up study confirmed the findings of this pilot study. Overall the impression is that the means are almost the same across the two studies and within the doses of the following study, and that also a small study was usable to get an estimation of the mean. However, the small study did not reflect the variability in the same way as the larger study. The data suggests that there is a dose-concentration relationship.

SAFETY

Naloxone is a medication frequently used in health care worldwide. When administering naloxone to opioid addicts, there is a concern regarding precipitation of withdrawal symptoms in the patient. However, when administered to healthy individuals it is well known for its safety. There have been case reports about pulmonary edema in postoperative patients, but the relationship is proven, and naloxone is generally considered a very safe medication.

When administering this new formulation of naloxone, the adverse effects are divided into the systemic effects of the drug and effects due to the route of administration. We did not expect any other systemic adverse reactions than those already known, but as the nasal spray contained high concentrations of naloxone, local discomfort or irritation such as itching, hurt or other nasal reactions could be precipitated.

This was a phase I pharmacokinetic study which also tested whether or not the test drug is tolerated in humans. It is a study in five healthy individuals and thus not designed to expose all side effects that possibly could be related to the drug. The participants were exposed to a marketed product once, Naloxon B. Braun 0.4 mg/ml for IV use, and once to the test product, intranasal naloxone 20 mg/ml.

The only events reported were taste sensation of the nasal spray. It was recorded in 4 out of the 5 administrations. The time period of reporting was from 1-19 minutes after nasal spray administration. The participants described the nasal spray as "bitter", "tasting like medicine". There were only mild taste sensations, and none of the participants reported that the test product was unpleasant or distasteful. No one described itch, hurt or burning sensations. These are considered related to the IMP and were therefore classified as adverse drug reaction. There were no other adverse events reported.

No clinically significant adverse event was observed. Moreover, the spray provoked no unexpected adverse events. The only reported adverse event was taste of the nasal spray. The safety of the spray therefore warrants further clinical investigation.

LIMITATIONS

The pilot study was a small study, conducted in few, healthy individuals free of other medications. They are not necessarily representative for the patients that will be treated with nasal naloxone for opioid overdose reducing external validity in this regard. The purpose of this study was to provide estimations of the bioavailability of nasal naloxone. Five subjects were chosen as it was expected to provide the data required for this preliminary estimation of bioavailability. This would be the foundation for deciding which concentrations (4, 8, 16 or 20 mg/ml) to proceed with. If this nasal spray showed potential for delivering a therapeutic dose in one spray actuation, this study would be followed by a more extensive study for a final determination the bioavailability of this nasal formulation. This follow-up study was recently published (30). From the combining data of the two studies we can say now that the estimation of a mean around 50% was pretty accurate, but the variation was not accurately described in this small study sample. The results from the comparison of the results from these two studies must be interpreted with caution due to the limitations with this analysis. 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 reported for low-concentration/high-volume formulations. This indicates that an optimised high-concentration/low-volume nasal spray of naloxone can deliver a therapeutic dose in one actuation. The nasal spray provided serum concentration that surpassed the IV after 10 min, and stayed above until 240 min. The spray did not elicit worrying side effects in the exposed subjects. The small study was reasonably accurate regarding central variables, but not regarding variability. The results are promising and further development of the product is warranted. The formulation will be used in further trials investigating the pharmacokinetics more closely, and also study the pharmacodynamic properties of the product. There is also a need for comparing the pharmacokinetic profile of intranasal and intramuscular naloxone.

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