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Thesis for the Degree of
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Sammendrag

Opioider, morfinlignende stoffer, fører til overdoser og dødsfall. I Norge dør rundt 250 mennesker hvert år. Disse dødsfallene kan i prinsippet forebygges. Det er økende fokus på skadereduserende tiltak, for eksempel sprøyterom og væresteder. Opioidforgiftning behandles med motgiften nalokson som har vært på markedet som injeksjon i over femti år. Verdens Helseorganisasjon anbefaler at alle som kan bli vitne til en opioidoverdose skal ha tilgang til nalokson, og flere har jobbet for at nalokson skal bli tilgjengelig som nesespray, ikke bare i sprøyteform.

Slik nesespray er brukt som førstehjelp i flere år. Sprayene har vært provisoriske løsninger uten godkjenning fra myndighetene og grunnleggende kunnskap om deres farmakologiske egenskaper har manglet. Denne ph.d. oppgaven tar sikte på å øke vitenskapelig forståelse av både forhold rundt opioidoverdoser og av farmakologiske egenskaper ved nalokson gitt intranasalt.

Vi har analysert kliniske data og oppfølgingen etter behandling i 1054 tilfeller av overdoser i Oslo Sentrum 2014-15. Vi har gjennomført to farmakokinetiske og farmakodynamiske studier (n= 12 og n= 22). I disse har friske frivillige deltagere fått nalokson intranasalt, intramuskulært og intravenøst. I ett studie fikk de bare nalokson, i den andre også opioidet remifentanil for å kunne måle virkningen av nalokson. Ved avansert modellering av resultatene beregnet vi en nasal dose som skulle være sammenlignbar med en effektiv sprøytedose.

Fra de 1054 overdosene fant vi at medianalderen for overdose var 35 år, og 79% er menn. Pasienter på Sprøyterommet og i private hjem var sykere enn de som ble behandlet på offentlige steder. Studiene i friske frivillige viste at intranasalt nalokson har en biotilgjenglighet sammenlignet med sprøyte på 0,50, men som økte til 0,75 ved samtidig bruk av et opioid. Måling av pupillstørrelse, men ikke smerteterskel, egnet seg til å vurdere virkningen av nalokson i friske frivillige . Etter beregning og utprøving fant vi at intranasal 1,4 mg nalokson må ansees som like god behandling som intramuskulært 0,8 mg. Vår nesespray på 1,4 mg er nå godkjent for bruk i 12 land i Europa.

Abstract

Deaths from opioid overdoses is described as an epidemic. The last decade has seen increasing focus on harm-reducing public health interventions and new treatment options. These are preventable deaths, and opioid intoxication is treated with naloxone. This antidote has been on the market as injection. The WHO recommends that everyone likely to witness an opioid overdose should have access to naloxone. Over many years, naloxone has been administered as a nasal spray, but without licence from authorities and with little pharmacologic knowledge available. This thesis aims to add to the scientific understanding regarding opioid overdoses and naloxone for intranasal use.

A cohort of 1054 cases of pre-hospital naloxone administration in Oslo in 2014-15 has been studied for clinical data and follow up after treatment. Two crossover pharmacokinetic (PK) and pharmacodynamic (PD) studies (n=12 and n=22) of naloxone at various doses administered intranasally (IN), intramuscularly (IM) and intravenously (IV) in healthy volunteers report central pharmacologic outcomes.

Median age for naloxone administration is 35 years old, and 79% were men. The level of consciousness and respiratory rate of patients treated vary between locations and are lowest at the Safe Injection Facility and in private homes. These patients are critically ill and in need of urgent medical attention. Naloxone IN 0.8 mg has a bioavailability of 0.75 when compared to IM under remifentanil co-administration. Pupillometry show that IM naloxone 0.8 mg elicits a larger change in pupil size than the same dose IN. Naloxone IN 1.4 mg is not statistically different from IM 0.8 mg in terms of AUC, C_{max} and T_{max}. Two doses of IN 1.4mg administered in the same nostril display dose proportionality.

Patients treated with naloxone out-side of hospital are gravely ill. Those treated in SIF and private homes have the most severe unconsciousness and respiratory arrest. IN naloxone 0.8 mg is inferior to the same dose IM, both in terms of PK and PD. IN 1.4 mg naloxone shows no statistically significant difference to IM 0.8 mg on central PK variables. IN 1.4 mg IN naloxone is well suited for titration.

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My first thanks must go to professor Ola Dale. I am immensely grateful for his role as a scientific mentor, and as a friend. This project, and the development of nasal naloxone in Norway, is unthinkable without him. He has boldly taken this all the way from idea and the laboratory to marketing approval and randomised clinical trial. Together with NTNU he has achieved a level of innovation rarely seen in academia. Anne-Cathrine Braarud and Fridtjof Heyerdahl has been excellent co-supervisors. Their support, ideas, and gentle correction has kept me on the straight and narrow, they are a joy to work with. Ida Tylleskär has been my partner-in-crime from day one. As researchers we have grown up together, and without her meticulous and clear thought and unlimited work capacity this thesis would not have been finished. All my supervisors have been available for discussions and teaching throughout, they have helped me see the bigger picture and pushed me to achieve beyond what I expected of myself.

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the field of addiction research and been helpful throughout. Study nurse Tomas Drægni have entered all the medical records into the cohort study database. Cooperating with AS Den Norske Eterfabrikk opened up the world of the pharmaceutical industry. It has been an exciting learning experience, and made words like “posology” part of my vocabulary. Their work on the nasal naloxone formulation changed the scope of this project. Together with Ola Dale and NTNU they have released the innovative potential in the naloxone formulation and brought it to the market. Professor Anders Åsberg, Department of Pharmaceutical Biosciences, UiO, has been an inspiring teacher in pharmacokinetics and excellent co-author. Outside of NTNU my employers and colleagues at the Department of Anaesthesiology and Critical Care and the Department of Air Ambulance at OUS have shown flexibility and patience beyond what can be expected, without this I would never have completed this thesis. My friends Erik Lundhaug and Stian Hårstad have made sure life in Trondheim has been filled not only with naloxone concentrations- thank you! For all of the research presented here to have happened, patients treated with naloxone have accepted the registration of their data, and volunteers have given of their time and blood to study the pharmacology of naloxone. Beyond raw data this has given me inspiration and motivation to go on with this project.

Without my family I would not be the person I am. My parents have always given me the freedom to find my own way; a skill and confidence that I have needed in each step of this work. Håkon, my husband, has been immersed in naloxone by-proxy for six years, and never complained. He has listened to endless babble and accepted distraction when my mind has wandered into some random detail regarding this thesis. Thank you. I love you.

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List of articles

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Abbreviations

AUC	Area Under the Curve
CI	Confidence Interval
C_{max}	Maximum Concentration
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
IM	Intramuscular
IMP	Investigational Medicinal Product
IN	Intranasal
IV	Intravenous
NoMA	Norwegian Medicines Agency
NTNU	Norwegian University of Science and Technology
PD	Pharmacodynamics
PK	Pharmacokinetics
REC	Regional Committees for Medical and Health Research Ethics
SD	Standard Deviation
SIF	Safe Injection Facility
$t_{1/2}$	Half-life
T_{max}	Time to maximum concentration
US	United States of America
WHO	World Health Organization

Definitions

Absorption	In pharmacokinetics it is the passage of a drug from the site of administration to the blood stream(4).
Agonism	The process where a substance initiates a response when combined with a receptor. Agonists have an affinity for a receptor and an observable biologic effect (4, 5).
Antagonism	Describes a situation where a drug binds to a receptor without activating it, and by doing so prevents the binding of the agonist. Antagonists have an affinity for a receptor with no efficacy (6).
Area Under the Curve	Description of total systemic exposure of a drug to the body.
Bioavailability	The fraction, or percent, of administered dose that is absorbed intact. Abbreviated *F*
Bioequivalence	Two drug products are said to be bioequivalent if the 90% confidence interval of the ratio of geometric means of the primary pharmacokinetic (PK) responses AUC and C_{max} (after log-transformation) is within the limits of 80% and 125% (7). Regulatory authorities accept a 20% difference in systemic exposure (AUC and C_{max}) as a standard without being clinically significant. The pharmacokinetic parameters for exposure (AUC and/or Cmax) are log-normally distributed. When transforming the symmetrical 20% to the natural logarithm from 100% we get the limits described.
C_{max}	Maximum concentration of systemic exposure of a drug to the body
Distribution	In pharmacokinetics distribution describes the reversible transfer of a drug from one location to another within the body (8).

EudraCT	European Union Drug Regulating Authorities Clinical Trials is the European Clinical Trials Database of all clinical trials of investigational medicinal products with at least one study site in the European Union or European Economic Area.
Excretion	The process by which metabolic waste is eliminated from an organism (9).
Half-life	Time for a concentration to fall by one half
Hypercapnia	An abnormal high amount of carbon dioxide in the blood.
Hypoxia	Deficiency in the amount of oxygen reaching the tissues.
Marketing authorisation	The approval to market a medicine in a county. This is a product licence granted by EMA or national competent medicinal authority such as NoMA. The licence is given after assessment of quality, efficacy and safety criteria from the applicant.
Metabolism	Drug metabolism is the breakdown of drugs by living organisms, usually through specialised enzymatic systems (10).
Miosis	Excessive constriction of the pupil of the eye (5)
Off-label use of drugs	Prescribing a marketed medication for an indication, at a dose or by a route of administration other than what is specified in the marketing authorisation given.
Opiates	Opiate is the older term classically used in pharmacology to mean a drug derived from opium (6).
Opioids	Opioid is used to designate all substances; agonist, partial agonists and antagonists, both natural and synthetic, that bind to opioid receptors (6). The term mainly describes the drugs that produce morphine-like effects and are blocked by naloxone (4).

Pharmacodynamics	The study of the relationship between a drug's concentration and the resulting effect. It describes what the drug does to the body, and measure physiological responses (11).
Pharmacokinetics	The study of the time-course of drug absorption, distribution, metabolism, and excretion. It describes the relationship between administered dose, the observed biological fluid/tissue concentrations of the drug, and time (12). It is sometimes described as what the body does to the drug.
Safe Injection Facilities	Legally sanctioned, medically supervised facilities designed to provide a low-threshold environment to use heroin hygienically and to access targeted safer injecting advice and intervention in case of overdose. "*The injection room will contribute to increased dignity for people with long-term drug addiction by providing a hygienic framework for injection"(13).* In Norway known as "Sprøyterom".
Take Home Naloxone	The community-based provision of naloxone to opioid users or others likely to witness an opioid overdose. Naloxone "kits" containing various naloxone formulations for IM or IN administration have been used. Initiated as grass-root activism in the 1990´s, but last decade promoted in many countries and is a WHO recommendation(14, 15).
T_{max}	Time to maximum concentration

1 Introduction

1.1 Background

When Homer lets Helen, daughter of Zeus, spike the drink of Telemachus with opium he is far from the first to describe the effects of the opium poppy *Papaver somniferum*. In the Odyssey it is described as "*a drug to quiet all pain and strife, and bring forgetfulness of every ill" (16).* For thousands of years it has been used to relieve pain and to bring comfort. Opium was often dissolved in alcohol and other ingredients such as saffron and musk to form a tincture. Thomas Sydenham's opium laudanum mixture recipe were produced from 1676 and well into the 20th century (17). In the early 1800s Friederich Sertürner isolated a potent chemical from the sap of unripe seed buds from the poppy plant. He named it morphine, after the ancient Greek god of dreams Morpheus (18). In the 200 years following, several natural opiates and synthetic opioids have been produced, and their use, both medical and recreational, has increased. In addition to analgesia and sense of well-being opioids reduce respiration by a number of mechanisms, and this is the prime worry in overdose (19). An overdose of opioids is recognized by pin-point pupils, reduced consciousness and reduced respiration. The reduced breathing lead to hypercapnia and hypoxia and culminate in cardiac arrest and death. In addition to acute overdose all opioids have the potential to cause dependence, i.e. the need to keep taking drugs to avoid a withdrawal syndrome and cause addiction characterised by intense drug craving and compulsive use (20).

The combination of desirable effects such as analgesia and euphoria on the one side, and addiction and life-threatening complications on the other makes the regulation of opioids both difficult and important. Illegal opioids, mainly heroin, has been misused for centuries. These alluring drugs have caused large scale war between imperial China and the British Empire in the 1800´s (21) and is today fuelling the ever cindering war in Afghanistan (22). Overdose deaths have been recognised as a serious public health threat for decades. Opioid addiction also cause havoc with non-fatal overdoses, new-borns with opioid withdrawal syndrome, crime, poverty and infection such as hepatitis and HIV.

1.1.1 Overdose epidemiology

In the last decade there has been a sharp rise in deaths from overdoses, see figure 1-1. Europe has seen an increase, albeit not as dramatic as the US. Norway has had stable numbers over 15 years, but these have been among the highest in Europe, both in total numbers and per capita (23, 24).

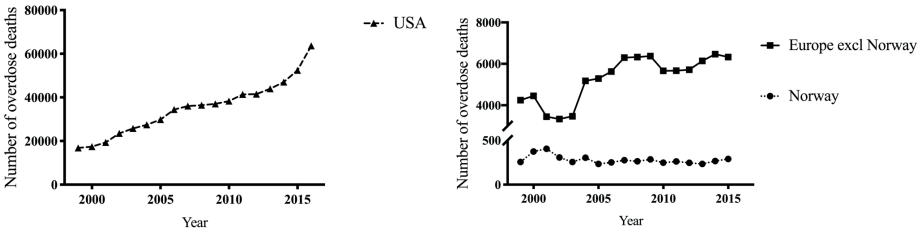

Figure 1-1: Overdose deaths from 1999- 2016. Note USA in ten thousands, and Europe in thousands. Source: CDC WONDER and EMCDDA

The use of prescription opioids have increased dramatically in use over the last 30 years, particularly in the US, and this has been accompanied by a massive increase in overdose deaths and an unprecedented reduction in life expectancy (25).

For each fatal opioid overdose there are many more non-fatal opioid overdose events. The exact ratio varies and is unknown, but is described to be as high as 30:1 for non-fatal: fatal events (26). Overall drug induced deaths (78% involving opioids) are more frequent in men (80%), and the average age of death by overdose is 39 years in Europe. Opioid users in Europe are 5 to 10 times more likely to die than their peers of the same age and gender (27), and this risk remains high for many years after non-fatal intoxications (28). The magnitude of the problem varies significantly between countries, with the US being exceptionally high, and Northern Europe having more overdoses than the south. The highest rates are in Estonia (127 per million) and Norway (70 per million), whereas Portugal only has 3 per million. These numbers are reported by The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (29, 30). There are some differences in national definitions of overdoses and practices regarding post mortem examinations, but these do not explain the differences in numbers between

European countries fully. The most recent estimate of the number of recreational opioid users in Norway is 9015 (6708 -13 977) persons, and approximately 3.000.000 hypodermic needles for drug injection are handed out annually in Norway. In Norway in 2016 a total of 17 925 people received addiction treatment primarily for opioid use and 7500 people was in opioid substitution therapy (29, 30).

There are also different populations within this patient group: some living on the streets injecting illegal drugs and/or in opioid substitution therapy, others living more stable lives using prescription opioids with or without illegal opioids in combination (31-34). Follow-up after naloxone treatment outside of hospital varies. After successful awakening many patients oppose further follow-up and are left by health professionals at the scene of the overdose. The safety of this practice is debated, but is found to be safe as rebound intoxication and short-term mortality is low (35, 36). However, it is obviously less than ideal to only treat the acute intoxication in patients who often have complex health and social needs. Somatic illnesses such as infections, and mental health issues should be treated. Access to addiction treatment and social services such as housing all need to be addressed after a non-fatal overdose.

Knowledge regarding the epidemiology of overdoses is important for the establishment of prevention- and treatment- services locally, public health policy on a wider scale and the development of new treatment options for antidote administration.

1.1.2 Overdose physiology

An opioid overdose is recognised by reduced consciousness, slow or absent breathing and miosis. The reduced breathing leads to hypercapnia and hypoxia. This in turn leads to cardiac arrest and death. Opioid receptors are G-coupled receptors in the cell membrane that elicit several responses when activated. There are four types of such receptors, designated mu (μ), delta (δ), kappa (κ), and the opioid receptor like-1 (ORL1) (37). The μ-opioid receptor is involved in analgesia and reduced breathing, and the one we are concerned about in overdose. The latter effect is mediated by activating the pre-Bötzinger complex, a respiratory rhythm-generating area in the pons reducing the sensitivity of the brain to generate breathing despite increased CO_2 and decreased O_2 in the blood (19, 38). The physiology and pharmacology are complex, as there is a wide

variety in the doses of opioid causing overdose. Several factors particular to the individual, the drug(s) and other properties act together and can culminate in an overdose.

I) The type and strength of opioid involved

All μ-opioid receptor agonists can cause overdose. Common drugs are natural- and semi synthetic-opioids such as morphine, heroin and oxycodone, and synthetic opioids such as fentanyl and methadone. M-opioid receptor partial agonists such as buprenorphine can also cause overdose, but the effects are more indiscriminate. For non-pharmaceutically produced and illegal drugs the exact drug type and concentration is unknown, and dosing is difficult. The introduction of potent fentanyl analogue opioids in the illegal market makes dosing even more challenging and have sparked increase in overdose-related deaths worldwide (39, 40).

II) Route of administration

The IV route is the most frequent route associated with overdoses. It leads to a high serum concentration almost impossible to create by other routes, and this rapid increase causes reduced breathing, without compensating mechanisms to ventilate CO_2 (19).

III) Tolerance to opioids

Tolerance is the effect of needing more drug to create the same response. Post mortem toxicology studies reveal that many who die from opioid overdose have serum concentrations far below what for others give no serious symptoms. An individual´s tolerance may change within days, and the dose that was safe before may be fatal after just a few days of abstinence (41).

IV) Use of other drugs and alcohol

The use of other drugs, especially with sedative effect such as benzodiazepines and alcohol greatly increase the risk of overdose and is a frequent toxicological finding post mortem (42, 43).

V) Concomitant disease

Autopsy and toxicological examination of deaths from opioid overdose often does not find a definite cause of death: Many are experienced opioid users and do not have serum concentrations above what may be considered safe. Drug users often have poor somatic health, with increased rates of hepatic and

pulmonary disease. They are also prone to infections. These factors all increase risk of dying from opioid intoxication (44, 45).

1.1.3 Overdose treatment and naloxone

The reduced consciousness and slow breathing of opioids may be counteracted by simply stimulating the patient, such as shouting to or pinching. In deeply unconscious patients, basic first aid, with airway control and ventilation will secure oxygenation and normalisation of carbon dioxide, thus preventing brain damage and cardiac arrest. However, unlike most poisonings, the opioids are antagonised by an efficient and safe antidote; naloxone. Naloxone is an opioid antagonist, a synthetic congener of oxymorphone. The chemical formula is $C_{19}H_{21}NO_4$ and the chemical structure is presented in Figure 1-2. Naloxone is a competitive antagonist of μ, δ and κ-opioid receptors, and it is most potent at the μ-receptor.

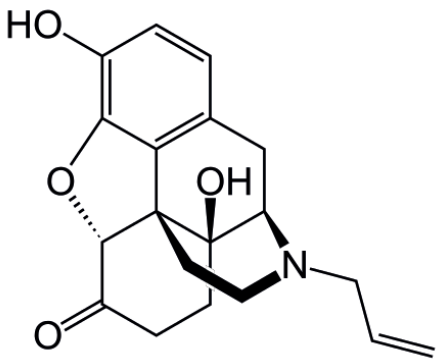

Figure 1-2: Chemical structure of naloxone

Source: https://en.wikipedia.org/wiki/Naloxone

Naloxone was first described in the early 1960s (46, 47). It was patented in 1966 and received an FDA approval in 1971. Naloxone is listed in the WHO list of "essential medicines", and rapidly reverses the effect of morphine and other opioids. Naloxone has not been shown to produce tolerance, or to cause physical or psychological dependence (48, 49). Naloxone by itself has few, and mostly mild side effects (50), and a wide range of safe dosing from 0.02-10 mg (51), with patients having received several thousand milligram/ 24-hours for research without toxic effects (52). Naloxone is traditionally licensed for intravenous, intramuscular and subcutaneous administration. Endotracheal and nebulized administration is described, but these are rare, and not relevant for routine clinical use (53-56). Naloxone is not

suited for per oral administration due to high first-pass metabolism in the liver where it is metabolised through glucuronidation.

Naloxone is widely used in both pre-hospital and hospital medicine. It has been available in various generic injectable forms for decades, most commonly in concentrations of 0.4 and 1.0 mg/mL it is considered being a low-cost drug (15). With renewed interest and more naloxone products entering the market the price has increased (57). Other compounds also act as antagonist on the opioid receptor and must be considered for treating opioid intoxication. Naltrexone is longer-acting but has a much slower uptake than naloxone and is therefore unsuited for emergency treatment. Its use in long term treatment for opioid dependence disorder is debated (58). Samidorphan is a novel antagonist under clinical testing for abuse deterrent and for depression (59, 60). Nalmefene has a much longer half-life than naloxone and is approved for opioid overdose reversal in the US and the treatment of alcohol dependence in Europe (61). It is not available in the market, and does not seem to have any industry support for expansion into the field of opioid overdose (62). Nalmefene has been shown to be as efficient as 2 mg naloxone IV in reversing acute opioid overdoses (63). Methylnaltrexone is designed not to enter the central nervous system. This antagonises peripheral opioid receptors and counteract effects such as opioid induced constipation (6).

Naloxone has also been considered for indications other than reversal of opioid overdose. There have been recent clinical trials in humans with IN naloxone for binge-eating disorder (64, 65) with no conclusive answers, and no approved expansion of indications in marketing authorisations. Naloxone has been given in humans in massive doses of several thousand milligram / 24 hours for assessment protection against neurological damage after spinal cord injury (52), again without finding rationale to investigate further.

The dose of naloxone needed to treat an opioid overdose varies. Titration, the incremental increase in drug dosage to a level of optimal therapeutic effect, is the cornerstone of treatment with this antidote. It has a wide therapeutic window in that it is safe and non- toxic. However, in opioid dependent patents it can trigger acute

withdrawal symptoms (66). Intramuscular administration gives less withdrawal than IV due to the lower maximum concentration. The medical literature reflects this dosing range and titration principle with recommendations for starting dose ranging all the way between 0.02 and 2.0 mg IV (67). This balancing act between too low and too high doses has implications both for local treatment protocols and also for new naloxone formulations or other treatment options to be investigated.

1.1.4 Developments in overdose treatment

Naloxone is traditionally a prescription drug, although this is changing in some jurisdictions. As it has been available in injection-only formulations, administrating naloxone has required formal training and specialised equipment for parenteral administration. Over the last decades there has been a tendency of changing several medicines from being prescription-only to over-the-counter drugs, and make them easily available for the patients or lay people. Examples such as adrenaline autoinjector, buccal midazolam and levonorgestrel for emergency contraception has proved safe and efficient (68-70). Naloxone is a safe antidote and treatment for a potential life-threatening condition, there has been a considerable push to make it more available close to the overdoses. The aim has been a safe and simple form of administration through Take Home Naloxone (THN) programs. Take Home Naloxone has become widespread over the last 10 years, and is now part of large public health programs across the world, in contrast to the early resistance by policy makers and industry 20 years ago (71). A thorough review using the Bradford- Hill criteria for causation shows that THN programmes can reduce overdose mortality among both programme participants and in the community, and have a low rate of adverse events (14). THN programs have used both naloxone for injection and for intranasal administration, with all IN naloxone use being “off-label”. The lack of basic pharmacologic knowledge of IN naloxone and no approved IN formulations led to increased research and development work in this field from 2010, see figure 1-3.

Non-injection routes were early identified as a potentially suitable alternative to injection of naloxone, as it requires little training, and remove any risk of sharps-injury or exposure to blood. The intranasal route has been favoured due to its simplicity, but sublingual administration is also explored (72, 73).

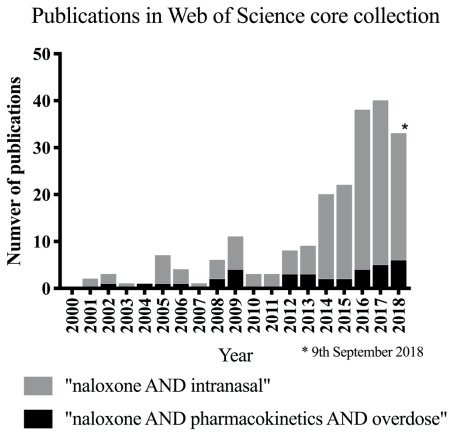

Figure 1-3: Published science 2000-2018

The British Medical Journal mentioned distributing naloxone as a harm-reducing strategy in the early 1990s, without discussing route of administration (74, 75). Activists and grass-root organisations in the addiction field started unofficial distribution of injectable naloxone at this time. In the next 20 years the field moved slowly, with several programs around the world handing out various naloxone formulations for IN use to drug-users, or others that may witness an opioid overdose. The “off- label” naloxone formulations had unknown uptake, duration of action, or type and frequency of adverse events. However, early studies indicated an effect (76). In general, such “off label” use is shown to increase adverse events, this has implications for patient safety (77, 78). It also raises ethical concerns exposing patients to undue risks (79). All the IN naloxone used was relatively low in concentration (1-2 mg/ mL) and large in volume (1- 5 mL). Such large volumes are unsuitable for IN administration as the nose can only take 0.1-0.2 mL of fluid for systemic uptake (80). Intranasal naloxone needs to be high-concentration and low-volume to secure rapid enough uptake and reverse the respiratory depression. IN naloxone must also have a duration long enough to reduce the risk of re-intoxication. Early studies indicated a very low bioavailability of IN naloxone, as little as 4% was reported in 2008 (81). However, the data were too weak to establish an authoritative nasal naloxone bioavailability. There was very little knowledge of the basic pharmacology of IN naloxone in opioid overdoses. Nevertheless, early epidemiological studies suggested a decrease in opioid mortality in areas IN naloxone were distributed to users (82) and open randomized trials of a dilute naloxone

formulation in Australia showed that it performed well compared to IM naloxone (76, 83). The WHO produced an expert report in 2014 (15) describing key research questions in the field of naloxone treatment of opioid overdoses outside of hospital. They concluded: *"People likely to witness an opioid overdose should have access to naloxone and be instructed in its administration…".* This recommendation was followed by a call for research regarding the optimal dosing and formulation for the intranasal route of administration. The WHO concludes that this could be addressed by a pharmacokinetic study, or tested in a randomised controlled trial.

In the last 10 years, more research has become available in the public domain, and this field of medicine has moved rapidly forward. Important bodies such as the FDA, WHO and others have actively been supporting new treatment options to be developed.

1.2 Drug development

New drugs, or new drug-formulations, need approval before market launch. In Europe this is governed by the European Medicines Agency (EMA), and in Norway the national competent authority is the Norwegian Medicines Agency (NoMA). Drugs also need to be produced within strict standards (Good Manufacturing Practice, GMP). Without involvement from the pharmaceutical industry, new products are unlikely to come to the market, as the know-how and production facilities are unavailable to academic, or other, institutions. Naloxone is a well-known drug, with an excellent safety profile. New routes of administration and new formulations for a similar indication can therefore be developed and approved on the basis of pharmacokinetic studies alone, omitting larger randomised control trials (15).

Such pharmacokinetic studies are common when approving generic products, with the same active drug delivered through the same route. The regulatory demand is that two such products can demonstrate bioequivalence. The most common pharmacokinetic values compared are AUC and C_{max}, but others can be added if clinically relevant. However, bioequivalence does not imply pharmaceutical equivalence, especially comparing two different routes of administration. To overcome this, the FDA concluded that any new IN naloxone product needed to match or exceed the pharmacokinetic

parameters of C_{max} and T_{max}, compared to IM, especially in the first few minutes after administration. This is important, as the indication for use is respiratory arrest, where rapid effect is paramount. In addition, new IN naloxone formulations need to show similar AUC to be approved (84).

1.2.1 Pharmacokinetics

The study of pharmacokinetics (PK) is fundamental in drug development.

Concepts central to PK are AUC, C_{max}, T_{max} and bioavailability. These are used to understand an individual drug, and to compare drugs with each other. Any new drug-formulation entering the market must evaluate these concepts as a part of the efficacy and safety evaluation. Other PK concepts such as clearance, volume of distribution and half-life are also interesting, but of less importance when comparing drugs already known.

Important aspects to the design of PK studies are the choice of administration (route and relevant clinical doses), the choice of subject (healthy volunteers or patients with relevant disorders) and choice of methodology (sampling and chemical analysis, study conduct, and statistics) (85).

Non-compartmental analysis and population PK modelling

By measuring the concentration of a drug in one part of the body (commonly blood), PK studies make presumptions about the time-course of the concentrations in the body as a whole, and at the site where it exerts its effect. This set of analysis is called non-compartmental analysis (NCA) and is based on algebraic equations. They represent the values from measurements in one individual, and can calculate maximum and total exposure (C_{max}, T_{max} and AUC), clearance, bioavailability, volume of distribution and half-life. Such calculations are quick to perform, as they are robust and simple, but they represent a very simplified model of the human body. However, the body is a much more complex system, with drugs passing through various tissues differently, some drugs hardly leave the blood stream (warfarin) and others rapidly diffuse everywhere (chloroquine). To reflect this, more complex models are developed in science.

Compartmental PK analysis is based on differential equations to describe the PK curve. This is accomplished by using theoretical “compartments”, such as blood, fatty tissue and brain, and the transfer-rates of drugs between these. They calculate the same parameters as non-compartmental analysis, and can be expanded to simple simulations. Both of these techniques are limited to one individual at the time, and extrapolations to larger populations are difficult. Modern computing has given rise to population PK analysis. This is less rapid and much more complex. More expertise is required for this analysis, but it has many advantages: Population analysis can be both descriptive and predictive, and alter between various routes of administration, and various dosing schedules. They can combine data from different studies for increased power. Population PK models do not need as rigorous a sampling schedule as NCA analysis, but can analyse on samples taken at different time points at each participant. Population analysis can be combined with PD data for comprehensive models of drug effects. Such models are continuously more complex, and increase our understanding of individual drugs. As more data is put in, often combined with parameters about the individuals tested, they can more precisely predict the PK values across populations.

1.2.2 Pharmacodynamics

Naloxone has no measurable physiologic effects in the absence of opioid agonists. To study the PD of naloxone, the co-administration of naloxone with an opioid agonist is necessary. Previous trials on naloxone PD shared a common limitation; antagonism was not studied under steady-state agonist influence. If the opioid agonist is given orally (86, 87) or as a bolus IV (88), the pharmacokinetics of the agonist will seriously confound the measured antagonistic effects. It is impossible to tell if the outcome measured is a result of antagonism by naloxone, or a result of the changed concentration of which ever opioid-agonist is used.

Opioids exert their effect in the central nervous system. Pain relief is the chief indication for their use in clinical practice. They cause respiratory depression, the prime worry in overdose. In addition, they cause miosis of the pupil in humans. These effects are all candidates for PD measurement. There are several modalities to study pain, such as the cold pressor test, pressure algometry electric stimulation and heat pain threshold (89-92). The study of pain is difficult, as it is a subjective sensation, and has several

confounding factors. The measure of respiratory effort is clinically highly relevant for opioid overdose research. Simple measurements, such as counting number of breaths per minute or oxygen saturation, are easily confounded, as any stimulation of the research subject may counteract the depressive opioid effect. More complex measurements, such as concentration of expired end-tidal carbon dioxide or intercostal muscle electromyography, may give a detailed picture (48, 93). With all studies of respiratory depression in volunteers there are safety concerns for participants. Pupil size is therapeutically somewhat irrelevant, but diagnostically essential, in opioid overdose (94). It is easily and non-invasively measured, and frequently used in the PD study of opioids (95-98).

In opioid-dependant individuals, revoking opioid agonism may precipitate acute withdrawal reactions. This can be achieved rapidly, by administering an antagonist, or more gradual, by not administering more agonist to someone dependent on opioids. This is recognised by nausea, diarrhoea, lacrimation, yawning, tachycardia, dilated pupils, agitation, restlessness and anxiety, and other symptoms (99). These affects are also candidates for PD outcomes, as they can be measured through subjective reporting or objective measurement. They have been used for research in the past (95, 100).

1.2.3 Good Clinical Practice and regulatory approval

All pharmacological studies on humans must rigorously conform to the standards set by the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH), and Good Clinical Practice (GCP) (101). This is an international ethical and scientific quality standard for design, conduct, performance, monitoring, auditing, recording, analysis and reporting of trials. It is also incorporated in the Norwegian and European statute books (102, 103). The purpose of these guidelines is both to protect the well-being of participants in trials, but also to safeguard scientific rigor and data credibility. It is mandatory for all trials that report to regulatory authorities such as NoMA, and it may also be applied to all clinical investigations. There are no differences in the demands put on pharmaceutical industry and academic institutions, in regard to adherence to central rules and regulations. NoMA provides both scientific and regulatory advice in drug development. Our research group has had several advisory meetings with NoMA, regarding study

protocols and various challenges relating to the studies reported in this thesis. Examples are sample size, early phase absorption of IN naloxone, repeat administration and adverse events.

1.2.4 Investigational Medicinal Product

The intranasal naloxone used in our trials is an innovation and had prior to June 2018 no approval for clinical use on patients. However, it received approvals for the use in clinical trials. We used 8 mg/mL naloxone hydrochloride delivered in 0.1 mL volume in article 2, and 14 mg/mL naloxone hydrochloride in 0.1 mL volume in article 3. In both studies we delivered the drug nasally with the Aptar Unit Dose device (Aptar, Louveciennes, France) (see figure 1-4). There are strict regulations as to the documentation for use of study drugs on humans. The formulation is designated as an Investigational Medicinal Product (IMP), distinguishing this formulation from medicine holding a Marketing Authorisation. For IMPs information outlining the chemical constituents, indication, dosing and administration, and adverse events of a drug must be provided by the trial's Sponsor in the form of a larger document, known as an Investigational Medicinal Product Dossier (IMPD). In addition to the IMPD produced by NTNU and the industry, IMPs used in clinical trials are required to have an Investigator's Brochure (IB). This holds clinical and non-clinical data on the IMP that are relevant to the study. Its purpose is to provide the investigators and others involved in clinical trials with this information, facilitated for a more general audience than the IMPD. The IB is distributed to the Ethics Committee, study sites and others. The IMPD holds extensive chemical-pharmaceutical data, and there are some overlaps between the two documents. The IB should provide information on the clinical management of

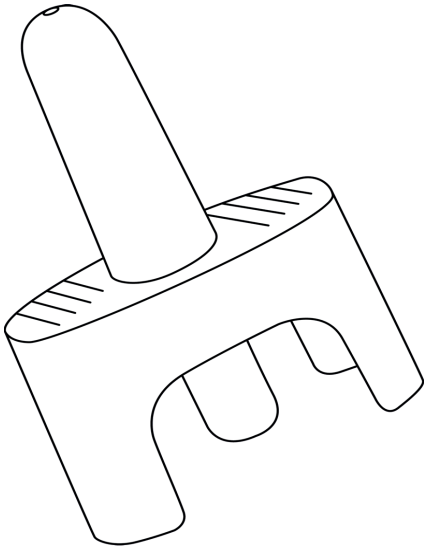

Figure 1-4: The Aptar Unit Dose device used for IN administration of naloxone.

Illustration: Øystein Horgmo, University of Oslo

subjects exposed to the IMP during a trial. Both PK studies in this thesis have produced IMPDs and IBs with updates—that is part of the body of scientific work not presented in this thesis

Article 2 and 3 answer central questions relating to the pharmacokinetics of naloxone, especially administered via the IN route. Article 2 also measures pharmacodynamic data. Article 3 is the study that formed the basis of the approval of Ventizolve naloxone nasal spray by the medicinal authorities (104).

2 Main research questions

We identified two main topics with corresponding research questions to be answered in this thesis:

I) Knowledge of the patients and the circumstances surrounding an overdose is important to prevent overdoses, develop harm-reducing strategies and new treatment options.

   i) *What are the characteristics of patients treated with naloxone by the Ambulance Service or others in Oslo and where is the locations of the overdose?*

   ii) *How are overdose patients followed up after treatment with naloxone?*

II) Pharmacokinetics and pharmacodynamics in human volunteers are essential for the development and approval of a new naloxone formulation.

   i) *How does 0.8 mg intranasal naloxone compare to 0.8 mg intramuscular in volunteers exposed to an opioid with respect to pharmacokinetic and pharmacodynamics outcomes?*

   ii) *Is the systemic exposure of an intranasal dose of 1.4 mg of naloxone equal to the intramuscular dose of 0.8 mg in healthy volunteers?*

3 Material and Methods

There are two main scientific methods included in this thesis work. The first study is an observational cohort study with methodology from the field of epidemiology. The second two studies are open, randomised, controlled, crossover trials of the IMP in healthy volunteers, a method well known within pharmaceutical science.

3.1 Article 1: Overdose cohort study

This study is a cooperation between NTNU, Oslo University Hospital and Norwegian Centre for Addiction Research (SERAF), University of Oslo. The database was established with the aim to characterise opioid overdoses, evaluating the distribution of naloxone from the Norwegian Directorate of Health (105), form the basis for the design of a randomised clinical trial of nasal naloxone in pre-hospital overdoses, and the long term coupling with the data with the Norwegian Cause of Death Registry (DÅR). Only the first of these four aims is a part of this thesis. The present article is aimed at informing the international debate regarding the use of Safe Injection Facilities. It is a cohort study, assessing outcomes in patients with one common exposure: being administered naloxone outside of hospital (sampling) (106, 107). This allows us to calculate relative risks in addition to epidemiological data (108). There are no external control group in this study. The cohort study is approved by REC (registration 2014/140), with data collection from 2014 trough 2018, and a 10-year coupling with DÅR until 2028.

3.1.1 Setting and participants

The patients included in this study are cases where naloxone has been administered by any route, and at any dose, and the patients are being treated by ambulance staff based at the Oslo City Centre Ambulance Station. Where bystanders have given naloxone, and this is recorded in the ambulance medical record, the case is included in the study. Participants below the age of 18 are excluded. Patients who were under the influence of opioids, but not administered naloxone, were excluded. This study had no sample size calculation. Data analysed in this thesis include cases in the years 2014 and 2015.

The case-collection has been consecutive in that all identified patients are included. Inclusion started 6th June 2014, with patients retrospectively identified being registered anonymously from 1st January the same year, and prospectively from inclusion start with patients registered de-identified, with an opportunity to withdraw from the database.

3.1.2 Data management, data sources and variables

The Oslo and Akershus Ambulance Service (renamed Oslo University Hospital Ambulance Service from November 2018) had a total of 130 884 missions in 2014, 29 260 of these from the Oslo City Centre Station. In 2015 the numbers were 140 474 and 27388 respectively. The service has a total of 15 stations, five in the city of Oslo. The majority of overdose cases are treated by ambulances being based at the Oslo City Centre Station. To ensure a feasible system for data collection, we focused our attention to this station. The service has a paper-based medical record system, with no electronic logging of interventions, administered medication or condition treated. Ambulance workers were instructed to file all medical records where naloxone had been involved in a separate collection box. All patients treated with naloxone were given an information letter with information about how to withdraw from the database. Withdrawal from the database were done by calling a telephone number and asking for the case not to be included in the database. Annually, members of the study team looked manually through all medical records for a given period, to identify misplaced naloxone medical records. Data sources in this database are medical records and the Medical Dispatch Centre registration for the identified ambulance mission. Collected data are manually entered into a database by a study nurse. This nurse received study-specific training, and data was extracted using pre-defined criteria. Missing data was not imputed. Ambiguous data were discussed and decided between study nurse and research team. No interrater reliability assessment was performed. The electronic data management system used was VieDoc version 4 ™ (PCG Solutions, Uppsala, Sweden). This system had a complete audit trail. Risk-based data monitoring and source data verification of key variables was conducted. The variables registered are:

i) Demographic: age and gender
ii) Tactical ambulance data: response times and total duration of dispatch

iii) Location and temporal data relating to overdose
iv) Clinical data relating to level of consciousness, respiratory effort before and after treatment with naloxone
v) The amount of naloxone given: number of doses and routes of administration
vi) Follow up after care
vii) Available information about type of opioid and intention of overdose, if known.

Date of birth and social security number are retained for later coupling with the Norwegian Cause of Death Registry. The database consists of a total of 377 unique variables.

3.1.3 The outcomes studied in Article 1

I) Demographic variable: age and gender of patients meeting the case definition

II) Temporal data regarding date and time of event

III) Decision to "Transport" or "not transport"

The decision by ambulance staff not to transport patients on to further care after naloxone treatment, are set out in their treatment guideline: *"Hospitalisation in case of overdose with long-acting opioids or if the patient does not wake up adequately. If the patient is in a bad general condition or is obviously ill for some reason, the patient should be hospitalised / referred to the emergency department. If suicidal attempt is suspected the patient should be evaluated by a physician" (109).* On this basis we included the dichotomisation of the follow-up variables, to catch a broad-spectrum of conditions and circumstances that were of value to evaluate the pre-hospital treatment, i.e. the naloxone effect and the clinical state of the patient.

IV) Places of overdoses and their definition

Rather than analysing geographically on postal codes or city districts, we have coded this variable as:

i) Public place-outdoor e.g. park or street
ii) Public place-indoor e.g. indoor car park, underground walk-way, public toilet

iii) The Safe Injection Facility
iv) Overnight shelters and other drug-user facilities and services
v) Health institutions and medical centres
vi) Private home
vii) Other
viii) Unknown

V) Clinical data

Patients were assessed clinically prior to naloxone treatment. The study variables relate to the toxidrome of opioids: reduced consciousness, reduced breathing and miosis. There is no clear set of clinical findings that trigger the administration of naloxone or define a level of intoxication in need of treatment. Life-threatening respiratory arrest and unconsciousness are obvious indications for naloxone treatment. There are also patients under opioid influence with adequate respiration, but inadequate ability to take care of themselves due to opioid induced stupor. This is often recognised in public areas where passers-by are concerned and alert emergency services, who sometimes administer naloxone, other times not. An increased severity of the clinical state indicates an urgent need for basic first aid and naloxone treatment. Deeper unconsciousness and slower respiration may be influenced both by the dose of opioid ingested, and the time from overdose to recognition and arrival of emergency staff. Clinical data are recorded as Glasgow Coma Scale (GCS), a clinical scoring system of level of consciousness. Points are given for response of eye movements (minimum 1 for no response and max 4 for open eyes), verbal response (minimum 1 for no response and 5 for orientated response) and motor response (minimum 1 for no response and maximum 6 for obeying commands). This gives a score of lowest 3/15 and highest 15/15 (110). A score below 8/15 is considered a critically low level of consciousness, and requiring urgent medical intervention (111). In patients where intoxications, rather than head injury or trauma, is presumed to be the cause of the reduced consciousness interventions such as endotracheal intubation may safely be held back (112). Respiratory frequency is reported as number of breaths per minute. A rate of less than 10 breaths per minute is classified as bradypnea, and could warrant medical intervention (11). In this dataset respiratory rate is reported based on the ambulance personnel's counting at the scene, where guidelines state that they should count for up to a minute.

If no spontaneous breaths are seen within the initial phase of observation with physical stimulation of the patient in the presence of a free airway, breathing support is advised (111).

3.2 Article 2 and 3: Methodology of pharmacological studies in volunteers

The crossover design is common in pharmaceutical sciences. As each person acts as his/her own control, the number of individuals included can be reduced while maintaining statistical power. By doing the same measurements in the same individual, with only the intervention differing, the results are easily compared. Article 2 and article 3 both report two separate trials, comparing naloxone administered through different routes and at different doses.

Article 2 reports a NTNU sponsored, open, randomised, two-way crossover study exploring pharmacokinetics and pharmacodynamics of nasal naloxone. Article 2 is REC approved as 2014/740 and by NoMA with EudraCT number 2014-001465-27. It was registered in ClinicalTrials.gov: NCT02307721.

Article 3 was sponsored by AS Den norske Eterfabrikk, and reports an open, randomised, four-way crossover study. Article 3 is REC approved as 2015/1285 and by NoMA with EudraCT number 2015-002355-10. It was registered in ClinicalTrials.gov: NCT02598856.

Both studies conformed to the ICH-GCP standard.

3.2.1 Choice of comparator

We have compared naloxone in the most commonly used routes of administration in clinical practice, IV, IM and IN. The dosing range on naloxone is wide, from 0.02 to 2.0 mg as starting doses. Regardless of this broad spectrum of dosing, 0.4 or 0.8 mg are common starting doses in today's clinical practice in Norway. We have used 0.8 mg as our IM comparator, and 0.4 mg as our IV comparator. IM 0.8 mg is the upper end of the

WHO recommended starting dose, and it is known to be successful in 88% of naloxone reversals at Oslo City Centre Station (15, 113).

3.2.2 Naloxone analysis

The basis of PK studies is the quantification of the concentration of the drug in question. The measurement of naloxone is not a routine test in most laboratories. Earlier published science has used methods more insensitive to the naloxone, a serious limitation to those reports (81). It is important to have a stable, accurate method with a low coefficient of variation, and that the limit of quantitation is lower than the clinically relevant concentrations. The NTNU laboratory at Proteomics and Metabolomics Core Facility (PROMEC) developed a validated high-performance liquid chromatography tandem mass spectrometry (LC-MSMS) method that is sensitive, and specific determination of naloxone in human serum used in article 2. This method is previously published in full (114). The study reported in article 3 had more strict regulatory demands, and PROMEC were not certified with the Good Manufacturing (GMP) and Good Laboratory Practices (GLP) required for laboratories that provide work to support an application for marketing approval. Naloxone analysis for article 3 was therefore performed by Vitas AS, Oslo, Norway, a GMP certified chemical analysis contract laboratory. This was also an LC-MSMS method, but used blood plasma, rather than blood serum as matrix for analysis. The precise analysis methodology is presented in the articles and their supplemental material.

3.2.3 PK Sampling schedule

Both studies have similar crossover designs and sampling schedule. When the same participants receive the same treatment several times it is important that the treatment periods are separated by a wash-out period sufficient to ensure that drug concentrations and effects from the first treatment are eliminated in all subjects at the beginning of the next period. Normally, at least 5 elimination half-lives are necessary to achieve this (115). The present studies had a minimum 72 hours washout period, which is about 50 half-lives of naloxone. To adequately describe the time-course of the drug concentrations in blood, samples need to cover about 80% of the AUC (115). This means that the time from the first to the last sample is long enough to capture almost all

of the excretion phase. At least three half-life times is necessary, our design has four. A calculation of the ratio $AUC_{0\text{-last}}$ to $AUC_{0\text{-}\infty}$ will show if the sampling time was long enough. Regarding sampling in the absorption phase, samples need to be closer together to capture the rapid shifts in concentrations during this phase. The distribution phase needs less frequent testing than the absorption, but closer than the late elimination phase samples. On the basis of all of this the sampling schedule in our trials have been 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240 and 360 minutes after naloxone administration, with one sample prior to administration. This sample prior to administration is to confirm adequate wash-out and to exclude any other signals in the mass spectrometry that may confound interpretation and analysis.

3.2.4 Non-compartmental analysis

Both articles make use of non-compartmental analysis for the central PK variables; AUC, C_{max}, T_{max}, half-life, clearance and volume of distribution. These are calculated in WinNonlin Standard version 6.4 (Pharsight Corporation, New Jersey, US) in Article 2 and Pmetrics (version 1.5.0, Laboratory for Applied Pharmacokinetics, California, US). in article 3. AUC was calculated using the trapezoidal rule in both articles. Article 2 and article 3 reports results for naloxone hydrochloride, rather than free base.

3.2.5 Population PK modelling Article 3

Article 3 has developed a non-parametric pharmacokinetic population model for IN and IM administration by using Pmetrics. This model is used to predict the PK curve for dosing regimens not studied directly in the article, and for discussions of various THN scenarios with both IN and IM naloxone at different doses administered at different times.

3.2.6 Steady state opioid agonism

Steady state opioid agonism is important in clinical practice, particularly in anaesthesia. The development of microprocessor-controlled syringe pumps and a target- controlled infusion (TCI) have created a system that achieves a pre-set drug concentration in a selected body compartment. Multicompartment pharmacokinetic-dynamic models are used by TCI systems to calculate the infusion rates required to achieve the *set target*

concentration (11). This system, using the potent and ultra-short acting opioid remifentanil has been used to study PD of opioids previously (116-118).

In article 2, the initial *set target concentration* of remifentanil was 2.5 ng/mL. This concentration was too high to elicit a clear response on the PD outcomes studied. This is particularly apparent in the hysteresis curve for 0.8 mg IN naloxone at the 2.5 ng/mL target in article 2, figure 5 (see attached article). This curve shows very small changes in pupil size, despite naloxone administration. As this was an explorative study the set target concentration was reduced to 1.3 ng/mL for five participants and 1.0 ng/mL for three participants. The crossover design was kept, and the protocol change approved by NoMA.

3.2.7 Pharmacodynamic measurements article 2

An optimal study design is one in which variables are chosen, so as to maximise the information that can be obtained. This increase the scientific yield and has an ethical dimension, in that fewer subjects are required (119). For this reason, we wanted to explore naloxone beyond the serum concentrations in the same study, and added PD outcomes to the PK outcomes.

Two PD measurements were chosen: Pupillometry and Heat Pain Threshold.

Pupillometry is a non-invasive, pain- and risk-free measurement. Miosis is a very important diagnostic marker for opioid overdose (94). It is frequently used in the PD assessment of opioids (95-98). Pupil size was measured using a Neuroptics VIP 200 Pupillometer, under similar, low ambient light-conditions, at all visits.

We used Heat Pain Threshold (HPT), as this had previously been used to study the PD effects of remifentanil (91, 92), and because analgesia is the prime indication for the use of opioids in clinical practice. Heat pain threshold were measured using the Somedic MSA Thermotest, an apparatus measuring the relationship between the intensity of controlled thermal stimulus, and the associated perception.

3.2.8 Adverse Events Recording

Adverse events (AE) were recorded in each study. The definition of adverse events and Serious Adverse Events (SAE) followed GCP definition. In article 2, adverse events were reported using the Common Terminology Criteria for Adverse Events version 4.0. Article 3 reported adverse events using Medical Dictionary for Regulatory Activities (MedDRA). In addition to self-reporting and normal clinical observation during the study period, participants in article 3 underwent anterior rhinoscopy by an ear, nose and throat specialist prior to, and after treatment with IN naloxone, to exclude any local damage to the nasal mucosa. Local irritation, pain or loss of smell was also specifically asked for in the AE assessment.

Comparator-naloxone for injection, and remifentanil, are drugs with marketing authorisation, and adverse events following their administration have been recorded in the same fashion as for the IMP.

3.3 Statistics and power calculation

We used different statistical methods in each article in this thesis. Descriptive statistics uses mean or median as description of central tendency, and 95% confidence interval, standard deviation or interquartile range (IQR) as measure of variability. Non-parametric tests are used when data are not normally distributed. A p-value of less than 5% was considered significant for all articles.

Article 1

In article 1 we used descriptive and comparative statistics as appropriate for the data.

Chi-square test was used to compare frequencies, and Fisher's Exact Test was reported when expected cell frequencies were less than five. The Mann Whitney U-test was used to compare each overdose location. Kruskal-Wallis test was used to compare continuous variables (Glasgow Coma Scale scores, respiratory rates, age, and time of the overdose) among each of the various locations. A logistic regression analysis was done to explore predictors for being transported for further medical treatment.

Article 2

We used descriptive statistics for demographics of participants. For bioequivalence, log-transformed data and a sample t-test is used. The PD data in article 2 is displayed based on a mixed linear model, shown in figure 2, 3 and 5 in the original paper, see article 2.

Mixed models divide variables into fixed and random effects. The fixed effects are the data of most importance, where we expect the variability and value to count the most. Time and treatment type were chosen in the current study as fixed effects. The random effects account for some variation but were of less importance. Participant ID were the random effect in this article.

The results of the mixed model were then compared between IM and IN treatment using a likelihood ratio test. The questions answered by the likelihood ratio test was whether PD data (pupil size or HPT measurements) changed from the point of naloxone administration (nadir) to t=90, for both IN and for IM and if the two treatments were different from each other. Time points prior to naloxone administration and after remifentanil cessation were not included in this analysis as we wanted to investigate the antagonistic effects of naloxone, and due to remifentanil´s ultra-short half-life we did not expect any effects after t=90 minutes. Where a statistical difference was found between IN and IM, a Wald test were conducted at that specific time point.

There was no power-calculation for the study reported in article 2, but the number 12 is a recommendation from EMA for such studies (120).

Article 3

In article 3, we followed the current bioequivalence guidelines from EMA (115), and used Analysis of variance (ANOVA) as its main statistical method, where appropriate for the data.

ANOVA is applied to compare where there are three or more groups. To pairwise compare treatments, Tukey’s honestly significant difference (HSD) test is used in

conjunction with ANOVA. A standard t-test was used to pairwise compare two groups, when only two groups were studied, such as for T_{max}.

Sample size for this study was based on bioequivalence criteria, which is based on calculation of the AUCs for the different administration methods. It was scaled to ensure the confidence interval would cross 100%, not be inferior (81%-99%) or superior (101%-124%). The calculation included the standard deviation for the IN:IM AUC ratio in article 2, and found it necessary to include 22 participants in the study

4 Results and summary of articles

4.1 Article 1

Ambulance-attended opioid overdoses: an examination into overdose locations and the role of a safe injection facility

This is a prospective cohort study, collecting cases where naloxone is administered in Oslo City Centre. The aim of the study is to describe patterns, severity, and outcomes of opioid overdoses, and compare these characteristics among various overdose locations, with particular focus on the Safe Injection Facility.

The 1054 overdoses cases analysed compromised 465 individual patients. The median age was 35, with 79 % being men.

I) Decision on further transport after naloxone

Overall 61% of patients treated with naloxone did not receive further care after being in contact with the ambulance service. However, there are significant difference in the follow-up based on the location of the overdose. 52.5 % of patients overdosing in public locations were transported onwards, whereas only 14.4 % of SIF overdoses were transported to additional care. Overdosing in public or outside of SIF opening-hours were strong predictors for being transported by the ambulance service.

II) Location

One third of overdoses occurred in the SIF, and half in public locations. Only 7.9% of the overdoses occurred in private homes. Clinical findings differed between various locations.

III) Clinical findings

Median GCS was 3/15 among all patients, with those in SIF and private homes being significantly lower than those in public places. Median respiratory rate was 6 breaths per minute, with the lowest rates being found in the SIF and private homes.

Conclusion

The main finding of article 1 is that the opening hours of the Oslo SIF impact on the location of overdoses in the city, and that patients overdosing in the SIF have a more severe clinical presentation than those found in public places. Patients treated with naloxone in private homes have the most severe clinical presentation of all cases studied.

4.2 Article 2

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

This was a phase I, randomised, open, two-way crossover study comparing naloxone given as 0.8 mg IM (2.0 mL) and 0.8 mg (0.1 mL) IN in healthy volunteers. The opioid remifentanil was administered as a Target Controlled Infusion (TCI) for 102 minutes. The aim of the study was to explore the pharmacodynamic profile of the antagonistic effects of naloxone, compared between IM and IN administration. Secondary endpoints were to estimate the relative bioavailability of IN to IM naloxone, and pharmacokinetic variables of IN naloxone under opioid influence. Remifentanil were administered at three different set target concentrations, subdividing the study population into three groups, with the aim of exploring which TCI target was best suited to study naloxone at the chosen doses.

Participants:
Nineteen volunteers were screened for inclusion; five did not meet the criteria, 14 were included. One withdrew consent, and one started medication that lead to exclusion; both prior to randomisation. Twelve participants were randomised and completed the trial. Six men and six women, with mean age of 23.8 years and mean body mass index of 22.3 kg/m^2.

Analysis

Pharmacodynamic measurements were pupillometry and heat pain threshold. Naloxone concentrations were measured in blood serum, using a validated liquid chromatography tandem mass spectrometry method, at the NTNU PROMEC laboratory.

Pharmacodynamics

Remifentanil TCI provides good conditions for studying the pharmacodynamics of naloxone, with miosis being induced in all groups. After naloxone administration, the reversal of miosis was seen in both treatment groups, but more prominent in the IM group. This effect was apparent in the whole dataset (n= 12) and in each remifentanil subgroup. Changes in in pupil size from nadir were seen for the whole dataset, and for the 1.3 ng/ml subgroup. Difference in pupil size between IN and IM was apparent in the data set as a whole, and in all the three subgroups.

Pupillometry showed that both IM and IN changed from the nadir, and displayed differences between the two forms of administration. Heat pain threshold showed no statistical difference neither with time, nor between IM and IN. The variability in the HPT data was large, and no difference was found between the two treatments.

Pharmacokinetics

The relative bioavailability of IN to IM naloxone was 0.75. The Tmax was 7.75 minutes for IM 0.8 mg, and 28 minutes for IN 0.8 mg. Cmax was 3.62 ng/mL for IM 0.8 mg, and 1.63 ng/mL for IN 0.8 mg. AUClast (min × ng/mL) was 244 for IM 0.8 mg, and 160 for IN 0.8 mg.

Conclusion

The overall conclusion is that an IN dose of 0.8 mg is inferior to the same nominal dose IM, even when the IN naloxone is administered in a low volume/high concentration formulation. Remifentanil TCI created good conditions to study naloxone pharmacodynamics, and pupillometry were superior to heat pain threshold as PD measurement.

4.3 Article 3

Pharmacokinetics of a novel, approved, 1.4 mg intranasal naloxone formulation for reversal of opioid overdose.

This was a phase I randomised, open, four-way crossover study, comparing IN naloxone 1.4 mg (0.1 mL) once, IN 1.4 mg twice, IM 0.8 mg (2.0 mL) and IV 0.4 (1.0 mL) mg in healthy human volunteers. The primary objective was to investigate the systemic exposure and pharmacokinetic profile of naloxone 1.4 mg, compared to injected naloxone. Secondary objectives were to investigate dose-proportionality, by administering IN naloxone 1.4 mg twice in the same nostril, and to investigate the safety and tolerability of nasal naloxone 1.4 mg/0.1mL.

Participants

44 subjects were screened, 24 of these subjects were included, and 22 completed all visits. Of these were 13 men and 11 women, with average age 25.9 years, and mean body mass index of 22.5 kg/m^2.

Analysis

A total of 1138 plasma samples from 22 subjects were analysed using a validated liquid chromatography tandem mass spectrometry method in blood plasma, from Vitas AS laboratory. Non-compartmental analysis was performed for standard PK measurements. A population-based PK model was developed in order to explore various treatment and titration scenarios between IN and IM naloxone, both as a Take Home Naloxone scenario, and as used by health professionals.

Pharmacokinetics

IN 1.4 mg naloxone showed similar pharmacokinetic values as IM 0.8 mg naloxone, with no statistically significant differences in C_{max}, T_{max}, AUC_{last}, $AUC_{0\text{-}inf}$ and half-life. IV 0.4 mg showed significantly lower systemic exposure of naloxone, compared to both IN 1.4 mg and IM 0.8 mg, and also higher variability in systemic exposure, compared to the other two administration forms. Naloxone showed dose proportionality, when administered as one and two IN 1.4 mg doses in the same nostril. Both absolute and

relative (compared to IM) bioavailability of IN 1.4 mg naloxone were approximately 0.50. Cmax was 2.36 ng/mL for IN 1.4 mg, and 3.73 for IM 0.8 mg. Two IN doses showed dose linearity, and achieved a C_{max} of 4.18 ng/mL. T_{max} was reached after 20.2 minutes for IN 1.4 mg, and 13.6 minutes for IM.

PK modelling

The model compares IN naloxone to both 0.8 mg IM and with 0.4 mg IM. The model shows that plasma concentrations following IN 1.4 mg naloxone remains above the concentrations obtained by IM 0.4 mg, when given as short as 2.25 minutes earlier. It also shows that the combination of IN 1.4 mg and IM 0.4 mg gives concentration levels comparable to IM 0.8 mg.

Conclusion

IN 1.4 mg naloxone provides adequate systemic exposure compared to IM 0.8 mg, without statistical difference on maximum serum concentration, time to maximum serum concentration or area under the curve. Simulations support that IN 1.4 mg naloxone has a place both as peer administered antidote and for titration of treatment by professionals.

5 Discussion

5.1 Methodological considerations research question I

Article 1 presents analyses on some of the variables in a large database, and patients in the first two years of what in total will collect data from 2014 through 2018. There are several articles reporting on similar data, and many focus on risk factors for overdose, such as concomitant drug use, or mortality after overdose reversal. The present study is unique in that it can present data from a Safe Injection Facility. With the opioid epidemic being declared a public health crisis in the US (121), there are current important calls for SIFs to be established there (122), and also expanded elsewhere. Data regarding overdose events in a SIF compared to other locations may inform policy makers in their decisions in this field. This is the rationale for early sub-group analysis prior to end of the data collection.

The ambulance medical record holds the information required to answer both questions regarding the characteristics of patients overdosing on opioids in Oslo City Centre, and the follow up directly after treatment with naloxone. The systematic collection and analysis of these records gather data not available through other means.

A medical record review of ambulance calls is common in emergency medicine. The record is a relatively simple document with both demographic and clinical data, regarding the whole spectrum of conditions seen in the pre-hospital environment.

5.1.1 Discussions on outcomes chosen in article 1

Age, gender, date and time are basic and non-controversial data for observational studies.

Decision to "transport" or "not transport" after naloxone

Follow-up after treatment is dichotomised into "transported" and "not transported". For the purpose of this thesis and its discussion this is useful. The degree of patients being left at the scene of the overdose impact on treatment and naloxone dosing. Patients not being transported are at the highest risk of critical re-intoxication, and any new treatment must minimise this risk. For this purpose, the identification of "not-

transported" is highly relevant. If patients are transported, the destination (to hospitals or other social services) is of less importance. Patient "not being transported" must be considered to be successfully reversed from the overdose and deemed competent to make informed choices regarding their own health care, which involves refusing further care. Three main reasons to "be transported" are: 1) non-successful reversal of the reduced respiration and/or continued unconsciousness 2) need for immediate medical attention despite successful reversal and/or 3) an awake patient who accepts the offer of further follow up by primary and social care. The analysis in Article 1 does not differ between these reasons to be "transported". Although such analysis can be of value, it does not add significantly to the research questions posed in this thesis, or inform the debate surrounding Safe Injection Facilities.

Place of overdose and their definition

This division allows comparisons between types of locations, which may represent different patients within the cohort, different drug-using behaviours or other factors not differentiated by clinical presentation or demographic data. The SIF stands out as a unique environment. Comparing the overdoses happening at the SIF to all other locations can increase the understanding of how such a facility is used and the overdoses happening there.
For statistical purposes these eight location types were combined to four categories, as category v) "Health institutions and medical centres", vii) "Other" and viii) "Unknown" had very few cases. The groups are then: "SIF", "public locations" (i and ii), "private homes" (vi) and "others" (iv, v, vii and viii).

Clinical data

We have chosen GCS and respiratory rate as our main clinical outcomes. Low GCS and slow respiration rate form the core of the opioid toxidrome, together with miosis. Other measurements such as heart rate and blood pressure are often omitted when treating opioid overdoses. Where the data is available it is recorded in the database, but we have not performed statistical analysis on these data for the research questions posed in article 1.

GCS is developed as a measurement of consciousness after head injury, not for the intoxicated patient. A more coarse scale, called AVPU may be better suited to these patients. In AVPU patients are grouped into four categories based on them being "Alert", "Responsive to verbal stimulus" "responsive to pain" or "unresponsive". However, GCS it is very commonly used by ambulance personnel, who are trained in this assessment. GCS is shown to correspond to the AVPU scale, and be of value in opioid overdose (94, 123).

The administration of naloxone to patients in cardiac arrest is debated. Current international and national guidelines state that there is no place for the administration of naloxone to patients in cardiac arrest (124, 125). This means that patients treated primarily for cardiac arrest by the ambulance service are not included in this database, even though ambulance personnel may suspect opioid overdose to be the cause of the arrest. This introduces a bias relating to the ratio of non-fatal: fatal events in the material, and we have therefor not made any assumptions regarding this.

5.1.2 Statistics and power calculation

There are no power calculations or sample size estimation in this study. It is an observational study for a given time period, a geographical limited area and with a clear case definition. For the analysis in article 1, the cases are not followed over time, but analysed at the time of entry into the cohort. Baseline demographic variables are calculated to compare for external validity.

The main results for the discussion of this thesis, the comparison of clinical data at presentation, and the follow up after naloxone treatment are based on simple frequency statistics. Central tendency is reported as median, and variability as interquartile range (IQR). This is chosen as the data are not normally distributed. Consequently, difference in clinical data as continuous variables are compared, using the Kruskal Wallis H test between locations.

Article 1, table 1 gives the frequency and percentage of the important "transported" or "not transported" outcome sorted between various locations.

A logistic regression analysis was done to explore predictors for being transported for further medical treatment. This analysis was done on cases from outside the SIF, and the SIF-cases were removed from the model not to violate the independence of the group-variable presented in article 1, table 3 (attached). Disposition "transported" was the designated outcome, dependent variable, in this analysis.

5.1.3 Limitations of this study

Case definition: No accepted and uniform definition of non-fatal opioid overdose, or death by opioid overdose exist. The Centre for Disease Control (CDC) in the US operates with a definition where several criteria need to be met. Probable cases are defined as "clinical suspicion of opioid exposure…or… diagnosis of drug poisoning or drug use and one or more clinical signs of central nervous system depression … or miosis". Confirmed opioid overdose cases are defined as cases which met the probable case definition, and in addition had a positive toxicology screening result for any drug of abuse (126) in addition. The CDC also use "naloxone administration" as criteria to identify ambulance records with suspected overdose. The European Monitoring Centre for Drugs and Drug Addiction, or the Norwegian Institute of Public Health, do not have any definitions of opioid overdose. Patients treated primarily for cardiac arrest were not included in this database.

The current study has a broad case definition: "*the administration of naloxone at any dose, by any route, by anyone and documented in the ambulance medical record*". This broad definition captures everyone that has been suspected to have overdosed on opioids, based on clinical or other findings, like the CDC definition, but does not exclude patients where the diagnosis was wrong, nor does it confirm cases by toxicological analysis.

Selection bias is a common type of bias in cohort studies, specially where selection is based on both exposure, i.e., in our case, given naloxone and outcome, for example "transported after treatment". Our study is at risk to this, in that we only sample cases that have been in contact with the ambulance service. There may be cases of opioid overdoses that are given naloxone outside of hospital, and no one alerting the

ambulance dispatch centre, thus excluding the patient from registration in this study. We can only hypothesise the magnitude of this bias knowing that there were distributed 645 nasal sprays in Oslo in and 684 in Bergen in 2015. These nasal sprays are reported to have been used in total 277 times from July 2014 to December 2015 (127). If we assume the reversal rate being the same in both cities, this gives us an estimated 138 cases that have not been available for inclusion by the Oslo Ambulance Service. We only select based on exposure, not on outcome, which compensates for this bias by a certain margin.

Rate of coverage: No reliable method exists to identify all eligible cases within the Oslo and Akershus Ambulance Service. The service handled 271358 cases 2014 and 2015. The medical records are collected at 15 different locations, and sorting out records meeting the case definition in the whole service were beyond the present research project. Even though we are certain the majority are treated in Oslo City Centre, the rate of cases missed cannot be calculated. 1/3 of overdoses cases are at the SIF, which is only a few hundred meters from the City Centre Station.

External validity: Overdose epidemiology in Oslo may differ from other cities, in Norway and abroad, both in terms of the patients typically seen, and the drugs commonly used. Results need to be compared across time and geography, with similar studies to evaluate this. Table 5-1 compares key demographic variables in article 1 with previous studies in Oslo and elsewhere. The age and gender ratios are similar, and this increases the external validity of our finding. The percentage transported from the scene varies much more.

Table 5-1: Comparison of demographic variables across overdose studies.

Year	2014-15	1998-99	2003	1994-2003	2016	2016
Reference	(1)	(50)	(36)	(128)	(31)	(29)
Place	Oslo	Oslo	Oslo	Copenhagen	USA	Europe
Cases selection	Non-fatal overdose	Non-fatal overdose	Non-fatal overdose	Non-fatal overdose	Fatal opioid overdose	First time entry to heroin addiction treatment
n=	1054	1192	691	4762	42 245	-
Age	Median 35	Mean 32	Median 33	Median 34.2	Median 40	Mean 34
Male/ female ratio	79/21	80/20	70/30	75/25	67.5/32.5	80/20
% transported from site of overdose	39%	15%	20%	30.7%	-	-

Regarding direct mistakes, either by ambulance personnel not documenting correctly, or at the point of database entry, two measures have been taken. First, ambulance personnel have received information and training throughout, that medical records involving naloxone will be thoroughly examined, and that extra diligence is expected in the filling in, and correct filing, of these records. Individual ambulance workers with poor documentation have been made aware that reports are sub-standard. Secondly, the study nurse that has entered data into the database, has received study specific training. The database has a complete audit trail, and risk-based source data monitoring has been conducted on 10% of all data. This means that a random sample of 10% of cases has been manually checked for consistency between original record (source data), and what is in the database. This did not reveal any systematic errors. The VieDoc database also has built-in logical checks to avoid error: all time-points have to be in chronological order, oxygen saturation cannot be higher that 100%, to mention two. These checks are put in place to increase the reliability of the data, and reduce errors.

There are also other methods used to examine non-fatal overdoses. Direct interview with drug-users, with linkage to ambulance medical records and/or other data sources

have been used previously, also in Oslo (129). Our design, with ambulance medical records as prime data source, catches a wider net, as the included individuals are assessed clinically as in need of naloxone (case-definition). In direct interviewing, selection bias will be introduced into where, and how, such individuals are recruited. The drug user population is diverse, and contact with the ambulance service is a common denominator across the entire population of patients using opioids.

In the future, electronic patient medical records with possibility for direct data harvesting will greatly increase the data yield, and precision levels, for studies such as this.

5.2 Methodological considerations research question II

Development of new treatment options, especially drugs or new formulations that require regulatory approval, have strict and detailed guidelines from medicinal authorities, regarding the methods used. For new substances particular care is needed, as safety concerns are strong. Naloxone is a well-known drug, with an excellent safety profile. The studies conducted in this thesis use the drug within the already approved dosing interval for injected naloxone. IN naloxone is also tested for use on the same indication as previously approved; the reversal of respiratory and/or central nervous depression, known or suspected to be caused by opioids. The approval of such medication can be done based on pharmacokinetic studies alone, thus requiring fewer participants than would otherwise have been demanded. As two different routes of administrations are compared, bioequivalence cannot be expected—and the aim is to show that IN naloxone can achieve sufficiently high serum concentrations fast enough, compared to standard IM treatment.

Healthy volunteers

Healthy participants are commonly used in PK and PD studies, particularly in phase I studies. This is a step up from animal testing, but still represent a difference from the patient most drugs will be used for. Disease specific conditions such as liver and kidney changes are missed and groups such as the elderly are excluded. Drug interactions are also missed as most studies ban the concomitant use of other drugs. This limits the extrapolation value of conclusions from PK studies in healthy volunteers. In the case of

naloxone, all patients will by definition also be exposed to an opioid. In many cases they will use illegal drugs, both opioids and others. As these are produced outside of the pharmaceutical industry, are impure and mixed with a large array of substances, complex drug interactions are likely to occur, which are not evaluated in volunteer studies.

Crossover studies

The European regulatory standard design for studies aimed at bioequivalence is a two-period, two-sequence single dose crossover design with no less than 12 participants (120). This has formed the basis of our study designs. Article 2 conforms to this exactly. Article 3 is a four-way crossover as it compares the IMP twice for dose proportionality, and the IMP to both IM and IV naloxone. In the crossover design each subject act as his or her own control, and the within-subject variability can be assessed. This analysis is performed on the present naloxone formulation earlier (114). An alternative to the crossover design would be parallel group design. A parallel group design would have required a much larger number of participants. This is both ethically and economically challenging.

Injected naloxone and comparator dose.

Naloxone is a drug of titration, and a non-toxic drug with a wide therapeutic window. The dose needed to reverse an overdose will vary, as the reasons for the individual overdose are divers as described in chapter 1.1.2. The most common dosing regimen in pre-hospital overdoses is 0.4- 0.8 mg IV or IM as a starting dose, with additional doses till effect. We use both these doses; 0.8 mg IM in article 2 and 3, and IV 0.4 mg in article 3, as comparators. Population-modelling allows us to investigate 0.4 mg IM naloxone compared to IN. The comparison to 0.4 mg IM is important, as other approved IN naloxone formulations have this dose as their main comparator (130, 131).

PD measurements

Ideally, new treatment options of naloxone for pre-hospital overdoses should be tested in that environment. However, it is not always suitable to conduct phase I studies on patients, particularly when the indication is a life-threatening condition. Efficacy and

adverse event profile are often not fully established in this early phase of drug development. The main indication for naloxone is central nervous and respiratory depression. Level of consciousness and respiratory effort measurements are the prime PD variables, with real-life clinical importance. However, there are challenges in recreating this in a research facility. To achieve significantly low GCS high doses opioid is needed, as this class of drugs has a low sedating potential alone. Respiratory depression is more easily achieved. There are safety and ethical concerns with a study design that wilfully reduce functions vital to life in healthy volunteers. Such risk-taking cannot be justified if alternatives exist. Various respiratory measurements, such as counting respiratory frequency, end-tidal CO_2 or O_2, parasternal intercostal muscle electromyogram, pulse oxymetry and airflow have been used in the past (93, 132). However, respiratory effort is influenced by stimulation, and the act of repeated blood sampling for PK analysis, would muddle any respiratory measurements.

We therefore hypothesised that pupillometry and/or pain measurement by HPT could be PD variables to study antagonism.

Concomitant opioid

To study the PD effects of naloxone, an opioid agonist must be co-administered, as naloxone has very little or no physiological effect by itself. The choice of agonist was influenced by several factors. Heroin would be ideally suited as it is the most common drug in the pre-hospital setting. This has been used in research in the UK, but only on patients already in heroin-assisted opioid substitution programs, not healthy volunteers. No such programs exist in Norway at this time. The individuals in such program are likely to be at risk of acute withdrawal if administered naloxone, and the inclusion into antidote-studies can be difficult. To study the pharmacological, antagonistic effects of naloxone, a steady state of the agonist must be achieved. Single agonist administrations would not achieve this, neither would standard infusion regimens. The first would have a fall in opioid concentration due to metabolism and excretion, and the latter would be more unpredictable; simple infusion regimens can give both an increase and a fall in agonist concentration. Remifentanil Target Controlled Infusion is specifically designed to achieve steady-state and we have shown that this is achieved in our model (118). Remifentanil is ideally suited for this as it has a rapid onset and short half-life. The co-

administration of an opioid also gives a chance to study any interaction with naloxone and other drugs. However, remifentanil is rare in pre-hospital overdoses, where opioids such as heroin, fentanyl analogues and oxycodone are more common. Any extrapolations regarding interactions must account for this limitation.

In this exploratory study the balance between dose of opioid (agonism) and dose of naloxone (antagonism) was hard to predict prior to study start. The initial set target concentration of remifentanil at 2.5 ng/mL was chosen based on previous studies (116, 117) and the naloxone dose of 0.8 mg was chosen based on clinical practice. After the first three participants it became clear that changes in the PD outcomes (pupillometry and HPT) were hardly measurable at the TCI dose chosen. This led to the conclusion that the remifentanil doses needed reduction, and we divided the participants into 3 subgroups, keeping the crossover design. This decreased the statistical power of the study. However, as an exploratory study it can guide future research into opioid antagonism with similar models being reproduced with higher accuracy.

5.2.1 Statistics and power calculations

Article 2

Sample size was not based on a formal power calculation, but follows the EMA guideline for such studies with 12 participants (120). Descriptive statistics for demographic variables and naloxone concentration are reported with mean as the central tendency as they are normally distributed. Dispersion is reported as 95% confidence intervals.

For PD outcomes, the data are displayed based on a mixed linear model, rather than just mean value at each time point. The mixed model is superior to pure descriptive mean values to make conclusions and perform statistical inference. This linear mixed model is useful for analysing repeated measurements of the same variable, and where the data is non-independent. This fits the dataset of pupil size and HPT well.

Calculations for pharmaceutical interactions between naloxone and remifentanil were conducted using log-transformed data, and a sample t-test was used and followed the guidelines for bioequivalence by EMA (115). Data from the present study where naloxone was given at the same time as remifentanil were compared with results (n=12)

from a previous study, giving the same IN naloxone formulation (114). The individual results for $AUC_{0-\infty}$ and C_{max} were compared, and a statistically significant difference was found on the AUC outcome, indicating an interaction.

Article 3

Sample size for this study was based on the bioequivalence criteria on AUC. It was scaled to ensure that the confidence interval would cross 100%, not be inferior (81%-99%) or superior (101%-124%). The calculation included the SD for the IN:IM AUC ratio in article 2, and found it necessary to include 22 participants in the study

The statistics of the central PK variables adheres to the current bioequivalence guideline from EMA (115) and uses ANOVA as its main statistical method where appropriate for the data. ANOVA is used for testing three or more groups, and functions similar to multiple two-sample t-tests. ANOVA only identifies that at least two groups are different and must be followed by closer comparisons between groups. We have used Tukey's honestly significant difference (HSD) test for these comparisons between two treatments for AUC and C_{max}. The Tukey test is common in conjunction with ANOVA. The HSD is the least amount that means must vary from each other to be significantly different from each other. After the ANOVA analysis returned its results, the Tukey HSD was used in a series of comparisons between the means for each treatment, identifying which were, and which were not, statistically significant different from each other.

The T_{max} outcome is only compared between IN 1.4 mg and IM 0.8 mg. With intravenous administration, T_{max} will always be the first sample, as this route of administration has no absorption phase, by definition. Article 3 studies IN 1.4 mg x 2 for dose proportionality, and T_{max} for this treatment arm were not considered relevant. The data comparing the two remaining treatment arms with a continuous dependant variable met the assumptions needed for a t-test, which was used.

5.2.2 Limitations PK and PD studies

PK studies extrapolate expected physiological effects from measurements of concentrations of a drug. The physiological response is more complex, with several unknown factors other than just concentrations in blood. There is also variation between subjects and within the same subject that is hard to capture in smaller studies. Non-compartmental analysis is a crude measurement with a very simple model of the human body. The compartmental computing models give a better view of the kinetics of a drug. Pharmacodynamic data should as far as possible measure disease related outcomes. In our study we have end-points with limited clinical relevance. The combination of PK and PD data in multi-compartmental and population-based computer models is a powerful tool that increases the yield from the data. We use this in Article 3.

The level of precision in the chemical analysis will limit the value of PK data. Any test used must have a low enough lower limit of quantification to capture minute concentrations and a high enough to capture the C_{max}. The method of analysis must be both specific and sensitive to the compound in question. In this thesis, two different analysis of naloxone are used, both fulfil accepted quality standards set out by regulatory authorities (133, 134). These standards describe optimising the conditions for and procedures surrounding the analytical method. Concepts such as method validation, limits of inaccuracy, reference standards and quality control samples are described.

Naloxone is a well-known drug with an excellent safety profile, it has a wide dosing interval and low risk of intoxication. The IN use for opioid overdose is within the existing indication for its approval as a drug for human consumption. This makes the studies in this thesis sufficient both for scientific and regulatory use for supporting an application to medicinal authorities for licencing of the drug. Studies to prove market entry for new substances, or change of indications for existing drugs, would require even higher scientific level of the study design, including toxicological animal studies.

5.3 Discussion and interpretation research question I

Article 1 reports a study that show external validity. The typical patient is a man in his mid-thirties. This is seen across Europe and the US. The overall conclusion is that the Safe Injection Facility reduces overdoses in other locations during its opening hours. This indicates that risky drug behaviour is moved from public spaces and private homes into a supervised environment. This is indeed one of the important rationales behind the establishment of a SIF and is not reported in Norway previously.

i) *What are the characteristics of patients treated with naloxone by the Ambulance Service or others in Oslo and where is the locations of the overdose?*

The clinical findings described in Article 1 confirm that the patients treated with naloxone are gravely ill. A median Glasgow Coma Scale of 3/15, and a respiratory rate of 6 breaths per minute, paint a picture of patients in a critical condition, who without rapid first aid and medical intervention may progress to cardiac arrest and death. The indications for naloxone administration may vary across locations and situations. In places with supervision, such as the SIF or other drug-user facilities, the tolerance for intoxicated people are different from than in the public spaces. Many patients in public spaces may not be in a life-threatening state, but are unable to take care of themselves. This is reflected in the data, as patients in public places had significantly higher GCS and respiratory score than other locations. Interestingly, the most seriously ill patients in our study are the ones found in private homes. They have a median GCS of 3/15, with a narrow interquartile range of 3-4. We know that the majority of patients dying from opioid overdoses in Norway are found in private homes (135). This information is highly relevant for our research question, as it has implications both for prevention strategies and new treatments. This finding, coupled with existing knowledge, means that the most ill patients—who are dying most frequently—are either alone at home, or in the company of lay people (family or friends). Consequently, these patients are the least likely to have any trained community worker or health professional nearby, in the event of an overdose. For prevention, this means educating people not to inject alone, or somewhere they cannot be found. In a harm-reducing setting, the training that the drug users' family or peers get in recognising an overdose, must focus on basic first aid, as

they are likely to meet deeply intoxicated patients. Take Home Naloxone is meant to cover precisely the patients outside of the reach of trained personnel. We show that these are the most ill, and this should have implications for the products launched in THN programs. These products need to have a rapid onset of action, to restore respiration and consciousness. Based on this, we have chosen to compare our nasal spray to IM 0.8 mg. We know this to be sufficient in 88% of overdoses in Oslo (113), and it is in the upper range of the WHO recommendation of 0.4-0.8 mg starting dose (15). We can argue that 0.4 mg naloxone is too low a starting dose for deeply intoxicated patients.

ii) How are overdose patients followed up after treatment with naloxone?

The follow up after naloxone treatment is widely debated. It is generally agreed upon that being left at the scene is suboptimal. The fear of immediate re-intoxication has been diminished, with findings from several studies indicating that this is rare. However, long-term results of different follow-up options are harder to describe. Indeed, immediate follow-up by ambulance personnel should be linked to wider care beyond the overdose, to address the problems underlying the opioid use and addiction. One interesting Oslo study shows that while immediate death after being treated by ambulance to be rare, these patients have a greatly increase risk of dying in the weeks and months, even up to five, years after being treated by ambulance staff for overdose (129). We show that being treated in the SIF almost always mean remaining there after the administration of naloxone, and that just under half remain at the scene in public spaces. The potential for intervention and follow-up is small for those left in public, but our finding should urge the SIF to establish a comprehensive post-overdose intervention plan for its users. 58% of patients treated with naloxone remain at home when treated there. Ambulance guidelines will ensure no one is left alone, but these patients are left without professional follow-up, despite their initial serious presentation to ambulance crew. The knowledge that the majority of patients remain outside of any health institutions after naloxone treatment, confirms the importance of any new naloxone product to have sufficient duration as standard IM naloxone administration. This is the only way we can secure that the practice of releasing against medical advice remain safe, even with new naloxone formulations.

5.4 Discussion and interpretation research question II

Overall PK comparison Article 2 and 3

A total of 1467 blood samples in 34 individuals compromise the result presented in this section. The main PK variables are presented in table 1, Article 2, and table 1, article 3 (attached).

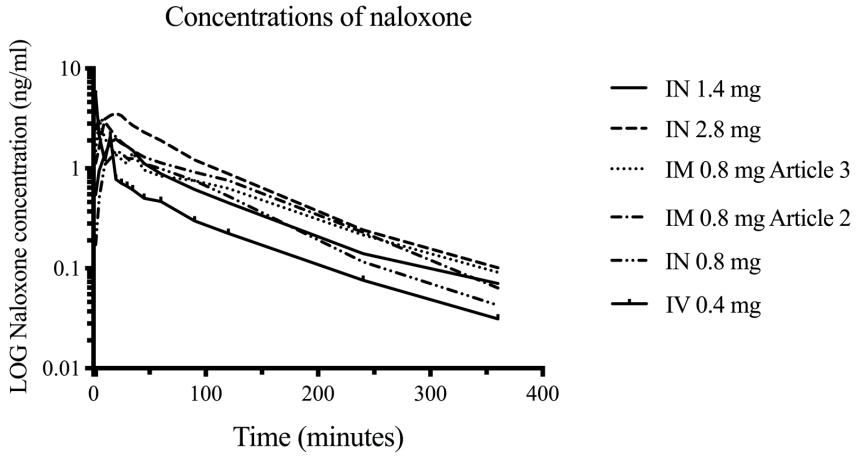

Figure 5-1: The time-course of mean naloxone concentration 0- 360 minutes in serum from article 2 and plasma from article 3.

Figure 5-1 shows how the time-course of naloxone IV, IM and IN are behaving in roughly the same manner. The elimination phase consists of the metabolization and excretion of the drug, and is similar across doses and forms of administrations. The IV administered is 0.4 mg, the lowest nominal dose. IV has the highest Cmax, but the lowest concentration in the elimination phase. Both IM and IN remain above IV. This graph shows that IN and IM are favoured over IV, at the doses common in today's clinical practice, in the distribution and elimination phase. It remains high, and can protect against re-intoxication.

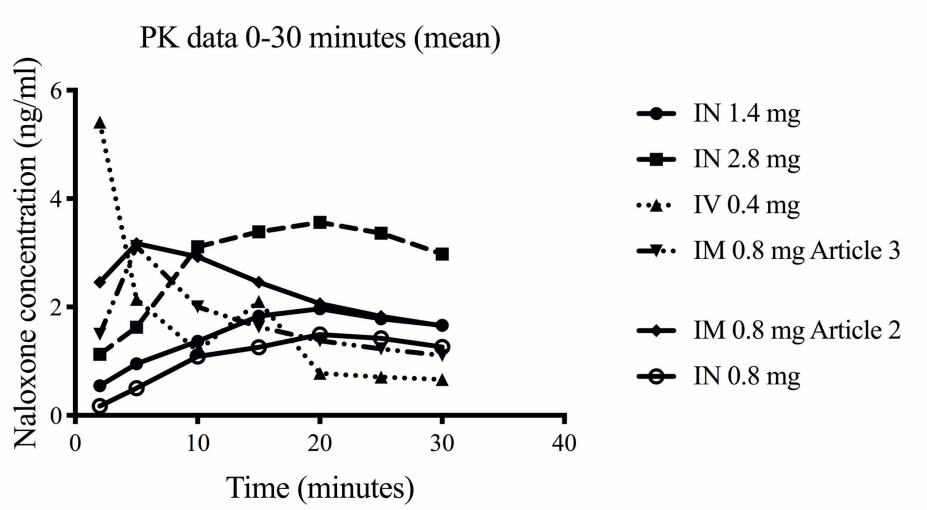

Figure 5-2: Mean naloxone concentrations 0-30 minutes from article 2 and 3. Variability across the mean is removed for clarity

Figure 5-2 is a magnification of the first 30 minutes after naloxone administration. It is the absorption and early distribution phase. This time period is of particular interest to drugs used in pre-hospital emergency medicine, as rapid effect is wanted. The IV route stands out with a C_{max} almost twice that of any of the other routes, despite the lowest nominal dose. This graph—and table 5-2—illustrate how the IV route probably increases the risk of precipitating acute withdrawal syndrome, by the initial high concentration. The C_{max} is four times higher than dose corrected IM. The large initial concentration does not increase the systemic exposure or duration of action proportionally. This makes the case for IM or IN over IV as the first choice pharmacologically. The IN administrations behave similar to IM, and—as article 3 shows—have no statistical difference in C_{max} and T_{max}.

Table 5-2: Central PK variables both direct data and dose corrected

	C_{max} (ng/mL)	Dose corrected C_{max}	AUC $_{0\text{-last}}$ (h*ng/ml)	Dose corrected AUC $_{0\text{-last}}$	T_{max} (minutes)	Half life (minutes)
IN 1.4 mg	2.36	1.69	2.62	1.87	20.2	73.0
IN 2.8 mg	4.18	1.49	5.23	1.87	20.7	69.8
IN 0.8 mg	1.62	2.00	2.66	3.33	28.0	69.7
IM 0.8 mg Article 2	3.62	4.52	4.06	5.08	7.80	63.7
IM 0.8 mg Article 3	3.73	4.66	3.09	3.86	13.6	84.8
IV 0.4 mg	7.44	18.6	1.84	4.60	3.50	74.3

Table 5-2 display the central PK variables in each article. C_{max} and AUC $_{0\text{-last}}$ have also been dose-corrected. This correction is simply to take the PK value in question and divide by the number of milligram naloxone given, e.g. Cmax 2.36/ 1.4 mg naloxone= 1.69. This is done to compare the values across different doses. The relative bioavailability of IN to IM in article 2 was reported as F= 0.75. This was higher than the absolute bioavailability of 0.54, reported for the same formulation by our group (114), and in article 3. The dose corrected IN C_{max} and AUC $_{0\text{-last}}$ is higher in article 2 than in article 3. A bioequivalence calculation (115) was therefore performed on IN data from the study in article 2, and data from the previous study. Bioequivalence was not demonstrated, as would be expected between two studies of the same formulation. On this basis, a drug-to-drug interaction can be postulated, in this case between naloxone and remifentanil. The calculation is exploratory only, and cannot answer by which mechanism this interaction may occur, if it is specific to remifentanil or would apply to other opioids. It is however, interesting to note, and has possible regulatory and clinical implications. If other opioids than remifentanil increase the C_{max} and AUC of naloxone,

this must be taken into account when interpreting PK studies, reporting a design where naloxone is given alone.

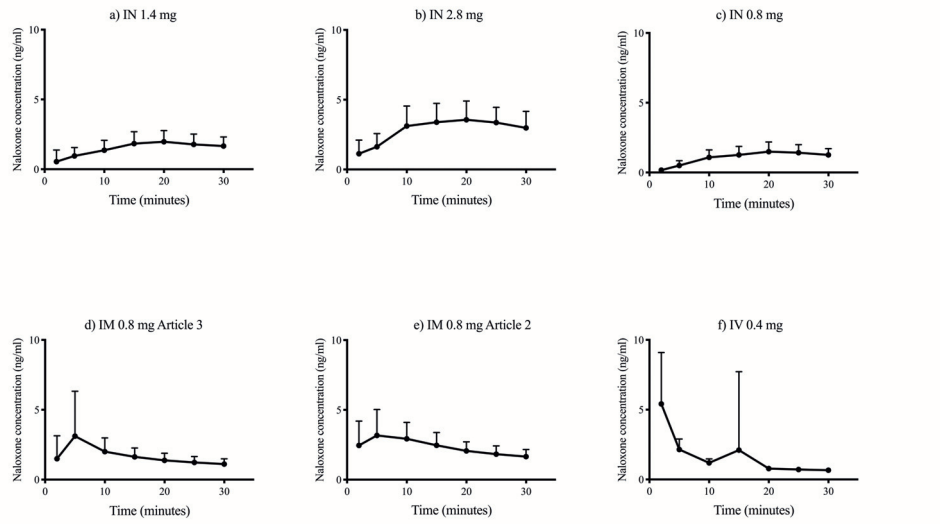

Figure 5-3 Mean naloxone concentration shown with standard deviation across treatments given.

For clarity, dispersion has been omitted in figure 5-1 and 5-2. Visually comparing the variability surrounding the mean value is interesting, as the largest spread seems to be in the IM and IV routes, not the IN route. Intranasal variability may be explained by anatomy, mucociliary clearance and blood flow. The IM variability is harder to explain, but injection close to, or accidentally in a vein, may explain some of the high values seen after 5 minutes in article 3 (figure 5-3 panel d) and e)). Panel f) displays surprisingly large variability of IV after 2 minutes. In IV administration there is no absorption phase, and rapid distribution. Whether the blood sample is actually drawn at one minute and 50 seconds, or two minutes and 10 seconds, will have impact on the concentration measured. For later samples this effect of precise sample timing is much smaller. The large variation seen in IV 0.4 mg sample after 15 minutes is explained by one single sample being set to 47.6 ng/mL, as it surpassed the upper limit of quantification. This result is biologically impossible, but kept in the analysis for regulatory purposes.

Adverse Events

The nasal naloxone formulation studied in article 2 and 3 is an IMP, and the recording of adverse events and drug reactions is important. No serious adverse events are reported in either trial. All adverse events reported were of mild severity, except for one in article 3, which was reported as moderate, but unrelated to treatment. The adverse events reported most by participants were headache and nasal congestion. These events were deemed to have a possible relationship to the test drug. Anterior rhinoscopy did not reveal any changes before or after administration of IN naloxone. The IN naloxone formulation was well tolerated at both 8mg/mL and 14 mg/mL. There were no reports adverse events from remifentanil or injected naloxone.

i) How does 0.8 mg intranasal naloxone compare to 0.8 mg intramuscular in volunteers exposed to an opioid with respect to pharmacokinetic and pharmacodynamics outcomes?

Pharmacokinetics

The comparison of equal doses naloxone in two formulations (IM and IN) provided a chance to calculate relative bioavailability and other key PK variables, for a high concentration/low volume naloxone nasal spray. The relative bioavailability was 0.75 in article 2. This is higher than the 0.54 absolute bioavailability reported for the same formulation (114) and the relative bioavailability reported in article 3 of 0.52. Both studies show much higher bioavailability than the 0.04-0.11 reported for other naloxone formulations (81, 136, 137) in the past.

Comparing the maximum concentrations between 0.8 mg IN and IM also shows a difference, with IM reaching twice the concentration as IN. As the C_{max} is important both for restoring respiration (high enough), and may precipitate withdrawal (not too high), this knowledge is important. The exact serum concentration, or range of concentrations, where naloxone safely reverses overdoses, is unknown. Compared to the same dose IM, the IN 0.8 mg has a C_{max} less than half. However, the C_{max} in this study which was 1.62 ng/mL for IN 0.8 mg, proves to be higher than the 0.90 ng/mL (130) and 1.42 ng/mL (131) reported for IM 0.4 mg—the reference naloxone in other studies.

As the indication for pre-hospital naloxone use is a life-threatening condition, the onset of action, and time to maximum concentration, is an important measurement. This is unlike most other drugs, where T_{max} is of less interest. The FDA specifically mentions that the new treatment options need to match naloxone exposure in the first few minutes after IM administration, and that T_{max} comparisons must be examined (138). There is a large difference between the T_{max} in Article 2, with IM reaching maximum concentration after only 7.8 minutes, and IN after 28. The IM T_{max} in article 2 is faster than what is seen in article 3 (13.6 minutes) and what is reported in other studies; 24 minutes IM 0.4 mg (130) and 10 minutes IM 0.4 mg (131). The variability in T_{max} is higher for IM than for IN. Intranasal reports are more consistent around 20-30 minutes in article 2 and 3, and with other IN formulations (130, 131). The pharmacological discussion surrounding T_{max} remain academic, in so far as naloxone starts working from the first molecule reaches the μ-opioid receptor in the brain stem. This means a patient may be completely awake prior to C_{max}. Comparing IN and IM 0.8 mg time to 50% and 80% of $C_{max,}$ we find that the difference between the routes of administration is less pronounced. IM 0.8 mg naloxone T_{max} of 7.8 minutes is only 27% faster than the intranasal 1.4 mg T_{max} which was 28 minutes. T_{50} IM equals 37% of IN.

Pharmacodynamics

The knowledge of a physiological response to naloxone is of importance, as there may be significant delays between serum concentrations and response. The patients invariably will be exposed to an opioid, PD studies may bridge the gap between healthy volunteers and real patients.

Out of the two PD measurements in Article 2, pupillometry gave good resolution, and showed statistically significant changes, both between the two naloxone formulations, between the nadir of pupil size and changes after naloxone administration. Heat Pain Threshold did not provide such results.

The PD results reflected the PK results, as the IM naloxone reversed the opioid induced miosis to a larger degree than did IN. This was particularly apparent in the early phase after naloxone administration. 35 minutes after naloxone administration there was no statistical difference in pupillometry between the two routes of administration.

The hysteresis display of PD response; article 2, figure 5 (attached), shows a time difference between the C_{max} and the maximum change in pupil size. For IM, the largest pupil diameter is at 15 minutes (T_{max} 7.75 minutes). For IN, the max effect is seen after 30-60 minutes (T_{max} 28 minutes). This clockwise hysteresis and delay of effect is seen in other opioid antagonists (88). The design cannot say whether this hysteresis shape is a distribution delay to the effect site, slow receptor kinetics or other mechanisms.

The present PD model of naloxone with remifentanil TCI can be used to study and compare other naloxone formulations to each other and to standard IV and IM treatment.

ii) Is the systemic exposure of an intranasal dose of 1.4 mg of naloxone equal to the intramuscular dose of 0.8 mg in healthy volunteers?

Article 3 reports a phase I four-way crossover study in 22 healthy volunteers. We have directly compared IN 1.4 mg naloxone given as one and two doses to IM 0.8 mg and IV 0.4 mg naloxone. Through population-based PK modelling we have compared IN 1.4 mg to IM 0.4 mg naloxone, and to IM 0.8 given at different times than direct comparison and NCA analysis.

The $AUC_{0\text{-}last}$ and $AUC_{0\text{-}inf}$ differ between IN, IM and IV, but there is no statistically significant difference between IN 1.4 mg and IM 0.8 mg naloxone. This shows that the total systemic exposure of naloxone is equal between the two administrations. It does not, however, ensure that the two administrations are similar, in terms of onset and effect. Neither the maximum concentration nor the time to maximum concentration differed significantly between IN 1.4 and IM 0.8 mg naloxone. Comparing these three core PK variables gives a comprehensive overview of the exposure to the body, of naloxone, and find the exposure to be similar both in the first few minutes after administration and throughout.

In addition, the article shows that IN 1.4 mg naloxone given in the same nostril three minutes apart shows dose proportionality. This is important, as more than one dose administration is anticipated. Saturation of the nasal mucosa, changes in blood flow or other factors could reduce uptake of the second dose and make IN a less favourable route in titration than IM or IV.

Population PK modelling extends the use of data obtained in the study, and article 3 can predict the PK curve for other naloxone doses in various routes of administration, and at different dosing intervals. This makes it possible to compare the IN 1.4 mg dose with data available through other studies of naloxone, notably IM 0.4 mg used as comparator to other high-concentration/low-volume naloxone sprays (130, 131).

5.5 Discussion on research ethics, Good Clinical Practice and regulatory aspects

All three studies reported in this thesis involve research on human subjects, an activity which requires particular care as to the conduct of the research. Both international and national laws and regulations (102, 139, 140), scientific standards and local guidelines at study sites, regulate and advice how this is carried out. Research involving people is important for society, however, can be morally challenging as research subjects are exposed to risks for the advancement of science (141). Both clinical practitioners and research investigators should be guided by the four basic principles of medical ethics in both science and clinical practice; do no harm, beneficence, justice and autonomy (141). To balance this, research must meet several conditions—consent alone is insufficient. There must be a pursuit of knowledge, and a reasonable prospect that the research will generate that knowledge. If the research involves human subjects, it must be considered necessary, with no reasonable alternative. There must be a favourable balance of benefits to the subjects and/or the society, over the risks to the subjects. The studies presented in this thesis all presented challenges regarding these principles, and a balance between benefits and risks.

5.5.1 Informed consent

The main rule of medical research is that of informed consent. The Norwegian Health Research act §14 (140) states that "*Consent must be informed, voluntary, expressed and documented. Consent must be based on specific information about a concrete research project…".*

The database in Article 1 contains data from patients who have not given informed consent prior to data being collected. At the time of naloxone treatment patients are to a varying degree unconscious, and not in a state where informed consent can be obtained. The database consists of identifiable data and will later be coupled with the national Cause of Death registry. Such registration normally requires consent. The risk to the individual subject in this case is to register in a database with highly sensitive information reading one's health. The benefit to the individual registered is non, except that a possible better treatment regimen developed through such research can make a

difference for the same person in a later overdose. The benefit to society may be considerable. Opioid overdoses are a massive health problem, and knowledge regarding emergency treatment, follow up and mortality in the long and short term may benefit future patients suffering the same conditions and benefit society at large. After consideration in REC, approval was given to create this database, on the condition that patients were informed about their registration and was given a chance to withdraw information registered in the database. To fulfil the REC requirements, patients receive written information regarding their inclusion in the database, and are given a mean to withdraw from registration. Ambulance staff handed out a one-page sheet with information and that people wanting to withdraw can contact the study team by telephone. During the years the study has been ongoing this has happened on only one occasion.

Participants in Article 2 and 3 have all given informed, written consent prior to randomisation. The written information they received was all approved by REC, and contained information about the intervention and measurements taken in the study, expected duration and adverse events.

5.5.2 Vulnerable group

The patients included in Article 1 will often be considered as a vulnerable patient group, and one that may deserve and require specific protection. The Helsinki declaration of medical ethics (142), article 19 states: "*All vulnerable groups and individuals should receive specifically considered protection",* and article 20: *"Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research."* The present article has no physical study intervention, change in treatment or exposure to other risks or harm. The study registers sensitive health information about each individual, with the risk of identification and the spread of this information. One way of reducing possible harm by spread of sensitive information was to completely anonymise the data. Anonymous data is defined as data that are impossible to link to an identifiable individual (143). However, this would reduce scientific yield from the data. Anonymising would make it impossible to link the

cohort with the Cause of Death registry later, and by doing this, reduce the beneficence future patients being treated for opioid overdose may have from this research. In the balance between benefit and risk the current solution with de-identified database and the possibility to withdraw was found ethically sound. The research may produce knowledge that benefit this exact group of patients in the future, by enabling tailored treatment and prevention strategies, thus fulfilling the Helsinki declaration, article 20.

5.5.3 Testing drugs for clinical trials

The inclusion of healthy human volunteers in medical and pharmaceutical trials are common, it is the basis of all modern drug development. However, it is not without risks. Both historic examples, such as the well-known thalidomide experiment (144), and the more recent phase 1 trial of TGN1412 (145), or sildenafil in new-borns (146) have shown that, despite pre-clinical testing or animal studies, human trails can have catastrophic consequences. To safeguard subjects in human trials in Norway the Health Research Act §5 clearly states that "*The participants' welfare and integrity shall have priority over scientific and social interests*." Similar sentiments and principles guide all medical practice both in law and in other declarations from Hippocrates to Helsinki. In our study two drugs were administered, in three different formulations:

i) Naloxone IN 0.8 mg as Naloxone nasal spray solution 0.1mL/ 8 mg/mL, produced by Norwegian Institute of Public Health, was an Investigational Medicinal Product, but again well within the dosing limit, and the nasal spray solution was constituted with well-known excipients. This was not a first-in-humans trial, the solution and strength had been tested before (114, 147, 148).
ii) Naloxone IN 1.4 mg as Naloxone nasal spray solution 0.1mL/ 14 mg/mL, produced by Sanivo Pharma AS. This also conformed to all standards set for the production and supply of drugs for clinical trials.
iii) Naloxone IM and IV as Naloxone B. Braun 0.4 mg/mL, supplied from the Hospital Pharmacy, holds a marketing authorisation, and was used well with its recommended dosing range (up to 10 mg).

The nasal naloxone underwent stability, efficacy of antimicrobial preservation and other tests by the manufacturers, and NoMA approved the drug for clinical trials. REC had no

ethical worries that any of the naloxone formulations posed a threat to participants' welfare. The last drug administered was remifentanil. This holds a marketing authorisation, and was used well below standard clinical doses. Both ethical and medicinal regulatory authorities agreed with us that the medicines administered were well within safety and ethical standards set—and all were approved.

5.5.4 Administration of opioids for research

The study presented in Article 2 posed several ethical and practical concerns regarding participants safety, welfare and wellbeing. Remifentanil is a potent opioid agonist. It has a rapid inset of action, short half-life, and is metabolised by blood esterases (149). As with all opioids it has a potential both for adverse reactions such as respiratory arrest in the immediate setting, and also for people to develop addiction (150, 151). The latter is of particular concern to the subject of this thesis; the underestimation of the abuse potential of all opioids has fuelled the overdose epidemic, particularly in the US (152). Our study involved the intravenous administration of this drug for 102 minutes twice, to healthy volunteers. The scientific rationale for this was that to study the antagonistic effects of naloxone, a state of opioid agonism must be created. Several models exist for this, and numerous studies have administered opioids to volunteers, both opioid naive participants (91, 117, 153) and to drug users (93, 154, 155). To justify this intervention we aimed at increasing the scientific value and decreasing the risk to our participants. By choosing remifentanil TCI we used a method proven to provide steady state of opioid influence (116). This increased the scientific value, in that any changes in pharmacodynamic data could be attributed to changes in naloxone alone, not confounded by changes in opioid serum concentrations. To minimise the risk to our participants we used two strategies; firstly through our exclusion criteria; potential participants with access to potent opioids in their workplace or a history of drug abuse or prolonged use were excluded. Secondly by asking the CAGE AID questions we screened for behaviour that could increase a participant´s risk of addiction (156). We were less worried that opioid users would be attracted to the study, as the administration of naloxone would precipitate an acute withdrawal reaction (95). For safety, participants were fasting prior to the study session, and under supervision by a trained anaesthetist. The fact that naloxone formed part of the study protocol also meant that the time

participants were exposed to full opioid agonism alone were far less than the 102 minutes the remifentanil TCI infusion lasted.

5.5.5 Reimbursement of participants

Participants in Article 2 were reimbursed with 1500 Norwegian crowns (NOK) per study visit in which they partook in (max NOK 3000). Participants in Article 3 were reimbursed for participation with NOK 1000 per treatment visit (max NOK 4000). The payment of money may pose an undue inducement, and make participants consent to research they would otherwise not agree to participate in. It is accepted that the remuneration of direct expenses (e.g. travel, parking and lost income during the time spent) can be offered without excessive pressure being put on participants (157). The payment should be adjusted to the time spent, and effort put in by participants (158). On this background the participants in Article 2, who were exposed to remifentanil and painful stimulus, were compensated higher than the ones in Article 3, who had a less invasive and less painful intervention. All our payments were approved by REC.

5.5.6 Conflict of interest

This project involves a cooperation between academic researchers and the pharmaceutical industry, building up the case-file and data necessary to file an application for Marketing Authorization for a drug for human consumption. Article 2 and 3 present data on an IMP, an innovation by Professor Ola Dale and NTNU. A licensing agreement regulating the ownership, sale and sharing of any profits from the nasal naloxone formulation have been agreed between NTNU, Technology Transfer Office and Farma Industri AS. According to this, NTNU remain in ownership of the innovation, Farma Industri AS have rights to commercialise the nasal spray. Any proceeds will be divided between dne Pharma AS, NTNU, TTO and Ola Dale. NTNU have full ownership of all data presented in article 2, whereas the data in article 3 are owned by AS Den Norske Eterfabrikk. NTNU has secured publishing rights for all results from all studies.

Arne Kristian Skulberg has signed a non-compete contract with AS Den Norske Eterfabrikk, limiting his rights to work for, or share data or know-how in IN naloxone

with other commercial entities, during his time as a research fellow at NTNU. He is not limited in his rights to publish results—including negative ones. He has no financial benefit, direct or in-kind, from any proceeds from sales of Ventizolve.

5.5.7 Good Clinical Practice and regulatory aspects

Article 2 and 3 reports trials that both conform to the ICH-GCP standards and are approved by NoMA. The study in article 2 was subject to a NoMA GCP audit in April 2015, and no serious breaches on GCP standards were found. The study reported in Article 3 has formed the basis of an application for Marketing Approval in 12 European countries. This approval was granted in June 2018. Having to obey and correspond to pre-set scientific standards, and regulations strengthens the results presented in these articles. From study design, participant inclusion, documentation, naloxone concentration and data analysis, and through to reporting, these studies have undergone the same regulatory demands as is seen in the pharmaceutical industry. The study in article 1 has no statutory need to conform to the same strict standards, as it is not a clinical drugs trial. However, the conduct of the study is inspired by GCP, which has strengthened the quality of the data presented here.

6 Conclusions

Based on the findings presented above, the research questioned posed can be answered.

i) *What are the characteristics of patients treated with naloxone by the Ambulance Service or others in Oslo and where is the locations of the overdose?*

Patients in need of naloxone treatment are gravely ill, with critically reduced level of consciousness and respiration, particularly in private homes or at the Safe Injection Facility. One third of the overdoses occur in the SIF and half in public places. The cohort studied in Oslo demographically matches previous findings around the world well, thereby increasing the external validity of the data.

ii) *How are overdose patients followed up after treatment with naloxone?*

Fifty percent of patients are not transported to further care after naloxone treatment, despite the seriousness of their clinical state at presentation to ambulance personnel. The rate of transport further vary considerably between locations.

i) *How does 0.8 mg intranasal naloxone compare to 0.8 mg intramuscular in volunteers exposed to an opioid with respect to pharmacokinetic and pharmacodynamics outcomes?*

Intranasal naloxone 0.8 mg is inferior to the same dose given intramuscularly, assessed by pharmacokinetic and by pharmacodynamic outcomes. Naloxone may have a pharmacological interaction with remifentanil.

ii) *Is the systemic exposure of an intranasal dose of 1.4 mg of naloxone equal to the intramuscular dose of 0.8 mg in healthy volunteers?*

Intranasal 1.4 mg naloxone provides systemic exposure of naloxone, and has a pharmacokinetic profile in the absorption phase, equal to intramuscular 0.8 mg. IN 1.4

mg naloxone as a 0.1 ml nasal spray is well tolerated by healthy volunteers, and suited for repeated dosing and titration.

In June 2018 naloxone hydrochloride 1.4 mg/0.1 mL nasal spray was granted a marketing authorisation under the trade name Ventizolve (Respinal in Sweden) in 12 European countries.

7 Future perspectives

The epidemiologic and pharmacologic knowledge presented in this thesis fits well with other science in the field of intranasal naloxone for opioid overdose. A continued examination of epidemiology of opioid overdoses is necessary, to guide local treatment protocols and harm-reduction policies. The patient group in question has multiple, and often unmet, health needs; follow-up beyond the pre-hospital treatment period is necessary.

This thesis only reports pharmacological data in healthy volunteers, with naloxone concentrations in blood as the main outcome. The pharmacodynamic outcomes are not directly clinically relevant for treatment. These are major limiting factors, and a randomised controlled, blinded phase III trial is warranted before intranasal naloxone can be safely considered non-inferior to injected naloxone.

References

1. Madah-Amiri D, Skulberg AK, Braarud A-C, Dale O, Heyerdahl F, Lobmaier P, et al. Ambulance-attended opioid overdoses: An examination into overdose locations and the role of a safe injection facility. Subst Abus. 2018;Online 27 Jun 2018.:1-6.
2. Skulberg AK, Tylleskar I, Nilsen T, Skarra S, Salvesen Ø, Sand T, et al. Pharmacokinetics and -dynamics of intramuscular and intranasal naloxone: an explorative study in healthy volunteers. Eur J Clin Pharmacol. 2018;74(7):873-83.
3. Skulberg AK, Aasberg A, Khiabani HZ, Røstad H, Tylleskar I, Dale O. Pharmacokinetics of a novel, approved 1.4, mg intranasal naloxone-HCL formulation for opioid overdose reversal, a clinical trial. Addiction In review (ADD-18-0843). 2018.
4. Rang HP. Rang and Dale's pharmacology. 6th ed. Philadelphia, PA: Churchill Livingstone/Elsevier; 2007. xiii, 829 p. p.
5. Stevenson A. Oxford dictionary of English. 3rd ed. / edited by Angus Stevenson. ed. Oxford: Oxford University Press; 2010.
6. Hemmings HC, Egan TD. Pharmacology and physiology for anesthesia : foundations and clinical application. Philadelphia, PA: Elsevier/Saunders; 2013. xiv, 690 p. p.
7. Chow S-C. Bioavailability and bioequivalence in drug development. Wiley Interdisciplinary Reviews: Computational Statistics. 2014;6(4):304-12.
8. Distribution (pharmacology) Wikipedia [cited 2018 1. november]. Available from: https://en.wikipedia.org/wiki/Distribution_(pharmacology).
9. Excretion: Wikipedia; [cited 2018 1. november]. Available from: https://en.wikipedia.org/wiki/Excretion.
10. Drug metabolism: Wikipedia; [cited 2018 1. november]. Available from: https://en.wikipedia.org/wiki/Drug_metabolism.
11. Miller RD. Miller's anesthesia. 8th ed. ed. Philadelphia, PA: Churchill Livingstone/Elsevier; 2015.
12. European Medicines Agency. Points to Consider have been developed to provide advice on selected areas relevant to the development of medicinal products in specific therapeutic fields. (CPMP/EWP/2655/99). 2000.
13. Helse og Omsorgsdepartementet. Lov om ordning med lokaler for injeksjon av narkotika (sprøyteromsloven). LOV-2011-06-24-30. 2012.
14. McDonald R, Strang J. Are take-home naloxone programmes effective? Systematic review utilizing application of the Bradford Hill criteria. Addiction. 2016;111(7):1177-87.
15. World Health Organization. Management of Substance Abuse Team, World Health Organization. Community management of opioid overdose. Geneva: World Health Organization,; 2014.
16. Homer, Murray AT. The Odyssey, with an English translation by A. T. Murray. London: William Heinemann ; New York : G. P. Putnam's Sons; 1919.
17. Sydenham T. Observationes medicæ circa morborum acutorum historiam et curationem. [The third edition, enlarged, of \201CMethodus curandi febres.\201D]. Londini: Typis A. C. [Andrew Clark]; impensis Gualteri Kettilby; 1676.
18. Sertürner FWA. Darstellung der reinen Mohnsäure (Opiumsäure) nebst einer Chemischen Untersuchung des Opiums mit vorzüglicher Hinsicht auf einendarin neu entdeckten Stoff und die dahin gehörigen Bemerkungen. . J Pharm f Arzte Apoth Chem. 1806;14:47–93.
19. Pattinson KT. Opioids and the control of respiration. Br J Anaesth. 2008;100(6):747-58.
20. Kosten TR, George TP. The neurobiology of opioid dependence: implications for treatment. Sci Pract Perspect. 2002;1(1):13-20.
21. Pletcher K. Opium Wars Encyclopædia Britannica: Encyclopædia Britannica, inc.; [Available from: https://www.britannica.com/topic/Opium-Wars.
22. Rasmussen SE. Afghanistan's booming heroin trade leaves trail of addiction at home. The Guardian. 17. November 2017.

23. Rudd RA, Aleshire N, Zibbell JE, Gladden RM. Increases in Drug and Opioid Overdose Deaths--United States, 2000-2014. MMWR Morb Mortal Wkly Rep. 2016;64(50-51):1378-82.
24. Folkehelseintsituttet. Narkotikautløste dødsfall i Norge i 2015 [cited 2017 26.08]. Available from: https://www.fhi.no/hn/statistikk/statistikk2/narkotikautloste-dodsfall-i-norge-i-2015/.
25. Ho JY, Hendi AS. Recent trends in life expectancy across high income countries: retrospective observational study. BMJ. 2018;362:k2562.
26. Darke S, Mattick RP, Degenhardt L. The ratio of non-fatal to fatal heroin overdose. Addiction. 2003;98(8):1169-71.
27. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). European Drug Report- Trends and Developments 2018. Luxembourg: Publications Office of the European Union, 2018; 2018. Report No.: 2314-9086.
28. Bjornaas MA, Jacobsen D, Haldorsen T, Ekeberg O. Mortality and causes of death after hospital-treated self-poisoning in Oslo: a 20-year follow-up. Clin Toxicol (Phila). 2009;47(2):116-23.
29. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). European Drug Report- Trends and Developments 2015. Luxembourg: Publications Office of the European Union, 2015; 2015.
30. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Norway Drug Report 2018 2018 [Available from: http://www.webcitation.org/72MIY2avS
31. Gomes T, Tadrous M, Mamdani MM, Paterson JM, Juurlink DN. The Burden of Opioid-Related Mortality in the United States. JAMA Network Open. 2018;1(2).
32. Bretteville-Jensen AL, Lillehagen M, Gjersing L, Andreas JB. Illicit use of opioid substitution drugs: Prevalence, user characteristics, and the association with non-fatal overdoses. Drug Alcohol Depend. 2015;147:89-96.
33. Martins SS, Sampson L, Cerda M, Galea S. Worldwide Prevalence and Trends in Unintentional Drug Overdose: A Systematic Review of the Literature. Am J Public Health. 2015;105(11):e29-49.
34. Elzey MJ, Barden SM, Edwards ES. Patient Characteristics and Outcomes in Unintentional, Non-fatal Prescription Opioid Overdoses: A Systematic Review. Pain Physician. 2016;19(4):215-28.
35. Kolinsky D, Keim SM, Cohn BG, Schwarz ES, Yealy DM. Is a Prehospital Treat and Release Protocol for Opioid Overdose Safe? J Emerg Med. 2017;52(1):52-8.
36. Heyerdahl F, Hovda KE, Bjornaas MA, Nore AK, Figueiredo JC, Ekeberg O, et al. Pre-hospital treatment of acute poisonings in Oslo. BMC Emerg Med. 2008;8:15.
37. Al-Hasani R, Bruchas MR. Molecular mechanisms of opioid receptor-dependent signaling and behavior. Anesthesiology. 2011;115(6):1363-81.
38. Boom M, Niesters M, Sarton E, Aarts L, Smith TW, Dahan A. Non-analgesic effects of opioids: opioid-induced respiratory depression. Curr Pharm Des. 2012;18(37):5994-6004.
39. Office for national statistics (UK). Deaths related to drug poisoning in England and Wales: 2017 registrations. Statistical Bulletin.6 August 2018.
40. U.S. drug overdose deaths continue to rise; increase fueled by synthetic opioids [press release]. Atlanta, March 29, 2018.
41. Dumas EO, Pollack GM. Opioid tolerance development: a pharmacokinetic/pharmacodynamic perspective. The AAPS journal. 2008;10(4):537-51.
42. Zador D, Sunjic S, Darke S. Heroin-related deaths in New South Wales, 1992: toxicological findings and circumstances. Med J Aust. 1996;164(4):204-7.
43. Frost J, Slørdal L, Vege Å, Nordrum IS. Forensic autopsies in a naturalistic setting in Norway: Autopsy rates and toxicological findings. Forensic Sci Int. 2012;223(1-3):353-8.
44. Warner-Smith M, Darke S, Lynskey M, Hall W. Heroin overdose: causes and consequences. Addiction. 2001;96(8):1113-25.
45. Darke S, Kaye S, Duflou J. Systemic disease among cases of fatal opioid toxicity. Addiction. 2006;101(9):1299-305.

46. Minakami H, Takagi H, Kobayashi S, Deguchi T, Kumakura S, Iwai I, et al. Morphine antagonistic actions of N-propargyl-14-hydroxydihydronormorphinone hydrochloride and related compounds. Life Sci. 1962;1(10):503-7.
47. Blumberg H, Dayton, H.B., George, M., Rapaport, D.N. N-allylnoroxynorphone; a potent narcotic antagonist. Fedn Proc Fedn Am Soc. 1961;20:311.
48. Dahan A, Aarts L, Smith TW. Incidence, Reversal, and Prevention of Opioid-induced Respiratory Depression. Anesthesiology. 2010;112(1):226-38.
49. Pasternak GW, Pan YX. Mu opioids and their receptors: evolution of a concept. Pharmacol Rev. 2013;65(4):1257-317.
50. Buajordet I, Naess AC, Jacobsen D, Brors O. Adverse events after naloxone treatment of episodes of suspected acute opioid overdose. Eur J Emerg Med. 2004;11(1):19-23.
51. B. Braun Melsungen AG. Naloxon B. Braun 0,4 mg/ml: Norwegian Medicines Agency; [updated 20.04.2012; cited 2015 12. March]. Summary of Product Characteristics]. Available from: http://slv.no/_layouts/Preparatomtaler/Spc/06-4660.pdf.
52. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, et al. A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. N Engl J Med. 1990;322(20):1405-11.
53. Weber JM, Tataris KL, Hoffman JD, Aks SE, Mycyk MB. Can nebulized naloxone be used safely and effectively by emergency medical services for suspected opioid overdose? Prehosp Emerg Care. 2012;16(2):289-92.
54. Baumann BM, Patterson RA, Parone DA, Jones MK, Glaspey LJ, Thompson NM, et al. Use and efficacy of nebulized naloxone in patients with suspected opioid intoxication. Am J Emerg Med. 2013;31(3):585-8.
55. Tandberg D, Abercrombie D. Treatment of heroin overdose with endotracheal naloxone. Ann Emerg Med. 1982;11(8):443-5.
56. Health Products Regulatory Authority Ireland. Summary of Product Characteristics Naloxone B Braun [cited 2016 17 March]. Available from: http://www.webcitation.org/6g4edopWl
57. Gupta R, Shah ND, Ross JS. The Rising Price of Naloxone - Risks to Efforts to Stem Overdose Deaths. N Engl J Med. 2016;375(23):2213-5.
58. Jarvis BP, Holtyn AF, Subramaniam S, Tompkins DA, Oga EA, Bigelow GE, et al. Extended-release injectable naltrexone for opioid use disorder: a systematic review. Addiction. 2018;113(7):1188-209.
59. Turncliff R, DiPetrillo L, Silverman B, Ehrich E. Single- and multiple-dose pharmacokinetics of samidorphan, a novel opioid antagonist, in healthy volunteers. Clin Ther. 2015;37(2):338-48.
60. Peckham AM, De La Cruz A, Dufresne RL. Kappa opioid receptor antagonism: Are opioids the answer for treatment resistant depression? Ment Health Clin. 2018;8(4):175-83.
61. Palpacuer C, Laviolle B, Boussageon R, Reymann JM, Bellissant E, Naudet F. Risks and Benefits of Nalmefene in the Treatment of Adult Alcohol Dependence: A Systematic Literature Review and Meta-Analysis of Published and Unpublished Double-Blind Randomized Controlled Trials. PLoS Med. 2015;12(12):e1001924.
62. Skolnick P. On the front lines of the opioid epidemic: Rescue by naloxone. Eur J Pharmacol. 2018;835:147-53.
63. Kaplan JL, Marx JA, Calabro JJ, Gin-Shaw SL, Spiller JD, Spivey WL, et al. Double-blind, randomized study of nalmefene and naloxone in emergency department patients with suspected narcotic overdose. Ann Emerg Med. 1999;34(1):42-50.
64. McElroy SL, Guerdjikova AI, Mori N, Keck PE, Jr. Psychopharmacologic treatment of eating disorders: emerging findings. Curr Psychiatry Rep. 2015;17(5):35.
65. Goracci A, di Volo S, Casamassima F, Bolognesi S, Benbow J, Fagiolini A. Pharmacotherapy of binge-eating disorder: a review. J Addict Med. 2015;9(1):1-19.

66. Clarke SF, Dargan PI, Jones AL. Naloxone in opioid poisoning: walking the tightrope. Emerg Med J. 2005;22(9):612-6.
67. Connors NJ, Nelson LS. The Evolution of Recommended Naloxone Dosing for Opioid Overdose by Medical Specialty. J Med Toxicol. 2016;12(3):276-81.
68. Sheikh A, Simons FER, Barbour V, Worth A. Adrenaline auto-injectors for the treatment of anaphylaxis with and without cardiovascular collapse in the community. Cochrane Database of Systematic Reviews. 2012(8).
69. McTague A, Martland T, Appleton R. Drug management for acute tonic-clonic convulsions including convulsive status epilepticus in children. The Cochrane database of systematic reviews. 2018;1:CD001905.
70. Shen J, Che Y, Showell E, Chen K, Cheng L. Interventions for emergency contraception. Cochrane Database of Systematic Reviews. 2017.
71. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Preventing opioid overdose deaths with take-home naloxone. European Monitoring Centre for Drugs and Drug Addiction; 2016.
72. Strang J, McDonald R, Alqurshi A, Royall P, Taylor D, Forbes B. Naloxone without the needle - systematic review of candidate routes for non-injectable naloxone for opioid overdose reversal. Drug Alcohol Depend. 2016;163:16-23.
73. Preston KL, Bigelow GE, Liebson IA. Effects of sublingually given naloxone in opioid-dependent human volunteers. Drug Alcohol Depend. 1990;25(1):27-34.
74. Strang J, Darke S, Hall W, Farrell M, Ali R. Heroin overdose: the case for take-home naloxone. BMJ. 1996;312(7044):1435-6.
75. Strang J, Farrell M. Harm minimisation for drug misusers. BMJ. 1992;304(6835):1127-8.
76. Kelly AM, Kerr D, Dietze P, Patrick I, Walker T, Koutsogiannis Z. Randomised trial of intranasal versus intramuscular naloxone in prehospital treatment for suspected opioid overdose. Med J Aust. 2005;182(1):24-7.
77. Eguale T, Buckeridge DL, Verma A, Winslade NE, Benedetti A, Hanley JA, et al. Association of Off-label Drug Use and Adverse Drug Events in an Adult Population. JAMA Internal Medicine. 2016;176(1).
78. McCarthy M. Off-label drug use is associated with raised risk of adverse events, study finds. BMJ. 2015;351:h5861.
79. Dale O. Ethical issues and stakeholders matter. Addiction. 2016;111(4):587-9.
80. Costantino HR, Illum L, Brandt G, Johnson PH, Quay SC. Intranasal delivery: physicochemical and therapeutic aspects. Int J Pharm. 2007;337(1-2):1-24.
81. Dowling J, Isbister GK, Kirkpatrick CM, Naidoo D, Graudins A. Population pharmacokinetics of intravenous, intramuscular, and intranasal naloxone in human volunteers. Ther Drug Monit. 2008;30(4):490-6.
82. Walley AY, Xuan Z, Hackman HH, Quinn E, Doe-Simkins M, Sorensen-Alawad A, et al. Opioid overdose rates and implementation of overdose education and nasal naloxone distribution in Massachusetts: interrupted time series analysis. BMJ. 2013;346:f174.
83. Kerr D, Kelly A-M, Dietze P, Jolley D, Barger B. Randomized controlled trial comparing the effectiveness and safety of intranasal and intramuscular naloxone for the treatment of suspected heroin overdose. Addiction. 2009;104(12):2067-74.
84. US Food and Drug Administration. Summary Review for Regulatory Action. Narcan nasal spray 2016 [Available from: http://www.webcitation.org/71ofIFafl
85. European Medicines Agency. PHARMACOKINETIC STUDIES IN MAN 1987 [Available from: http://www.webcitation.org/71oky75OY
86. Gufford BT, Ainslie GR, Padowski JM, Layton ME, White JR, Paine MF. A novel human model to assess reversal of opioid effects. Clin Pharmacol Ther. 2015;97:S13-S4.
87. Stoops WW, Lofwall MR, Nuzzo PA, Craig LB, Siegel AJ, Walsh SL. Pharmacodynamic profile of tramadol in humans: influence of naltrexone pretreatment. Psychopharmacology (Berl). 2012;223(4):427-38.

88. Shram MJ, Silverman B, Ehrich E, Sellers EM, Turncliff R. Use of Remifentanil in a Novel Clinical Paradigm to Characterize Onset and Duration of Opioid Blockade by Samidorphan, a Potent mu-Receptor Antagonist. J Clin Psychopharmacol. 2015;35(3):242-9.
89. Wolf S, Hardy JD. Studies on Pain. Observations on Pain Due to Local Cooling and on Factors Involved in the "Cold Pressor" Effect. J Clin Invest. 1941;20(5):521-33.
90. Staahl C, Olesen AE, Andresen T, Arendt-Nielsen L, Drewes AM. Assessing analgesic actions of opioids by experimental pain models in healthy volunteers - an updated review. Br J Clin Pharmacol. 2009;68(2):149-68.
91. Gustorff B, Felleiter P, Nahlik G, Brannath W, Hoerauf KH, Spacek A, et al. The effect of remifentanil on the heat pain threshold in volunteers. Anesth Analg. 2001;92(2):369-74.
92. Kim TE, Kim KP, Shin D, Chung YJ, Price J, Mistry P, et al. Assessment of the analgesic effect of remifentanil using three pain models in healthy Korean volunteers: a randomized, controlled study. Basic Clin Pharmacol Toxicol. 2012;110(6):518-23.
93. Jolley CJ, Bell J, Rafferty GF, Moxham J, Strang J. Understanding Heroin Overdose: A Study of the Acute Respiratory Depressant Effects of Injected Pharmaceutical Heroin. PLoS One. 2015;10(10):e0140995.
94. Friedman MS, Manini AF. Validation of Criteria to Guide Prehospital Naloxone Administration for Drug-Related Altered Mental Status. J Med Toxicol. 2016;12(3):270-5.
95. Loimer N, Hofmann P, Chaudhry HR. Nasal administration of naloxone for detection of opiate dependence. J Psychiatr Res. 1992;26(1):39-43.
96. Meissner K, Avram MJ, Yermolenka V, Francis AM, Blood J, Kharasch ED. Cyclosporine-inhibitable blood-brain barrier drug transport influences clinical morphine pharmacodynamics. Anesthesiology. 2013;119(4):941-53.
97. Rollins MD, Feiner JR, Lee JM, Shah S, Larson M. Pupillary effects of high-dose opioid quantified with infrared pupillometry. Anesthesiology. 2014;121(5):1037-44.
98. Kharasch ED, Francis A, London A, Frey K, Kim T, Blood J. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimal and noninvasive probes for hepatic and first-pass CYP3A induction. Clin Pharmacol Ther. 2011;90(1):100-8.
99. Latt N. Addiction medicine. Oxford ; New York: Oxford University Press; 2009. xxxi, 459 p. p.
100. Loimer N, Hofmann P, Chaudhry HR. Nasal Administration of Naloxone Is as Effective as the Intravenous Route in Opiate Addicts. Int J Addict. 1994;29(6):819-27.
101. THE COMMISSION OF THE EUROPEAN COMMUNITIES. COMMISSION DIRECTIVE 2005/28/EC laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products. Official Journal of the European Union,. 2005;9.4.2005(L 91/13).
102. Forskrift om klinisk utprøving av legemidler til mennesker, FOR-2009-10-30-1321 (2009).
103. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, (2002).
104. Medicines and Healthcare products Regulatory Agency UK. Summary of Product Characteristics, Ventizolve 1.26 mg 2018 [cited 2018 31.08]. Available from: http://www.webcitation.org/7254sSO6n
105. Helsedirektoratet. «Ja visst kan du bli rusfri – men først må du overleve» [updated April 2014. Available from: http://helsedirektoratet.no/publikasjoner/nasjonal-overdosestrategi-20142017/.
106. Mathes T, Pieper D. Clarifying the distinction between case series and cohort studies in systematic reviews of comparative studies: potential impact on body of evidence and workload. BMC Med Res Methodol. 2017;17(1):107.

107. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg. 2014;12(12):1500-24.
108. Dekkers OM, Egger M, Altman DG, Vandenbroucke JP. Distinguishing case series from cohort studies. Ann Intern Med. 2012;156(1 Pt 1):37-40.
109. Karr B BA. Medisinsk Operativ Manual. Versjon 7 ed: Oslo Universitetssykehus HF; 2012.
110. Teasdale G, Jennett B. Assessment of Coma and Impaired Consciousness. The Lancet. 1974;304(7872):81-4.
111. National Association of Emergency Medical Technicians (U.S.). Advanced Medical Life Support Committee. AMLS : advanced medical life support : an assessment-based approach. Second edition. ed. Burlington, MA: Jones & Bartlett Learning; 2017. xvii, 466 pages p.
112. Duncan R, Thakore S. Decreased Glasgow Coma Scale score does not mandate endotracheal intubation in the emergency department. J Emerg Med. 2009;37(4):451-5.
113. Skulberg AK, Heyerdahl F, Dale O, Clausen T, Braarud AC. Dosering av nalokson prehospitalt i Oslo 2014 og 2015. NAForum. 2017;Vol 30(3).
114. Tylleskar I, Skulberg AK, Nilsen T, Skarra S, Jansook P, Dale O. Pharmacokinetics of a new, nasal formulation of naloxone. Eur J Clin Pharmacol. 2017;73(5):555-62.
115. European Medicines Agency. GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE London2010 [cited 2018 20.08]. CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **:[Available from: http://www.webcitation.org/71ok9096A.
116. Lenz H, Raeder J, Draegni T, Heyerdahl F, Schmelz M, Stubhaug A. Effects of COX inhibition on experimental pain and hyperalgesia during and after remifentanil infusion in humans. Pain. 2011;152(6):1289-97.
117. Comelon M, Raeder J, Stubhaug A, Nielsen CS, Draegni T, Lenz H. Gradual withdrawal of remifentanil infusion may prevent opioid-induced hyperalgesia. Br J Anaesth. 2016;116(4):524-30.
118. Tylleskar I, Skulberg AK, Skarra S, Nilsen T, Dale O. Pharmacodynamics and arteriovenous difference of intravenous naloxone in healthy volunteers exposed to remifentanil. Eur J Clin Pharmacol. 2018;74(12):1547-53.
119. Aarons L, Ogungbenro K. Optimal design of pharmacokinetic studies. Basic Clin Pharmacol Toxicol. 2010;106(3):250-5.
120. European Medicines Agency. Note on guidance on the investigation of bioavalability and bioequivalence. CPMP/EWP/QWP/1401/98. London; 2001.
121. Taking Action on Drug Addiction and the Opioid Crisis Whitehouse.govOctober 27. 2017 [cited 2018 3. november]. Available from: http://www.webcitation.org/73eXwClNp
122. Wood E. Strategies for Reducing Opioid-Overdose Deaths - Lessons from Canada. N Engl J Med. 2018;378(17):1565-7.
123. Kelly CA, Upex A, Bateman DN. Comparison of consciousness level assessment in the poisoned patient using the alert/verbal/painful/unresponsive scale and the Glasgow Coma Scale. Ann Emerg Med. 2004;44(2):108-13.
124. Lavonas EJ, Drennan IR, Gabrielli A, Heffner AC, Hoyte CO, Orkin AM, et al. Part 10: Special Circumstances of Resuscitation: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132(18 Suppl 2):S501-18.
125. Norsk Resuscitasjonsråd. Retningslinje 2015 AHLR på voksne 2015 [cited 2018 29. october].
126. Massey J, Kilkenny M, Batdorf S, Sanders SK, Ellison D, Halpin J, et al. Opioid Overdose Outbreak - West Virginia, August 2016. MMWR Morb Mortal Wkly Rep. 2017;66(37):975-80.
127. Madah-Amiri D, Clausen T, Lobmaier P. Rapid widespread distribution of intranasal naloxone for overdose prevention. Drug Alcohol Depend. 2017;173:17-23.

128. Nielsen K, Nielsen SL, Siersma V, Rasmussen LS. Treatment of opioid overdose in a physician-based prehospital EMS: frequency and long-term prognosis. Resuscitation. 2011;82(11):1410-3.
129. Gjersing L, Bretteville-Jensen AL. Are overdoses treated by ambulance services an opportunity for additional interventions? A prospective cohort study. Addiction. 2015;110(11):1767-74.
130. Krieter P, Chiang N, Gyaw S, Skolnick P, Crystal R, Keegan F, et al. Pharmacokinetic Properties and Human Use Characteristics of an FDA-Approved Intranasal Naloxone Product for the Treatment of Opioid Overdose. The Journal of Clinical Pharmacology. 2016;56(10):1243-53.
131. McDonald R, Lorch U, Woodward J, Bosse B, Dooner H, Mundin G, et al. Pharmacokinetics of concentrated naloxone nasal spray for opioid overdose reversal: Phase I healthy volunteer study. Addiction. 2018;113(3):484-93.
132. Olofsen E, van Dorp E, Teppema L, Aarts L, Smith TW, Dahan A, et al. Naloxone reversal of morphine- and morphine-6-glucuronide-induced respiratory depression in healthy volunteers: a mechanism-based pharmacokinetic-pharmacodynamic modeling study. Anesthesiology. 2010;112(6):1417-27.
133. US Food and Drug Administration. Bioanalytical Method Validation Guidance for Industry 2018 [Available from: http://www.webcitation.org/74477mhvE
134. INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. GOOD MANUFACTURING PRACTICE GUIDE FOR ACTIVE PHARMACEUTICAL INGREDIENTS Q7 2000 [Available from: http://www.webcitation.org/7446y0smx
135. Norwegian Institute of Public Health (FHI). Narkotikautløste dødsfall i Norge i 2016 [Available from: http://www.webcitation.org/72S4pfo5C
136. McDonald R, Danielsson Glende O, Dale O, Strang J. International patent applications for non-injectable naloxone for opioid overdose reversal: Exploratory search and retrieve analysis of the PatentScope database. Drug and alcohol review. 2018;37(2):205-15.
137. Edwards E, Kessler C, Kelley G, Gapasin A, Mardari G, Goldwater R. PAINWeek Abstract Book 2016: Pharmacokinetics of 2.0 mg intranasal and intramuscular naloxone HCL administration and the impact of vasoconstrictor use on the bioavailability of intranasal naloxone HCL. Postgrad Med. 2016;128(sup2):46.
138. US Food and Drug Administration. Joint Meeting of the Anesthetic and Life Support Drugs Advisory Committee and Drug Safety & Risk Management Advisory Committee 2016 [updated September 9, 2016. Available from: http://www.webcitation.org/70vfcWrJ2
139. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use., European Counsil.(2001).
140. The Norwegian Parliament. Act on medical and health research (the Health Research Act): Lovdata.no; 2008 [cited 2016 22. june]. ACT 2008-06-20 no. 44:[Available from: http://www.webcitation.org/6iSKfNSUj
141. Beauchamp TL, Childress JF. Principles of biomedical ethics. 4th ed. New York: Oxford University Press; 1994. x, 546 p. p.
142. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
143. The European Parliament and The Council Of The European Union. Regulation (EU) 2016/679 Of The European Parliament And Of The Council (General Data Protection Regulation). Official Journal of the European Union,. 2016;L 119/1.
144. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today. 2015;105(2):140-56.

145. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med. 2006;355(10):1018-28.
146. Reuters News Agency. Dutch trial with Viagra halted after 11 babies die on line2018 [updated 24 July; cited 2018 23. August]. Available from: http://www.webcitation.org/71stq7wEG
147. Tylleskar I, Skulberg AK, Nilsen T, Skarra S, Jansook P, Dale O. Biotilgjengelighet av nalokson som nesespray - grunnlaget for fremtidig prehospital bruk. NAForum. 2014;Vol 27(3).
148. Tylleskar I. Nasal naloxone - A pilot study of the pharmacokinetics of a concentrated formulation. Trondheim, Norway: Norwegian University of Science and Technology 2017.
149. Ultiva: Norwegian Medicines Agency; [updated 13.05.2013; cited 2014 31.03]. Summary of Product Characteristics]. Available from: http://slv.no/_layouts/Preparatomtaler/Spc/1995-03195.pdf.
150. Levine AI, Bryson EO. Intranasal self-administration of remifentanil as the foray into opioid abuse by an anesthesia resident. Anesth Analg. 2010;110(2):524-5.
151. Baylon GJ, Kaplan HL, Somer G, Busto UE, Sellers EM. Comparative abuse liability of intravenously administered remifentanil and fentanyl. J Clin Psychopharmacol. 2000;20(6):597-606.
152. Seth P, Rudd RA, Noonan RK, Haegerich TM. Quantifying the Epidemic of Prescription Opioid Overdose Deaths. Am J Public Health. 2018;108(4):500-2.
153. Gustorff B, Hoerauf KH, Lierz P, Kress HG. Comparison of different quantitative sensory testing methods during remifentanil infusion in volunteers. Br J Anaesth. 2003;91(2):203-8.
154. Middleton LS, Nuzzo PA, Lofwall MR, Moody DE, Walsh SL. The pharmacodynamic and pharmacokinetic profile of intranasal crushed buprenorphine and buprenorphine/naloxone tablets in opioid abusers. Addiction. 2011;106(8):1460-73.
155. Setnik B, Sommerville K, Goli V, Han L, Webster L. Assessment of pharmacodynamic effects following oral administration of crushed morphine sulfate and naltrexone hydrochloride extended-release capsules compared with crushed morphine sulfate controlled-release tablets and placebo in nondependent recreational opioid users. Pain Med. 2013;14(8):1173-86.
156. Brown RL, Leonard T, Saunders LA, Papasouliotis O. The prevalence and detection of substance use disorders among inpatients ages 18 to 49: an opportunity for prevention. Prev Med. 1998;27(1):101-10.
157. Council for International Organizations of Medical Sciences., World Health Organization. International Ethical Guidelines for Biomedical Research Involving Human Subjects. Geneva: CIOMS; 2002.
158. Den nasjonale forskningsetiske komité for medisin og helsefag (NEM). Betaling til deltakere i medisinsk eller helsefaglig forskning, En veiledning laget av Den nasjonale forskningsetiske komité for medisin og helsefag (NEM). 2009.

Article 1

Article 2

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CLINICAL TRIAL

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

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Abstract

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

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

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

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

Trial registration clinicaltrials.gov: NCT02307721

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

Introduction

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

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

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

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

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

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

Methods

Ethics

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

Subjects

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

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

Design

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

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

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

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

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

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

Safety

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

Pharmacodynamic measurements

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

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

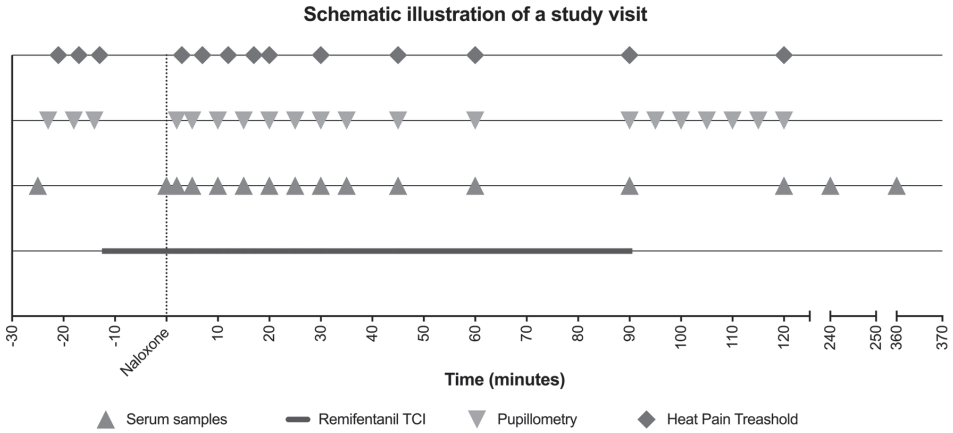

Fig. 1 Schematic illustration of samples and measurements and sequence of events in the protocol for each session. Remifentanil infusion lasts 102 min and naloxone is administered at $t = 0$

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

Blood samples and analysis

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

Statistics

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

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

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

Results

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

Changes in pupillary size

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

Difference in pupil size from nadir

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

Difference in pupil size between IN and IM

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

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

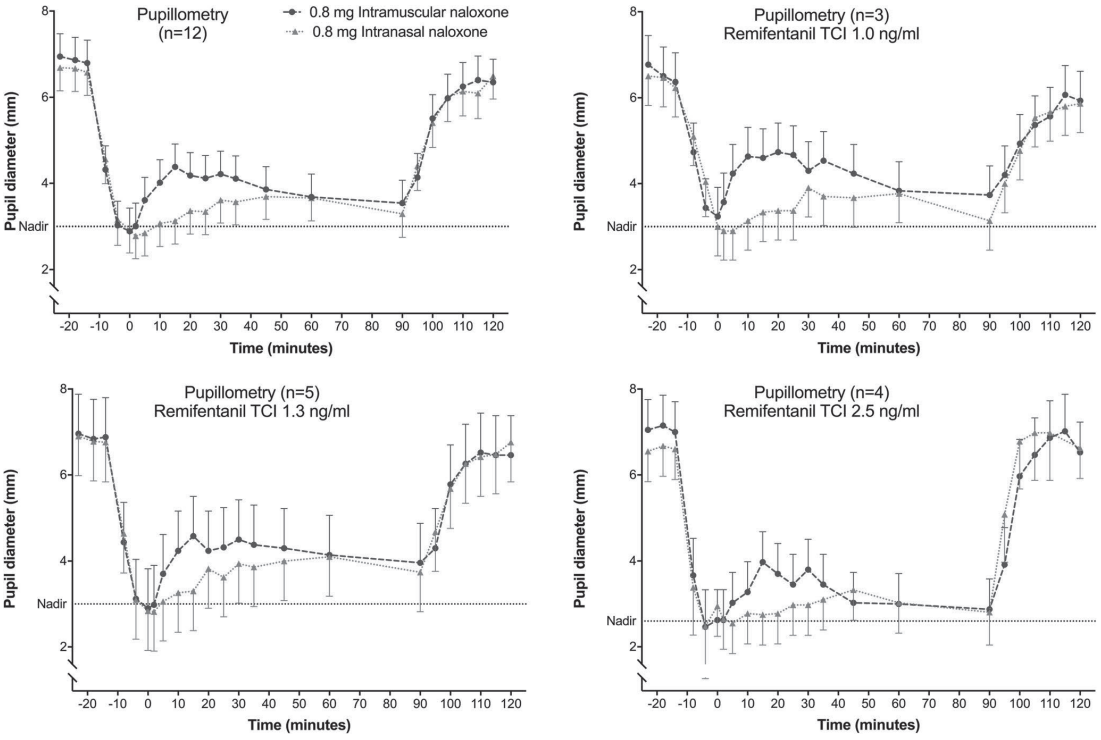

Fig. 2 Time course of variation of pupil size (mean, 95% confidence interval). Pupils are adapted to low ambient light and remifentanil TCI is administered between $t = -12$ and $t = 90$ min; 0.8 mg naloxone is administered at $t = 0$ as either nasal spray (triangles) or intramuscular injection (circles)

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

Heat pain threshold

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

Pharmacokinetic variables

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

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

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

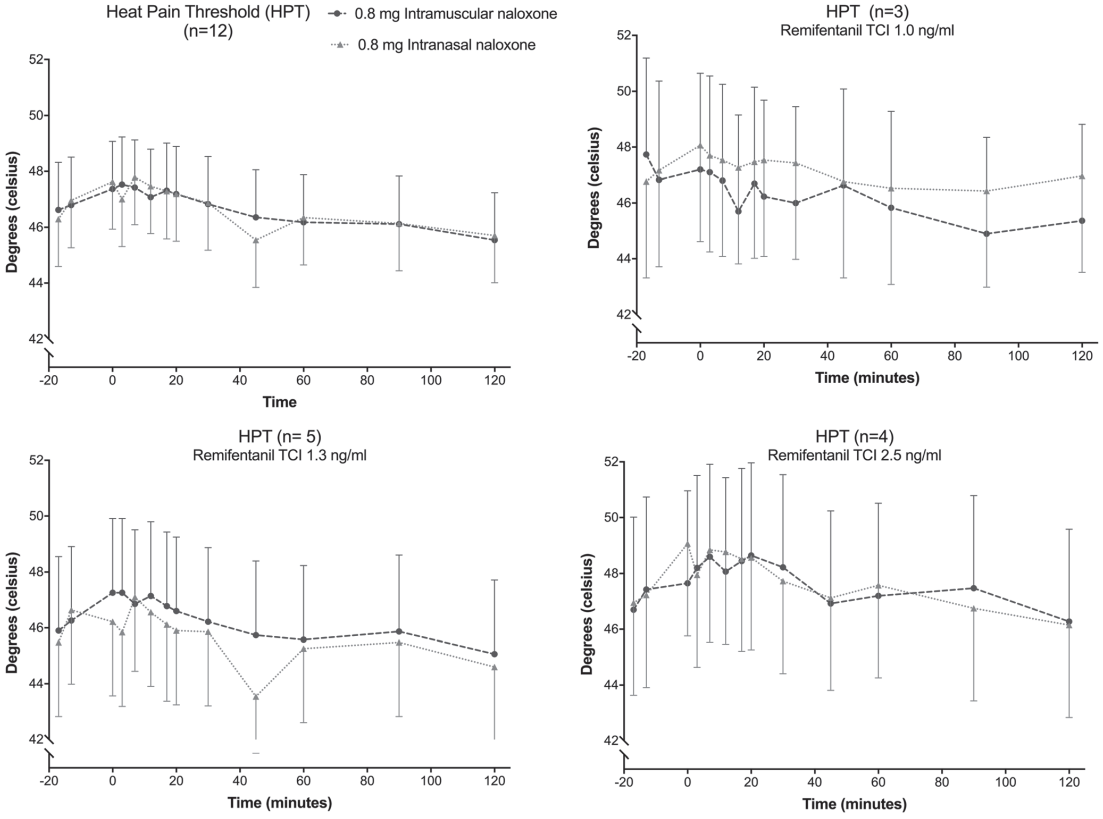

Fig. 3 Time course of variation of heat pain threshold (mean, 95% confidence interval). Remifentanil TCI is administered between $t = -12$ and $t = 90$ min and naloxone administered at $t = 0$; 0.8 mg naloxone is administered at $t = 0$ as either nasal spray (triangles) or intramuscular injection (circles)

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

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

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

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

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

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

PK/PD comparison

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

Safety

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

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

Discussion

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

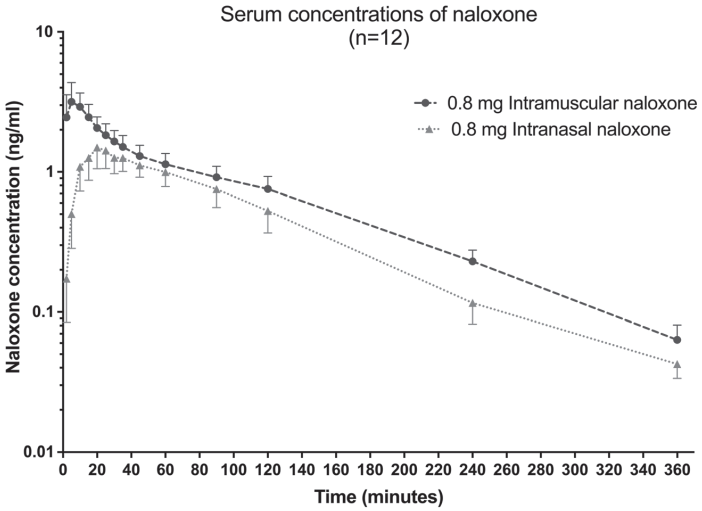

Fig. 4 Time course of serum concentrations of naloxone (ng/ml) mean and 95% confidence interval after intranasal or intramuscular administration of 0.8 mg naloxone in healthy human volunteers receiving a remifentanil infusion ($n = 12$). Triangles represent the nasal spray and circles the intramuscular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

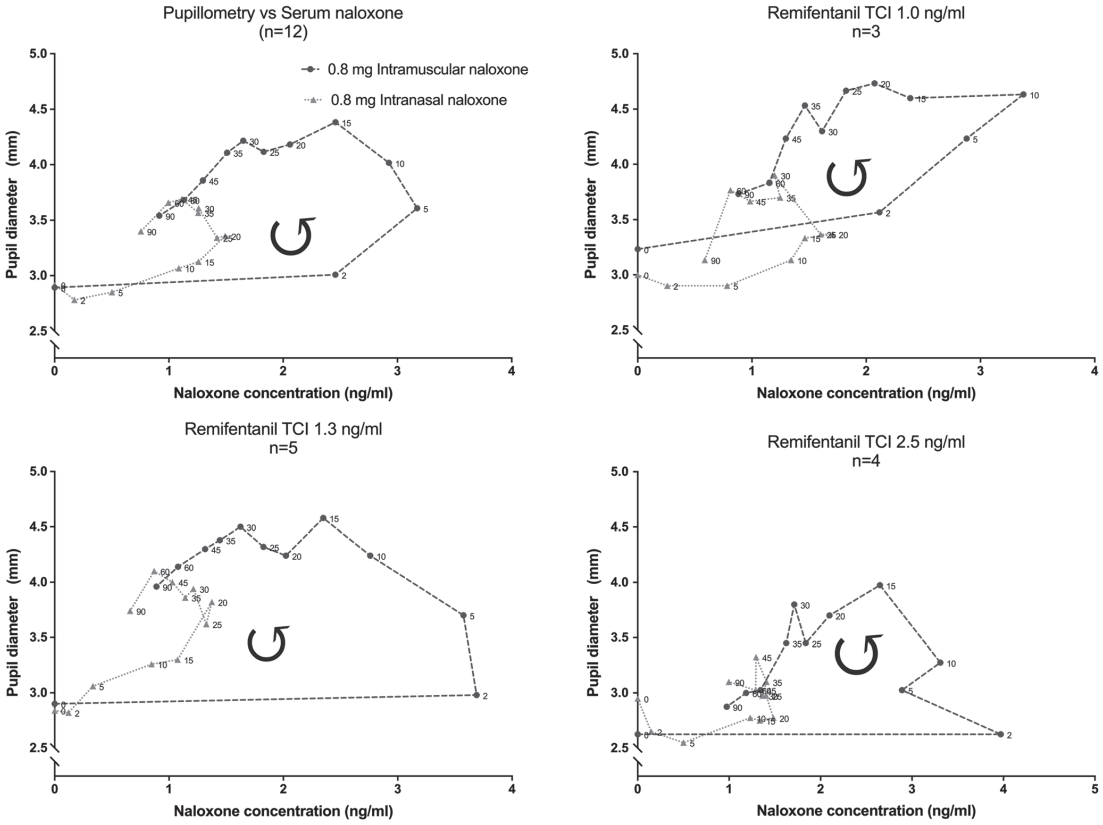

Fig. 5 Hysteresis plot of pupil diameter and naloxone concentration during a stable remifentanil infusion. Each point is numbered corresponding to time it was measured. Error bars are removed for clarity. The arrow indicates the direction of time. Triangles represent the nasal spray and circles the intramuscular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

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

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

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

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

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

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

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

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

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

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

Limitations

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

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

Compliance with ethical standards

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

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

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

References

1. United Nations Office on Drugs and Crime (2016) World drug report. United Nations publication, Sales No. E.16.XI.7
2. Rudd RA, Aleshire N, Zibbell JE, Gladden RM (2016) Increases in drug and opioid overdose deaths—United States, 2000–2014. MMWR Morb Mortal Wkly Rep 64(50–51):1378–1382. https://doi.org/10.15585/mmwr.mm6450a3
3. Clarke SF, Dargan PI, Jones AL (2005) Naloxone in opioid poisoning: walking the tightrope. Emerg Med J 22(9):612–616. https://doi.org/10.1136/emj.2003.009613
4. World Health Organization (2014) Community management of opioid overdose Substance Use:
5. 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 111(4):574–582. https://doi.org/10.1111/add.13209
6. Dale O (2016) Ethical issues and stakeholders matter. Addiction 111(4):587–589. https://doi.org/10.1111/add.13267
7. 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. https://doi.org/10.1097/FTD.0b013e3181816214
8. 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. https://doi.org/10.1111/j.1360-0443.2009.02724.x
9. 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. https://doi.org/10.1016/j.ijpharm.2007.03.025
10. 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. https://doi.org/10.1067/mcp.2002.128386
11. Pond SM, Tozer TN (1984) First-pass elimination. Basic concepts and clinical consequences. Clin Pharmacokinet 9(1):1–25. https://doi.org/10.2165/00003088-198409010-00001
12. ADAPT Pharma NARCAN® (naloxone hydrochloride) nasal spray—PRESCRIBING INFORMATION. http://www.narcannasalspray.com/pdf/NARCAN-Prescribing-Information.pdf. Accessed 22. FEBRUARY 2016 2016
13. Tylleskar I, Skulberg AK, Nilsen T, Skarra S, Jansook P, Dale O (2017) Pharmacokinetics of a new, nasal formulation of naloxone. Eur J Clin Pharmacol 73:1–8. https://doi.org/10.1007/s00228-016-2191-1
14. Macleod DB, Habib AS, Ikeda K, Spyker DA, Cassella JV, Ho KY, Gan TJ (2012) Inhaled fentanyl aerosol in healthy volunteers: pharmacokinetics and pharmacodynamics. Anesth Analg 115(5):1071–1077. https://doi.org/10.1213/ANE.0b013e3182691898
15. Harris SC, Perrino PJ, Smith I, Shram MJ, Colucci SV, Bartlett C, Sellers EM (2013) Abuse potential, pharmacokinetics, pharmacodynamics, and safety of intranasally administered crushed oxycodone HCl abuse-deterrent controlled-release tablets in recreational opioid users. J Clin Pharmacol 54:468–477. https://doi.org/10.1002/jcph.235
16. Staahl C, Upton R, Foster DJ, Christrup LL, Kristensen K, Hansen SH, Arendt-Nielsen L, Drewes AM (2008) Pharmacokinetic-pharmacodynamic modeling of morphine and oxycodone concentrations and analgesic effect in a multimodal experimental pain model. J Clin Pharmacol 48(5):619–631. https://doi.org/10.1177/0091270008314465
17. Gufford BT, Ainslie GR, Padowski JM, Layton ME, White JR, Paine MF (2015) A novel human model to assess reversal of opioid effects. Clin Pharmacol Ther 97:S13–S14. https://doi.org/10.1002/cpt.48
18. Stoops WW, Lofwall MR, Nuzzo PA, Craig LB, Siegel AJ, Walsh SL (2012) Pharmacodynamic profile of tramadol in humans: influence of naltrexone pretreatment. Psychopharmacology 223(4):427–438. https://doi.org/10.1007/s00213-012-2739-4
19. Shram MJ, Silverman B, Ehrich E, Sellers EM, Turncliff R (2015) Use of remifentanil in a novel clinical paradigm to characterize onset and duration of opioid blockade by Samidorphan, a potent mu-receptor antagonist. J Clin Psychopharmacol 35(3):242–249. https://doi.org/10.1097/JCP.0000000000000320
20. Loimer N, Hofmann P, Chaudhry HR (1992) Nasal administration of naloxone for detection of opiate dependence. J Psychiatr Res 26(1):39–43
21. Meissner K, Avram MJ, Yermolenka V, Francis AM, Blood J, Kharasch ED (2013) Cyclosporine-inhibitable blood-brain barrier drug transport influences clinical morphine pharmacodynamics. Anesthesiology 119(4):941–953. https://doi.org/10.1097/ALN.0b013e3182a05bd3
22. Rollins MD, Feiner JR, Lee JM, Shah S, Larson M (2014) Pupillary effects of high-dose opioid quantified with infrared pupillometry. Anesthesiology 121(5):1037–1044. https://doi.org/10.1097/ALN.0000000000000384
23. Kharasch ED, Francis A, London A, Frey K, Kim T, Blood J (2011) Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimal and noninvasive probes for hepatic and first-pass CYP3A induction. Clin Pharmacol Ther 90(1):100–108. https://doi.org/10.1038/clpt.2011.59
24. Friedman MS, Manini AF (2016) Validation of criteria to guide prehospital naloxone administration for drug-related altered mental status. J Med Toxicol 12(3):270–275. https://doi.org/10.1007/s13181-016-0549-5
25. Gustorff B, Felleiter P, Nahlik G, Brannath W, Hoerauf KH, Spacek A, Kress HG (2001) The effect of remifentanil on the heat pain threshold in volunteers. Anesth Analg 92(2):369–374
26. Kim TE, Kim KP, Shin D, Chung YJ, Price J, Mistry P, Jang IJ, Yu KS (2012) Assessment of the analgesic effect of remifentanil using three pain models in healthy Korean volunteers: a randomized, controlled study. Basic Clin Pharmacol Toxicol 110(6):518–523. https://doi.org/10.1111/j.1742-7843.2011.00849.x
27. Lenz H, Raeder J, Draegni T, Heyerdahl F, Schmelz M, Stubhaug A (2011) Effects of COX inhibition on experimental pain and hyperalgesia during and after remifentanil infusion in humans. Pain 152(6):1289–1297. https://doi.org/10.1016/j.pain.2011.02.007
28. Comelon M, Raeder J, Stubhaug A, Nielsen CS, Draegni T, Lenz H (2016) Gradual withdrawal of remifentanil infusion may prevent opioid-induced hyperalgesia. Br J Anaesth 116(4):524–530. https://doi.org/10.1093/bja/aev547
29. American Society of Anasthesiologists. ASA Physical Classification System. http://www.webcitation.org/6fl0gQVDp
30. Brown RL, Leonard T, Saunders LA, Papasouliotis O (1998) The prevalence and detection of substance use disorders among inpatients ages 18 to 49: an opportunity for prevention. Prev Med 27(1):101–110. https://doi.org/10.1006/pmed.1997.0250

31. Health Products Regulatory Authority Ireland Summary of product characteristics Naloxone B Braun. http://www.webcitation.org/6g4edopWl
32. Boyer EW (2012) Management of opioid analgesic overdose. N Engl J Med 367(2):146–155. https://doi.org/10.1056/NEJMra1202561
33. GlaxoSmithKline UK Ltd Ultiva Injection SmPC. Datapharm. http://www.webcitation.org/6rX0zM3Bd. Accessed 27 june 2017
34. Minto CF, Schnider TW, Egan TD, Youngs E, Lemmens HJ, Gambus PL, Billard V, Hoke JF, Moore KH, Hermann DJ, Muir KT, Mandema JW, Shafer SL (1997) Influence of age and gender on the pharmacokinetics and pharmacodynamics of remifentanil. I. Model development. Anesthesiology 86(1):10–23
35. AAGBI Safety Guidelines, Pre- operative Assessment and Patient Preparation (2010). The Association of Anaesthetists of Great Britain and Ireland,
36. US Food and Drug Administration (2001) Statistical approaches to establishing bioequivalence. Guidance for industry
37. Olofsen E, van Dorp E, Teppema L, Aarts L, Smith TW, Dahan A, Sarton E (2010) Naloxone reversal of morphine- and morphine-6-glucuronide-induced respiratory depression in healthy volunteers: a mechanism-based pharmacokinetic-pharmacodynamic modeling study. Anesthesiology 112(6):1417–1427. https://doi.org/10.1097/ALN.0b013e3181d5e29d
38. 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. https://doi.org/10.1111/j.1360-0443.2011.03424.x
39. Yu EH, Tran DH, Lam SW, Irwin MG (2016) Remifentanil tolerance and hyperalgesia: short-term gain, long-term pain? Anaesthesia 71(11):1347–1362. https://doi.org/10.1111/anae.13602
40. Rosow CE, Gomery P, Chen TY, Stefanovich P, Stambler N, Israel R (2007) Reversal of opioid-induced bladder dysfunction by intravenous naloxone and methylnaltrexone. Clin Pharmacol Ther 82(1):48–53. https://doi.org/10.1038/sj.clpt.6100164
41. Kharasch ED, Hoffer C, Whittington D (2004) Influence of age on the pharmacokinetics and pharmacodynamics of oral transmucosal fentanyl citrate. Anesthesiology 101(3):738–743
42. 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 56(10):1243–1253. https://doi.org/10.1002/jcph.759
43. McDonald R, Lorch U, Woodward J, Bosse B, Dooner H, Mundin G, Smith K, Strang J (2017) Pharmacokinetics of concentrated naloxone nasal spray for opioid overdose reversal: phase I healthy volunteer study. Addiction 113:484–493. https://doi.org/10.1111/add.14033
44. McDonald R, Danielsson Glende O, Dale O, Strang J (2017) International patent applications for non-injectable naloxone for opioid overdose reversal: exploratory search and retrieve analysis of the PatentScope database. Drug Alcohol Rev 37:205–215. https://doi.org/10.1111/dar.12571
45. Evzio Full prescribing information (2014)

Article 3

Pharmacokinetics of a novel, approved, 1.4 mg intranasal naloxone formulation for reversal of opioid overdose- a randomised controlled trial.

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Declarations of Interest

The formulation described has been approved by 12 European countries from 16th June 2018 under the name of Ventizolve (Respinal in Sweden), produced by Sanivo Pharma AS. Sponsor of this trial was AS Den Norske Eterfabrikk.

Ola Dale (OD) was engaged by AS Den Norske Eterfabrikk as Principle Investigator in this study for which OD receives no personal honorarium. OD´s employer Norwegian University of Science and Technology (NTNU) and its subsidiary Technical Transfer Office have signed

cooperation and licensing contracts with dne pharma as to seek commercialisation of this nasal naloxone formulation. This regulates potential royalties for OD through NTNU. dne pharma as has compensated OD for business travels from Trondheim to Oslo.
Arne Kristian Skulberg (AKS) has signed a non- compete contract with AS Den Norske Eterfabrikk lasting the duration of his PhD program at NTNU (estimated 2018). This does not limit AKS right to publish results. AKS has received no honorarium from AS Den Norske Eterfabrikk or dne pharma as and will receive no financial benefit from the licence agreement between dne pharma as and NTNU.
Anders Åsberg (AÅ) has received consultant honorarium from dne pharma as in relation to the naloxone formulation presented.
Other authors declare they have no conflicts of interest.

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Abstract

Introduction

Intranasal (IN) naloxone is established as treatment for opioid overdose. Anyone likely to witness an overdose should have access to the antidote. This study presents data on a new formulation of naloxone for this use, recently approved in 12 European countries.

Methods

Open, randomised four-way crossover trial in human volunteers (n=22). One and two doses of IN 1.4 mg naloxone compared to intramuscular (IM) 0.8 mg and intravenous (IV) 0.4 mg naloxone. Quantification of plasma naloxone was performed by liquid chromatography tandem mass spectrometry. Pharmacokinetic non-compartment analyses were used for the main analyses. A non-parametric pharmacokinetic population model was developed for Monte Carlo simulations of different dosing scenarios.

Results

$AUC_{0\text{-}last}$ for IN 1.4 mg and IM 0.8 mg were 2.62 ±0.94 and 3.09 ±0.64 h*ng/mL, respectively (p=0.33). C_{max} was 2.36 ±0.68 ng/mL for IN 1.4 mg, and 3.73 ±3.34 for IM 0.8 mg (p=0.72). Two IN doses showed dose linearity, and achieved a C_{max} of 4.18 ±1.53 ng/mL. T_{max} was reached after 20.2 ±9.4 min for IN 1.4 mg and 13.6 ±15.4 min for IM (p=0.098). The absolute bioavailability for IN 1.4 mg was 0.49 (±0.24), while the relative IN/IM bioavailability was 0.52 (±0.25).

Conclusion

IN 1.4 mg naloxone provides adequate systemic concentrations compared to IM 0.8 mg, without statistical difference on maximum serum concentration, time to maximum serum concentration or area under the curve. Simulations support that it has a place both as peer administered antidote and for titration of treatment by professionals.

Key words

Administration, intranasal; Administration, Intravenous; Administration, intramuscular; Drug Overdose, Substance-Related Disorders, Naloxone, Narcotic Antagonists, Antidotes

Introduction

The increasing number of deaths from opioid overdoses is extensively documented (1-3). Opioid overdoses are reversed by naloxone. The maximum recommended initial dose of naloxone is 2.0 mg, but starting doses of 0.4 - 0.8 mg intramuscularly are favoured. The WHO guideline of 2014 warns that start doses exceeding 0.8 mg may increase the risk of triggering acute opioid withdrawal (2). Acute opioid withdrawal is rarely fatal, but is harmful to the patients. Withdrawal may hinder the further medical and social follow up required by these patients. Restoring ventilation and oxygenation, as well as careful titration of naloxone without overshooting the mark, are the goals of naloxone reversal (4, 5). The lowest safe naloxone dose should be administered initially, with rapid escalation as warranted by the clinical situation (6).

Originally initiated by activist organisations, the distribution of naloxone to lay people has now become an important public health care strategy (7). Intranasal naloxone has been preferred due to its simple administration and reduced risk of exposure to blood. After years of using various off-label, improvised, naloxone formulations without marketing authorisation, several intranasal (IN) naloxone formulations are now licenced in Europe and the US. They are all low volume/high concentration, and are characterised by absorption rates that deliver systemic exposure within the recommended range in one actuation.

In this setting—treatment of a life-threatening condition where titration is the cornerstone—pharmacokinetic (PK) knowledge of the formulation used is important to optimise dosing. The previous use of various dilute naloxone formulations given IN in improvised devices has been criticised (8, 9). Dilute Take Home Naloxone (THN) formulations typically have low bioavailability, ranging from 0.10 to 0.15 (10, 11). The corresponding dose absorbed of a 2.0 mg dose would then be 0.2-0.3 mg; 50-75 % of the lowest recommended starting dose (12). The off-label use of IN naloxone was the only alternative, until FDA approved the Narcan 40 mg/mL nasal spray in 2015, with later additions to the market, both in the US and Europe.

Other approved IN sprays (Narcan Nasal® and Nyxoid®) both deliver systemic exposure of naloxone higher than 0.8 mg IM. There are two reasons for the development of high-dose IN

sprays. In order to receive regulatory approval, the FDA has required that administration forms alternative to 0.4 mg IM must demonstrate similar or higher blood concentrations, especially in the initial absorption phase (13). There is also concern that the naloxone doses that worked in past may be insufficient, as the opioid epidemiology changes, with the introduction of potent synthetic opioids such as fentanyl (14, 15). A meeting in the FDA in 2016 narrowly voted to increase the minimum acceptable naloxone exposure from 0.4 mg (16). The 0.8 mg naloxone comparator is the higher spectrum of the WHO recommendation, and provides increased safety for successful reversal without sparking off avoidable acute withdrawal.

The present study was conducted to demonstrate that a novel formulation delivering 1.4 mg naloxone hydrochloride would achieve systemic exposure comparable to that of 0.8 mg IM. The IN dose was chosen on the basis of previous studies with the same formulation (17-19). The formulation contains the stabiliser EDTA, the mucoadhesive substance povidone and the humectant glycerol. The licensed product will be delivered with two sprays per pack for dose titration.

Material and methods

This study was a two-centre randomised, open label, four-way crossover trial in healthy human volunteers, with 72 hours wash-out.

It was approved by the Regional Committee of Medical and Health Research Ethics (2015/1285) and by the Norwegian Medicines Agency (EudraCT number: 2015-002355-10). All procedures were in accordance with the ethical standards of the Helsinki declaration and the ICH Good Clinical Practice guidelines. The study was registered in clinicaltrials.gov (NCT02598856). Participants were insured trough the Drug Liability Association, Norway, and compensated for each treatment visit with 1000 NOK (110 Euro/120 USD). The trial was conducted at Clinical Trials Units at St. Olavs Hospital, Trondheim, and at Rikshospitalet, Oslo, Norway between October 28th, 2015 and September 30th, 2016. Smerud Medical Research Group operated as clinical Contract Research Organisation.

The primary pharmacokinetic outcome variables were: Area under the plasma concentration versus time curve (AUC) from administration to last measured concentration ($AUC_{0\text{-}last}$), AUC from administration to infinity ($AUC_{0\text{-}inf}$), maximum plasma concentration (C_{max}), and time to C_{max} (T_{max}), compared for single dose IN, IM and IV naloxone. Secondary outcome variables were dose proportionality, by comparing systemic exposure following one and two doses of 1.4 mg of IN naloxone, and absolute and relative bioavailability.

Eligibility criteria for participants.

Healthy men and women aged 18-45 years with haemoglobin, creatinine, aspartate transaminase (AST), alanine transaminase (ALT) and gamma glutamyl transferase within reference values and a normal electrocardiogram (ECG) were eligible for inclusion. Regular use of medications, including herbal, were not allowed. Female participants required a negative pregnancy test, the use of high efficacy contraception from inclusion, and could not be breastfeeding during the study period. Participants with a history of previous nasal surgery, a history of drug allergies or drug addiction were excluded. A full list of inclusion and exclusion criteria is presented in the supplementary material.

Interventions:

There were six study visits, first a screening visit for consent and eligibility criteria, and last for safety follow up. The four visits in between involved the administration of study medicine. All participants were set to receive all treatments. Treatment A: Single dose IN naloxone 1.4 mg: Administered as 0.1 mL 14.0 mg/mL (1.4 mg naloxone HCl) by Aptar Unit dose device as one puff in one nostril. Treatment B: Double dose IN naloxone 1.4 mg: Administered as 2 x 0.1 mL 14.0 mg/mL (2.8 mg naloxone HCl) by Aptar Unit dose device as two puffs in the same nostril, three minutes apart. Treatment C: IM naloxone 0.8 mg: Administered as 2.0 mL Naloxon B. Braun 0.4 mg naloxone HCl/mL in the deltoid muscle. Treatment D: IV naloxone 0.4 mg administered as 1.0 mL Naloxon B. Braun 0.4 mg naloxone HCl/mL. Adverse events were monitored at all visits. All participants underwent anterior rhinoscopy at the screening and the follow up visit.

Randomisation:

This was performed by a computerised procedure from the clinical research organization Smerud, using block randomisation without stratification. Subjects were randomised to treatment order of the four naloxone administrations.

Study procedures:

Participants were reclined fully as they received naloxone. They were monitored with oxygen saturation, ECG and non-invasive blood pressure. Participants who had taken any concomitant medication during the study period had their treatment visit rescheduled to a time where at least five half-lives of the medication had passed, or minimum 7 days, if no half-life was known.

Blood samples were drawn within 10 minutes prior to administration of naloxone, and then at 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240 and 360 minutes after administration of study drug from an IV cannula placed in the antecubital fossa. Six mL blood were collected in glass tubes with K_2EDTA anticoagulant, gently mixed and centrifuged for 20 minutes at 1300 g, and 0.5 mL plasma was decanted into cryotubes and immediately frozen at -20 ºC, and stored at -80 ºC before the end of the day and until analysis.

Naloxone Analysis:

1320 (88 sessions) plasma samples were to be analysed for naloxone using liquid chromatography tandem mass spectrometry. Only subjects contributing with datasets from all visits were included in the statistical analyses. Twenty-one plasma concentrations were missing, and these were not replaced. Of the 1,299 measured plasma concentrations of naloxone, 161 were below the limit of quantification and one was above upper limit of the calibration curve. The latter was set at 47.6 ng/mL and included in analysis. Results below LOQ were not included in the analyses. Two concentrations measured before dose administration showed values above LOQ, and these two were set to zero in the analyses. In total, 182 (7.3%) were either below LOQ or missing, thus a total of 1,138 plasma naloxone concentration measurements were used in the analyses.

The bioanalyses were performed by Vitas AS, Oslo, Norway. The analytical method used was validated in accordance with the European Medicines Agency guideline for bioanalytical

method validation (EMEA/CHMP/EWP/192217/2009). 200 µL plasma was precipitated using methanol containing a stable isotope labelled internal standard (naloxone d5). Precipitated samples were filtered using Impact protein precipitation plates (Phenomenex, Torrance CA, USA). Analysis was performed using an Agilent 1260 LC system coupled to an Agilent 6460 QQQ detector (Agilent Technologies, Palo Alto CA, USA). Separation was performed on a Phenomenex Kinetex EVO C18 (100mm x 3,0 mm x 2,6 µm) column. Quality control samples analysed in duplicate at four levels of analyte were included in each analytical run. QC samples were prepared from pools of human plasma and spiked with naloxone at levels 0.05, 0.26, 15.32 and 38.5 ng/mL. Limit of quantification (LOQ) was <0.02 ng/mL for 26 samples, <0.05 ng/mL for 41 samples and <0.1 ng/mL for 94 samples.

Drug Supply:

Nalokson DnE 14 mg/mL nasal spray was manufactured by AS Den norske Eterfabrikk, Oslo, Norway. Naloxon B. Braun 0.4 mg/mL (B. Braun Melsungen AG, Melsungen, Germany) was supplied from the Hospital Pharmacy in Trondheim, Norway.

Statistics and sample size

The significance level was set to 5%, and the sample size was scaled to not accept bioequivalence of an inferior or superior drug. The data used to assess the anticipated variation in the naloxone data were from previous studies of the same IN formulation. Based on this, it would be necessary to include 22 participants. See Supplementary Material for details. All planned analyses of the efficacy and safety variables were described in the Clinical Trial Analysis Plan. Analysis of variance were preformed using SAS software, version 9.4 (SAS Institute Inc., Cary, USA).

Pharmacokinetic calculations and simulations

Non-compartmental analysis (NCA) was applied assuming a salt factor of 1.0. Time zero concentration for IN and IM administered naloxone was set to zero, and for intravenous naloxone first measured concentration was used also as concentration at time zero. The elimination rate constant (k_{el}) was assessed from at least three concentrations in the semi logarithmic linear elimination phase. $AUC_{0\text{-}last}$ was assessed by the trapezoidal rule. $AUC_{0\text{-}inf}$ was calculated according to the following formula: $AUC_{0\text{-}last} + C_{last}/k_{el}$. Terminal half-life was

calculated as LN(2)/k_{el} and bioavailability (F) as ($AUC_{test,0\text{-}inf}/AUC_{reference,0\text{-}inf}$)* ($DOSE_{reference}/DOSE_{test}$), where test was either IN or IM and reference either IV or IM administered naloxone. Clearance (CL) was calculated as DOSE*F/$AUC_{0\text{-}inf}$.

A non-parametric pharmacokinetic population model was developed for intranasal administration, and one for intramuscular administration, using Pmetrics (version 1.5.0, Laboratory for Applied Pharmacokinetics, Los Angeles, CA) (20). Details on model development, validation and simulation are presented in Supplementary Material.
The population model was used to evaluate different dosing scenarios presented in figures 3 and 4.

Results

Patients: 44 subjects were screened and gave informed consent to participate. 20 were not included, while 24 were randomised, two were withdrawn from the study after randomisation, one because of an adverse event, and one started with medication, leading to exclusion. Twenty-two participants (12 men and 10 women) were included in the final analysis, all providing evaluable data from all four visits. The two participants that received study drug and later withdrawn, were included in the safety analysis. Median age was 25.8 years (min 20.7, max 30.7) and a body mass index of median 22.5 kg/m^2 (min 20.7, max 26.0).

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The mean time-course of the plasma concentrations (0-360 min) for the IN, IM and IV administrations is seen in fig 1. As expected, the distribution and elimination phases are similar in all administrations, with both IN and IM staying above IV after 20 minutes. The real-data absorption phase is magnified in figure 2.1. The absorption rate of IM 0.8 is higher compared to IN, but plasma concentrations following IN 1.4 mg and 2.8 mg administration surpass IM after 15 and 10 minutes, respectively. Figure 2.2 shows the simulated absorption phase, comparing IN 1.4 mg to both IM 0.4 mg and 0.8 mg. Concentrations after IN 1.4 mg exceeds concentrations after IM 0.4 mg after 7.5 minutes, and remains above.

----------Please insert table 1 here----------

C_{max} (table 1) was significantly different between the three administration routes (P=0.031, ANOVA), however IM 0.8 mg and IN 1.4 mg did not differ significantly (P=0.72, TukeyHSD). There was no interaction of treatment sequence on C_{max} (P=0.90, ANOVA).

$AUC_{0\text{-}last}$ (table 1) was significantly different between the three routes (P=0.0025, ANOVA). Significant differences between both IV 0.4 mg and IM 0.8 mg (P=0.008, TukeyHSD) and IN 1.4 mg (P=0.050, TukeyHSD) were seen, but not between IN 1.4 mg and IM 0.8 mg (P=0.33, TukeyHSD). Treatment sequence did not show any significant interaction with the effect (P=0.80, ANOVA). Data analysed as $AUC_{0\text{-}inf}$ showed similar differences as the $AUC_{0\text{-}last}$ data, but in these data IV 0.4 mg and IN 1.4 mg only tended to be significantly different (P=0.059, TukeyHSD). The applied sampling strategy assured coverage of 92% ±6%, 96% ±2%, 90% ±8%, 87% ±11% of the systemic exposure of $AUC_{0\text{-}last}$, compared to $AUC_{0\text{-}inf}$ for IN 1.4 mg, 2xIN 1.4 mg, IM 0.8 mg and IV 0.4 mg, respectively.

T_{max} (table 1) was not significantly different between IM 0.8 mg and IN 1.4 mg (p=0.098, t-test). Mean time to 50% of C_{max} was 10.1 min for IN 1.4 mg naloxone and 6.5 for IM 0.8 mg (p=0.061, t-test). On average, naloxone concentrations following both IN 1.4 mg and IM 0.8 mg were above 0.5 ng/mL at the first sample at 2 minutes (Figure 2).

Mean **terminal elimination half-lives** (table 1) of naloxone ranged from 73-85 min, and were not significantly different between the different administration forms (P=0.11, ANOVA). In the elimination phase 0.5 ng/mL has been suggested as a minimum effective concentration of naloxone (21). Figure 1 shows how IN 1.4 mg maintained its concentration above this for 88 minutes and IN 2.8 mg 118 minutes, IM 0.8 mg 118 minutes and IV 0.4 mg 45 minutes.

The absolute **bioavailability** for IN 1.4 mg in this study was 0.49 ±0.24, while the relative bioavailability to IM 0.8 mg was 0.52 ±0.25.

----------Please insert figure 3 here----------

Dose proportionality assessed by systemic exposure ($AUC_{0\text{-}last}$) between IN 1.4 mg and 2 x IN 1.4 mg naloxone was on average 1.09 ±0.53, and for C_{max} 1.27 ±0.57.

Results from PK simulations

A two-compartment model with five transit compartments in the absorption phase described the data well. The model was parameterised using differential equations with rate constants and volume of distribution in the central compartment, scaled for centralised (median) body weight. No covariates were retained in the final models. The intranasal and intramuscular models had 42 and 41 support points, respectively. A more detailed presentation of model development and validation is presented in supplemental material.

Simulations:

Simulation of the absorption phase in a "standard" person weighing 70 kg from respective population pharmacokinetic model, i.e. the IN- and IM-model separately, is presented in figure 2.2. IM administration is simulated as 0.8 mg and 0.4 mg. The major observation is that the lag in achieved plasma concentrations during the absorption phase between IN 1.4 mg and IM 0.4 mg is, as expected, far smaller than when compared with IM 0.8 mg.

The model is used to visualise clinical scenarios where 1.4 mg IN naloxone is administered prior to, or in addition to, injected naloxone.

----------Please insert figure 4 here----------

Panel 4.1 shows IN 1.4 mg naloxone administered 10 minutes prior to injected IM naloxone, a common scenario in THN. Plasma concentrations following IN 1.4 mg remain above the concentrations obtained by IM 0.4 mg, during the whole period. They do not reach the levels obtained by IM 0.8 mg within this 20-minutes period. Panel 4.2 simulates the shortest time IN 1.4 mg could be administered prior to IM 0.4 mg, and constantly provides higher plasma concentrations. That time is 2.25 minutes. Panel 4.3 simulates the opposite; the injection of IM 0.4 mg naloxone, 10 minutes after IN 1.4 mg is given. The C_{max} in this scenario is 3.15 ng/mL, lower C_{max} than what we find for IM 0.8 mg in our real data.

Safety and adverse events: At anterior rhinoscopy at the follow-up visit, one had abnormal colour and swelling of mucosa, one had abnormal amount and colour of secretion and one had presence of concha inferior swelling, not present prior to the study. One participant had a clinically significant increased value of ALT after treatment with IN 1.4 mg, and was withdrawn. This increase of the ALT value was deemed possibly related to the study drug. A total 31 adverse events were reported for 14 participants in the study. All adverse events reported were of mild severity, except for one abnormal haemoglobin, which was reported as moderate, but unrelated to treatment. The adverse events reported most by participants were headache and nasal congestion. For questions related to irritation in the nose, no events were reported for rhinorrhoea, itching and loss of smell sensation. Intranasal administration of 1.4 mg naloxone was found to be safe and well tolerated by healthy volunteers.

Discussion

The major finding in this study was that the absorption of 0.8 mg naloxone administered IM was slightly faster than for the IN 1.4 mg. There were no statistically significant differences between IN 1.4 mg and IM 0.8 mg in $C_{max,}$ $T_{max,}$ or $AUC_{0\text{-}last}$. IN naloxone showed dose linear increase in systemic exposure for two doses to the same nostril separated by three minutes, indicating that it is suited for repeated administration and titration. Simulations showed that IN 1.4 mg naloxone compares well with 0.4 mg IM naloxone, providing higher concentrations within 7.5 minutes. The present IN formulation was safe in healthy volunteers, and has received regulatory approval in 12 European countries under the trade name Ventizolve® (Respinal® in Sweden).

This study builds on two previously published studies of a similar naloxone formulation (17, 18). The formulation shows similar dose corrected C_{max} across these studies. The absolute bioavailability was also similar, but the relative bioavailability compared to IM was lower compared to when naloxone was given together with remifentanil (18).

Several new naloxone formulations have come to the market in recent years. Nyxoid 1.8 mg IN naloxone by Mundipharma (Cambridge, UK) (22) and Narcan Nasal 2.0 mg and 4.0 mg IN

naloxone (Adapt Pharma, Inc. Radnor, PA, USA) (23) are now available. These formulations and the present 1.4 mg have several pharmacokinetic characteristics in common. They can all deliver a therapeutic dose (corresponding to 0.4-2.0 mg IM) by one actuation of a 0.1 mL volume by the Aptar Unit dose device. They all have a relative bioavailability of about 50%, similar average T_{max} of 21 minutes (min 15, max 30), and similar dose corrected C_{max} (1.52 ± 0.16 ng/mL, n=9). Although the absolute C_{max} of IN 1.4 mg was 82 % and 76% of Nyxoid and Narcan, respectively, IN 1.4 mg C_{max} was 186 % compared to that of 0.4 mg IM (22). $AUC_{0\text{-}inf}$ for IN 1.4 mg was 85% of that of IM 0.8 mg, but again this exceeds by far the published AUC values of 0.4 mg IM (157% and 134% of (Narcan Nasal and Nyxoid,) respectively.

Questions have been raised about different uptake and interactions with opioids or other drugs used by patients in overdose. In a previous study of this IN naloxone formulation administered with the opioid remifentanil, the relative bioavailability to IM was 75% (18). This led to the conclusion that there may be an interaction between naloxone and remifentanil. Further studies in this direction can bridge the gap between healthy volunteers and patients presenting with opioid overdose.

The current formulation was compared with IM 0.8 mg naloxone as reference, as it represents the safe upper end of the start-dose recommendations, without undue risk of triggering withdrawal. As other regulatory studies relate to 0.4 mg IM, a population kinetic simulation was developed to examine the relations between 1.4 mg IN and 0.4 mg IM. Modelling is also used to compare different treatments in a Take Home Naloxone scenario, where peer administered naloxone may substitute or be combined with injected naloxone by ambulance personnel. Titration is the core principle in naloxone reversal of overdose, and these simulations can guide clinical use. Part of the rationale of THN is to shorten the time from an opioid overdose is suspected to the administration of antidote. Calling for help, dispatch and transport times for ambulance personnel, securing the workplace, establishing airway and breathing control, and preparing and injecting naloxone takes considerable time. As shown in Figure 4.1; when naloxone was given 10 minutes prior to naloxone injected, the THN administration of the present formulation delivered serum concentrations above IM 0.4 mg at all times, however, below IM 0.8 mg. Calculations showed that when IN 1.4 mg was given as close as 2.25 min before IM injection of 0.4 mg, it still provided higher blood

concentrations (figure 4.2). This indicates a clinical benefit by this IN formulation, even by ambulance personnel, as 2.25 minutes is comparable to the time it takes to prepare an IM injection site, fill a syringe and inject naloxone, or to establish IV access (24). Figure 4.3 shows a simulation where ambulance personnel administer 0.4 mg IM naloxone 10 minutes after 1.4 mg is given as THN. This would be relevant if a patient remained unresponsive after one dose IN, and ambulance personnel suspected opioid intoxication to be a possible cause. The C_{max} in this scenario is almost identical to the arithmetic mean of Nyxoid 1.8 mg, and is reached 5 minutes after ambulance personnel administered IM naloxone. The early administration of antidote is the rationale behind THN, and the simulations show that IN 1.4 mg has a place in this treatment model and is well suited for titration.

The safe initial dose of naloxone is debated (13), and will remain a balancing act between safe reversal and the precipitation of acute withdrawal reactions (4). Dilute formulations have shown to provide relatively low rate of repeat naloxone dosing in the field (25-27). Previously approved nasal formulations deliver systemic exposure similar to 1.0 and 2.0 mg injected naloxone, which is above the upper initial dose recommended by the WHO (2). A high initial dose will increase the likelihood of provoking acute withdrawal; the symptoms are well described (28), and experiencing withdrawal is feared among opioid abusers (29). Withdrawal and inadequate follow up may lead to death (30). Withdrawal is a part of what leads to early discharge or being left at the scene against medical advice. Both must be seen as less than ideal follow-up after non-fatal overdoses. Being left at the scene of the overdose has been debated over the years and found to be relatively safe, as death immediately after is rare (31, 32). This may change in the future with the arrival of more potent opioids, and vary between the location and other circumstances of the overdose (33). There is conflicting evidence regarding the fentanyl-like opioids and the need for potent naloxone formulations (34, 35), but basic first aid with ventilation and antidote titration will remain treatment gold standard.

Limitations

This is study is conducted in healthy volunteers, that may differ from patients being treated for opioid overdose. Our participants did not use concomitant medication, so interactions

with other drugs, prescription or illegal, are not assessed. The conclusions in this study is based on plasma concentrations, not relevant clinical end-points.

Conclusion

IN 1.4 mg naloxone provides adequate systemic concentrations compared to IM 0.8 mg, without statistical difference on maximum serum concentration, time to maximum serum concentration or area under the curve. The naloxone exposure following administration by this formulation far exceeds more dilute "off-label" formulation often used in Take Home Naloxone programs. Compared to the higher doses in other nasal sprays, IN 1.4 mg can reduce the risk for withdrawal, while still safe, as it reaches relevant plasma concentrations fast. It exceeds IM 0.4 mg after 7.5 minutes. Simulations support that it has a place both as peer administered antidote and for titration of treatment by professionals. However, only randomised clinical trials on real opioid overdoses can determine whether IN naloxone can compare with IM naloxone.

Acknowledgements

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Figure legends

Figure 1: Time course of plasma concentrations 0-360 minutes (mean ±SD) of naloxone after intranasal (1.4 and 2.8 mg), intramuscular 0.8 mg and intravenous (0.4 mg) administration in healthy human volunteers (n=22). Dashed horizontal line indicates 0.5 ng/ml, a proposed minimum effective concentration in the elimination phase.

Figure 2:

2.1: Time-course of plasma concentrations 0-30 minutes (mean values, variability removed for clarity) of naloxone after intranasal (1.4 and 2.8 mg), intramuscular 0.8 mg and intravenous (0.4 mg) administration in healthy human volunteers (n=22).

2.2: Simulated time course of plasma concentrations 0-30 minutes (mean ±SD as shaded area) of naloxone after intranasal 1.4 mg and intramuscular 0.4 mg and 0.8 mg.

Figure 3: Box plot of absolute and relative bioavailability of IN 1.4 mg naloxone. Bold line is median, box is 75% percentiles, and whiskers are 95% percentiles.

Figure 4: Simulated time courses of mean naloxone concentrations (line) and standard deviations as shaded area. 0 minutes indicate a time of administration of injected naloxone.

4.1 shows IN 1.4 mg naloxone administered 10 minutes prior to injected naloxone (0.4 and 0.8 mg).

4.2 shows the simulation of the shortest time (2.25 min) beneficial to give IN, rather than wait for naloxone to be injected.

4.3 simulates a situation where IM 0.4 mg naloxone is injected to a patient already given IN 1.4 mg 10 minutes before.

References

1. Rudd RA, Aleshire N, Zibbell JE, Gladden RM. Increases in Drug and Opioid Overdose Deaths--United States, 2000-2014. MMWR Morb Mortal Wkly Rep. 2016;64(50-51):1378-82.
2. World Health Organization. Management of Substance Abuse Team, World Health Organization. Community management of opioid overdose. Geneva: World Health Organization,; 2014.
3. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Preventing opioid overdose deaths with take-home naloxone. European Monitoring Centre for Drugs and Drug Addiction; 2016.
4. Clarke SF, Dargan PI, Jones AL. Naloxone in opioid poisoning: walking the tightrope. Emerg Med J. 2005;22(9):612-6.
5. Boyer EW. Management of Opioid Analgesic Overdose. N Engl J Med. 2012;367(2):146-55.
6. Goldfrank LR, Flomenbaum NE, Lewis NA, Howland MA, Hoffman RS, Nelson LS. Goldfrank's Toxicologic Emergencies. 7th ed: McGraw Hill; 2002.
7. McDonald R, Strang J. Are take-home naloxone programmes effective? Systematic review utilizing application of the Bradford Hill criteria. Addiction. 2016;111(7):1177-87.
8. Strang J, McDonald R, Tas B, Day E. Clinical provision of improvised nasal naloxone without experimental testing and without regulatory approval: imaginative shortcut or dangerous bypass of essential safety procedures? Addiction. 2016;111(4):574-82.
9. Dale O. Ethical issues and stakeholders matter. Addiction. 2016;111(4):587-9.
10. McDonald R, Danielsson Glende O, Dale O, Strang J. International patent applications for non-injectable naloxone for opioid overdose reversal: Exploratory search and retrieve analysis of the PatentScope database. Drug and alcohol review. 2018;37(2):205-15.
11. Edwards E, Kessler C, Kelley G, Gapasin A, Mardari G, Goldwater R. PAINWeek Abstract Book 2016: Pharmacokinetics of 2.0 mg intranasal and intramuscular naloxone

HCL administration and the impact of vasoconstrictor use on the bioavailability of intranasal naloxone HCL. Postgrad Med. 2016;128(sup2):46.
12. McDonald R, Dale O, Kral AH, Strang J. Use of take-home naloxone for the emergency management of opioid overdose. Drugs. 2018, in review.
13. US Food and Drug Administration. Joint Meeting of the Anesthetic and Life Support Drugs Advisory Committee and Drug Safety & Risk Management Advisory Committee 2016 [updated September 9, 2016. Available from: http://www.webcitation.org/70vfcWrJ2
14. Suzuki J, El-Haddad S. A review: Fentanyl and non-pharmaceutical fentanyls. Drug Alcohol Depend. 2017;171:107-16.
15. Fairbairn N, Coffin PO, Walley AY. Naloxone for heroin, prescription opioid, and illicitly made fentanyl overdoses: Challenges and innovations responding to a dynamic epidemic. International Journal of Drug Policy. 2017;46:172-9.
16. US Food and Drug Administration. Summary Minutes of the Joint Meeting of the Anesthetic and Analgesic Drug Products Advisory Committee and the Drug Safety and Risk Management Advisory Committee October 5, 2016 www.fda.gov2016 [cited 2018 4. november]. Available from: http://www.webcitation.org/73g7tOzsK
17. Tylleskar I, Skulberg AK, Nilsen T, Skarra S, Jansook P, Dale O. Pharmacokinetics of a new, nasal formulation of naloxone. Eur J Clin Pharmacol. 2017;73(5):555-62.
18. Skulberg AK, Tylleskar I, Nilsen T, Skarra S, Salvesen Ø, Sand T, et al. Pharmacokinetics and -dynamics of intramuscular and intranasal naloxone: an explorative study in healthy volunteers. Eur J Clin Pharmacol. 2018;74(7):873-83.
19. Tylleskar I. Nasal naloxone - A pilot study of the pharmacokinetics of a concentrated formulation. Trondheim, Norway: Norwegian University of Science and Technology 2017.
20. Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. Ther Drug Monit. 2012;34(4):467-76.
21. Tylleskar I, Skulberg AK, Skarra S, Nilsen T, Dale O. Pharmacodynamics and arteriovenous difference of intravenous naloxone in healthy volunteers exposed to remifentanil. Eur J Clin Pharmacol. 2018.
22. McDonald R, Lorch U, Woodward J, Bosse B, Dooner H, Mundin G, et al. Pharmacokinetics of concentrated naloxone nasal spray for opioid overdose reversal: Phase I healthy volunteer study. Addiction. 2018;113(3):484-93.
23. Krieter P, Chiang N, Gyaw S, Skolnick P, Crystal R, Keegan F, et al. Pharmacokinetic Properties and Human Use Characteristics of an FDA-Approved Intranasal Naloxone Product for the Treatment of Opioid Overdose. The Journal of Clinical Pharmacology. 2016;56(10):1243-53.
24. McDermott C, Collins NC. Prehospital medication administration: a randomised study comparing intranasal and intravenous routes. Emerg Med Int. 2012;2012:476161.
25. Kerr D, Kelly A-M, Dietze P, Jolley D, Barger B. Randomized controlled trial comparing the effectiveness and safety of intranasal and intramuscular naloxone for the treatment of suspected heroin overdose. Addiction. 2009;104(12):2067-74.
26. Klebacher R, Harris MI, Ariyaprakai N, Tagore A, Robbins V, Dudley LS, et al. Incidence of Naloxone Redosing in the Age of the New Opioid Epidemic. Prehosp Emerg Care. 2017;21(6):682-7.
27. Weiner SG, Mitchell PM, Temin ES, Langlois BK, Dyer KS. Use of Intranasal Naloxone by Basic Life Support Providers. Prehosp Emerg Care. 2017;21(3):322-6.
28. Buajordet I, Naess AC, Jacobsen D, Brors O. Adverse events after naloxone treatment of episodes of suspected acute opioid overdose. Eur J Emerg Med. 2004;11(1):19-23.
29. Neale J, Strang J. Naloxone--does over-antagonism matter? Evidence of iatrogenic harm after emergency treatment of heroin/opioid overdose. Addiction. 2015;110(10):1644-52.
30. Darke S, Larney S, Farrell M. Yes, people can die from opiate withdrawal. Addiction. 2017;112(2):199-200.

31. Willman MW, Liss DB, Schwarz ES, Mullins ME. Do heroin overdose patients require observation after receiving naloxone? Clin Toxicol. 2016;55(2):81-7.
32. Rudolph SS, Jehu G, Nielsen SL, Nielsen K, Siersma V, Rasmussen LS. Prehospital treatment of opioid overdose in Copenhagen--is it safe to discharge on-scene? Resuscitation. 2011;82(11):1414-8.
33. Madah-Amiri D, Skulberg AK, Braarud AC, Dale O, Heyerdahl F, Lobmaier P, et al. Ambulance-attended opioid overdoses: An examination into overdose locations and the role of a safe injection facility. Subst Abus. 2018;Online 27 Jun 2018.:1-6.
34. Bell A, Bennett AS, Jones TS, Doe-Simkins M, Williams LD. Amount of naloxone used to reverse opioid overdoses outside of medical practice in a city with increasing illicitly manufactured fentanyl in illicit drug supply. Subst Abus. 2018:1-4.
35. White JM, Irvine RJ. Mechanisms of fatal opioid overdose. Addiction. 1999;94(7):961-72.

Figure 1

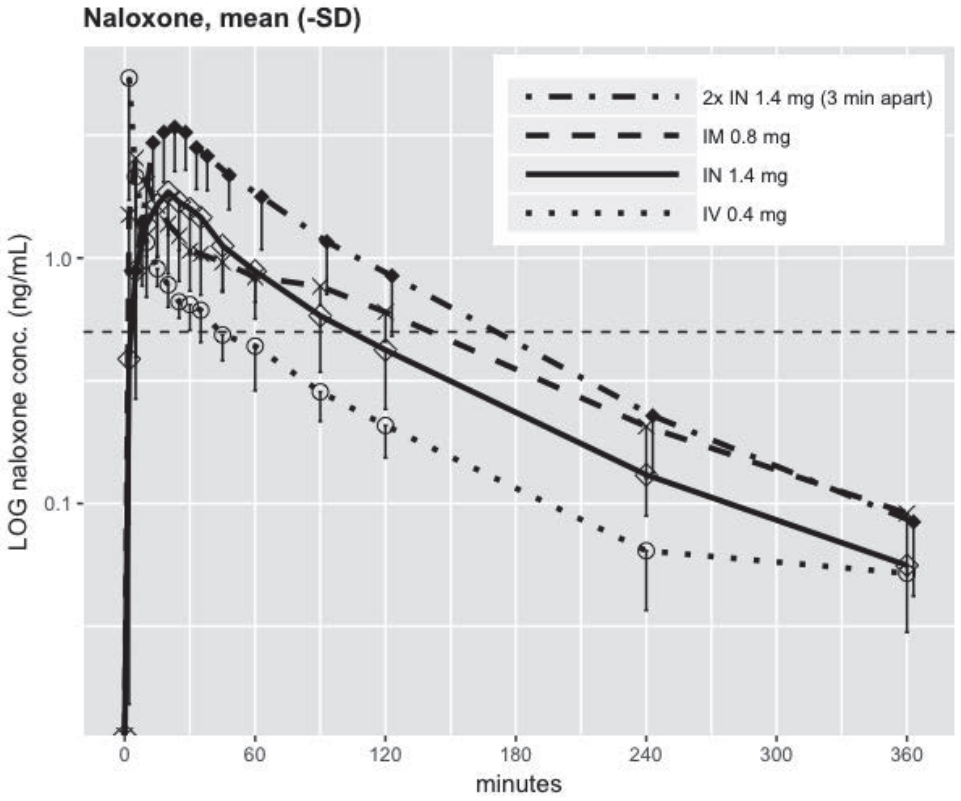
Naloxone, mean (-SD)
2x IN 1.4 mg (3 min apart)
IM 0.8 mg
IN 1.4 mg
IV 0.4 mg
LOG naloxone conc. (ng/mL)
1.0
0.1
0
60
120
180
240
300
360
minutes

Figure 2

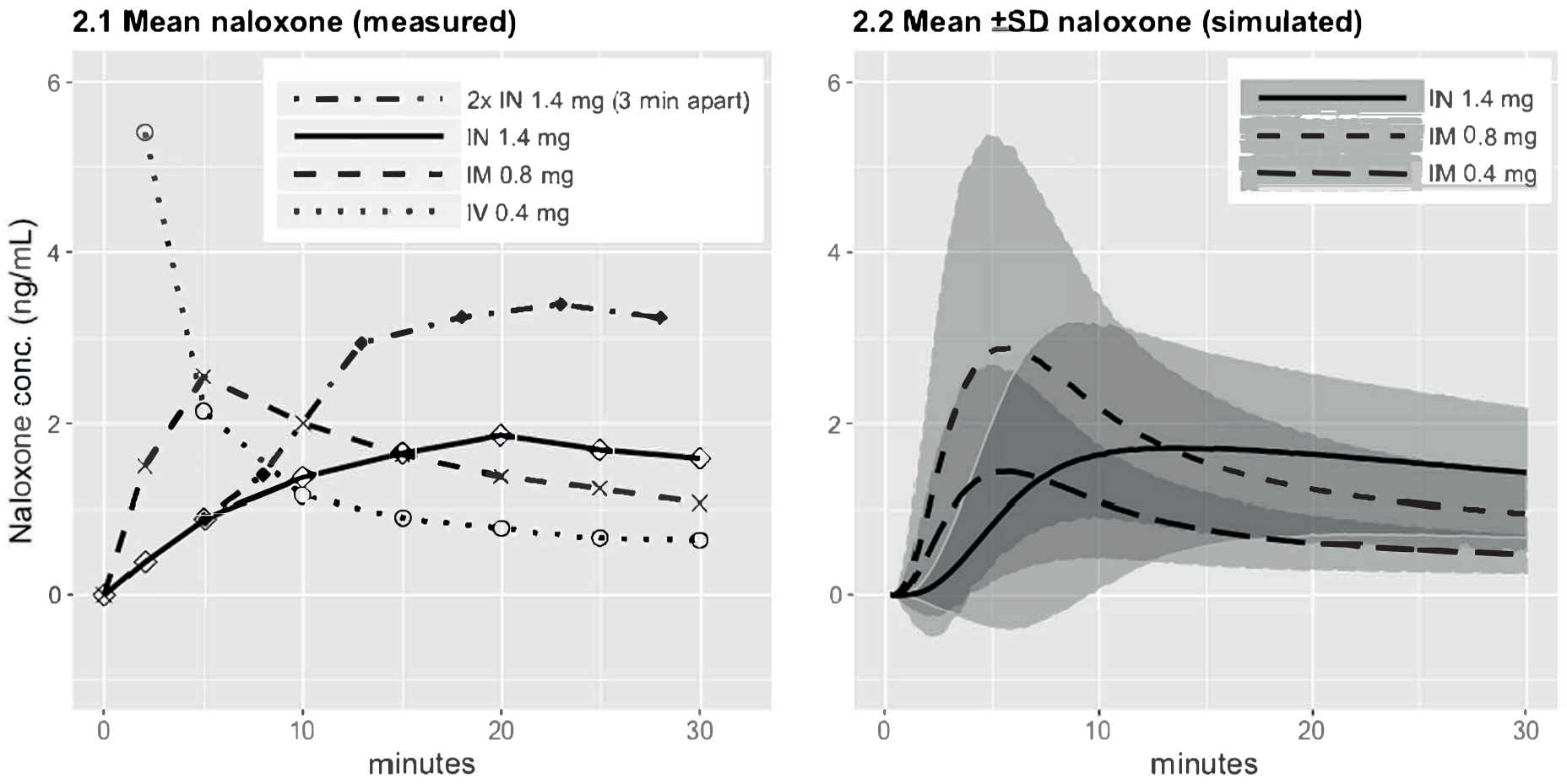
2.1 Mean naloxone (measured)
2x IN 1.4 mg (3 min apart)
IN 1.4 mg
IM 0.8 mg
IV 0.4 mg
Naloxone conc. (ng/mL)
0
2
4
6
0
10
20
30
minutes
2.2 Mean ±SD naloxone (simulated)
IN 1.4 mg
IM 0.8 mg
IM 0.4 mg
0
2
4
6
0
10
20
30
minutes

Figure 3

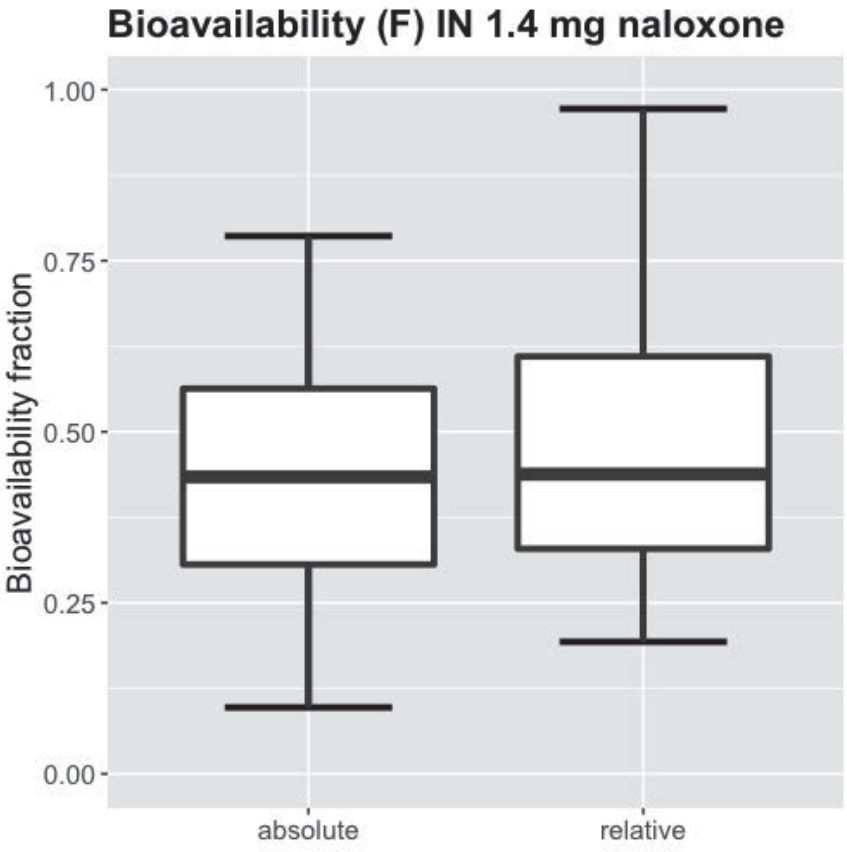
Bioavailability (F) IN 1.4 mg naloxone
Bioavailability fraction
1.00
0.75
0.50
0.25
0.00
absolute
relative

Figure 4

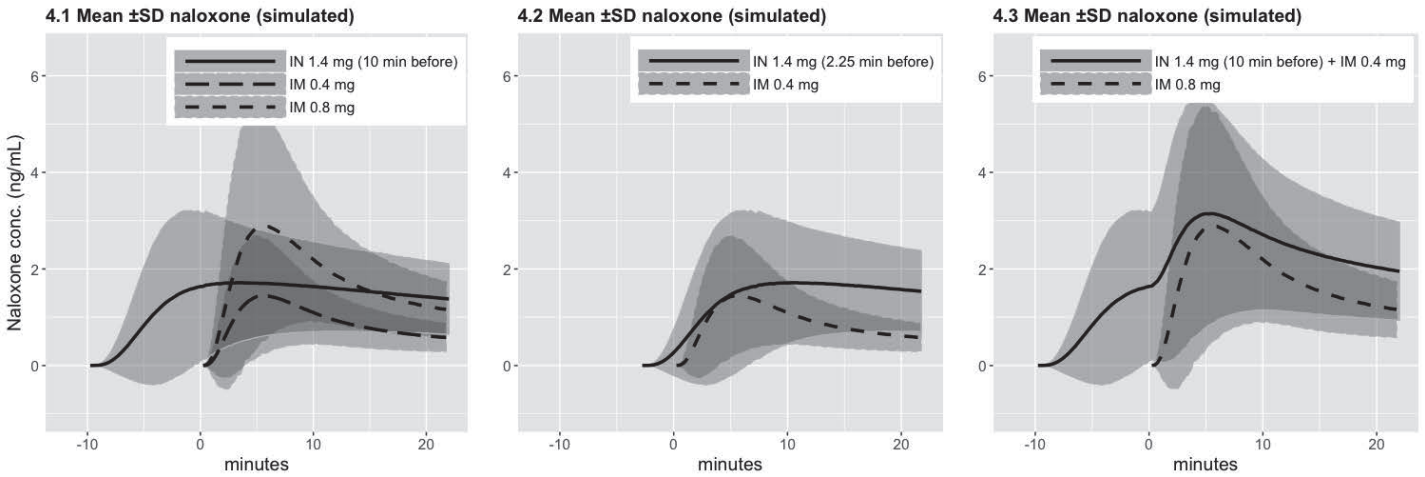
4.1 Mean ±SD naloxone (simulated)
IN 1.4 mg (10 min before)
IM 0.4 mg
IM 0.8 mg
Naloxone conc. (ng/mL)
6
4
2
0
-10
0
10
20
minutes
4.2 Mean ±SD naloxone (simulated)
IN 1.4 mg (2.25 min before)
IM 0.4 mg
6
4
2
0
-10
0
10
20
minutes
4.3 Mean ±SD naloxone (simulated)
IN 1.4 mg (10 min before) + IM 0.4 mg
IM 0.8 mg
6
4
2
0
-10
0
10
20
minutes

Table 1. Pharmacokinetic variables (mean values ±SD) n= 22 healthy volunteers after intranasal, intravenous and intramuscular administration of naloxone in an open, randomised four-way-way crossover trial.

Treatment	Cmax (ng/ml)	Tmax (min)	AUClast (h*ng/ml)	AUC0- inf (h*ng/ml)	Terminal half-life (min)	Cl (L/h)
1.4 mg IN	2.36 ±0.68[§]	20.2 ±9.4[§]	2.62 ±0.94[§]	2.84 ±0.94[§]	73.0 ±20.2[§]	239 ±68
2.8 mg IN	4.18 ±1.53	20.7 ±9.54	5.23 ±1.79	5.47 ±1.89	69.8 ±12.8	250 ±66
0.8 mg IM	3.73 ±3.34	13.6 ±15.4	3.09 ±0.64	3.43 ±0.66	84.8 ±26.5	236 ±68
0.4 mg IV	7.44 ±9.67	3.5 ±4.2	1.84 ±1.49	2.09 ±1.47	74.3 ±32.1	223 ±58

Abbreviations: Cmax: maximum concentration, Tmax: time to maximum concentration,
AUClast: area under the curve until last measurement. AUC0- inf: area under the curve until infinity, Cl: clearance
IN 2.8 mg is administered as IN 1.4 mg naloxone 3 minutes apart in the same nostril
[§]not statistically significant different to 0.8 mg IM ($p>0.05$)

Supplementary material

Pharmacokinetics of a novel, approved, 1.4 mg intranasal naloxone formulation for reversal of opioid overdose.

Arne Kristian Skulberg, Anders Åsberg, Hasse Zare Khiabani, Hilde Røstad[2], Ida Tylleskar, Ola Dale

1 Study participation

Inclusion criteria

In order to participate in this study the subjects had to meet all of the following inclusion criteria:

1. Provision of a signed written informed consent.
2. Healthy men and women aged 18- 40 years
3. ECG without any pathological abnormalities
4. Have a BMI range of 18.5- 26.0 kg/m2
5. Female subject with child bearing potential must use high efficacy contraception. For the purpose of this study acceptable contraception is defined as oral contraceptives, patch, implants, vaginal ring, hormonal IUD, copper IUD, sterilization through out the study until the last visit.
6. Laboratory values within reference values for the following haematology and biochemistry tests:

a. Haemoglobin, b.Creatinine, c. AST, d.ALT, e.Gamma GT

Exclusion criteria

In order to participate in the study subjects could not meet any of the following exclusion criteria:

1. Subjects using medication on a regular basis, including regular use of nasal spray of any form.
2. History of prior drug allergy.
3. Subject having local nasal disease or nasal surgery for the last 2 months.

4. Pregnant or breast feeding women. A serum HCG below 3 U/L must be demonstrated in females of child-bearing potential at Screening Visit.

5. Current drug or alcohol abuse which in the opinion of the Investigator should preclude participation in the study.

6. Has received another new medical chemical entity (defined as a compound which has not

been approved for marketing) or has participated in any other clinical study that included drug treatment within 3 months of the administration of investigational product in this study.

7. Hypersensitivity to naloxone or any of its excipients.

8. Investigator considers subject unlikely to comply with study procedures, restrictions and or other requirements.

2 Sample size calculation:

The significance level was set to 5% and the sample size was scaled to not accept bioequivalence and at the same time be significantly different from unity, i.e. included the number of patients that would provide a 90% CI of the AUC-ratio that was as wide as the acceptance range so it was not constrained within the 80 to 100% (or 100 to 125 %) acceptance ranges. The data used to assess the anticipated variation in the naloxone data was from previous studies of the same IN formulation. Scaled AUC-data from Skulberg et al 2018 to an IM dose of 0.8 mg and an IN dose of 1.4 mg was used in the power calculation. The SD for the IN:IM AUC-ratio (LN-scale) in the Skulberg et al 2018 data were 0.41 and based on this it would be necessary to include 22 participants using the formula; $N=(1.645*SD/0.11155)^2$. The point estimate for the AUC-ratio was 1.31.

3 Pharmacokinetic population model – development/validation

A non-parametric population model was developed for intranasal administration and one for intramuscular administration using Pmetrics (version 1.5.0, Laboratory for Applied Pharmacokinetics, Los Angeles, CA) (21). All concentrations fulfilling the criteria for being included in the analyses as outlined was included in the development of the models and the intravenous data were used in both models to parameterize it with absolute bioavailability.

Time zero concentrations were not imputed. Data from different investigation days in the same patient were treated as separate individuals during the model development and validation. The absorption phase was estimated both with lag-time, gamma distribution as well as a range of transit compartments. Both the additive lamda and multiplicative gamma error models in Pmetrics were tested during the model development, using an assay error polynomial obtained from the laboratory (C0=0.006033624, C1=0.03669370, C2=0.0009973684, C3=-0.00002028414). As many multiples of 80,021 grid points as possible were applied (limited by hardware storage capacity), with uniform initial distribution, and the analyses were run on a MacBook Pro (2.7 GHz Intel Core i7 Duo processor, 16 GB 1600 MHz DDR3 memory and running OS X, version 10.13.5; Apple Inc, Cupertino, CA, USA). The apparent distribution volume of the central compartment was scaled to centralized body size; testing body weight, body mass index (BMI) and fat-free mass. Covariates were scaled to the median population values and continuous covariates were extrapolated between observations. Covariates were included stepwise, followed by a reduction of the resulting model by taking one covariate out of the model. Available covariates for testing in the model were; sex, age, height, body weight, BMI and the following blood/plasma variables; haemoglobin, creatinine, ASAT, ALAT and gamma GT. Model selection was based on comparison of the Akaike information criterion (AIC), the fit of both the population and individual observed vs. predicted plots and biological plausibility. The model was evaluated for its predictive accuracy on an external validation data set consisting of 29 new patients and in total 971 naloxone concentrations obtained from previous studies (14, 17-19). From the Bayesian prior model parameter joint density, Pmetrics calculated the Bayesian posterior joint density for each subject in the external validation set without cycling. The median marginal parameter values of each posterior density were used to calculate the predicted naloxone concentrations, given individual naloxone dosing and patient covariates. The following statistics were computed: median PE (predicted minus observed concentrations) and relative PE (100*(predicted minus observed concentrations)/observed concentrations). These statistics in the external validation set were compared to the same statistics in the model development participants.

Pharmacokinetic population model - results: A 2-compartment model with 5 transit compartments in the absorption phase described the data well. The model was parameterised using differential equations with rate constants and volume of distribution in the central compartment scaled for centralised (median) body weight. Plasma creatinine tended to be associated with the elimination rate constant but was not retained in the final model. The multiplicative gamma error model gave Hessian error with the model why the additive lamda function was used as error model. Both the intranasal and intramuscular model was initiated with 10 x 80,021 support points. The intranasal model converged after 8,145 cycles with 42 support points and an AIC of 451. The intramuscular model converged after 2,988 cycles with 41 support points and an AIC of -553. Parameter values for respective model are shown in **Table S1 and S2** and observed versus predicted plots in **Figure S3 and S4** together with absolute and relative predicted error for the two models are shown in **Table S5** for both the development dataset as well as the external validation dataset.

Table S1. Parameter values for the final intranasal model.

	Mean	SD	CV%	Var	Median	Shrink%
Vx	123.342	107.839	87.431	11629.304	91.620	8.857
FAx	0.431	0.151	35.060	0.023	0.414	7.655
K70	5.061	4.967	98.135	24.671	2.938	3.261
K78	23.593	19.807	83.951	392.301	18.537	13.597
K87	13.600	10.751	79.048	115.582	9.388	4.341
Ktrx	39.124	26.623	68.047	708.783	33.871	7.455

Table S2. Parameter values for the final intramuscular model

	Mean	SD	CV%	Var	Median	Shrink%
Vx	146.146	118.474	81.065	14036.050	129.201	1.092
FAx	0.713	0.247	34.706	0.061	0.827	0.505
K70	4.548	5.456	119.973	29.773	1.646	0.039
K78	13.543	13.935	102.899	194.189	10.849	1.168
K87	6.959	7.762	111.527	60.243	3.131	0.292
Ktrx	96.692	71.800	74.257	5155.298	67.550	0.279

Figure S3. OP-plot intranasal

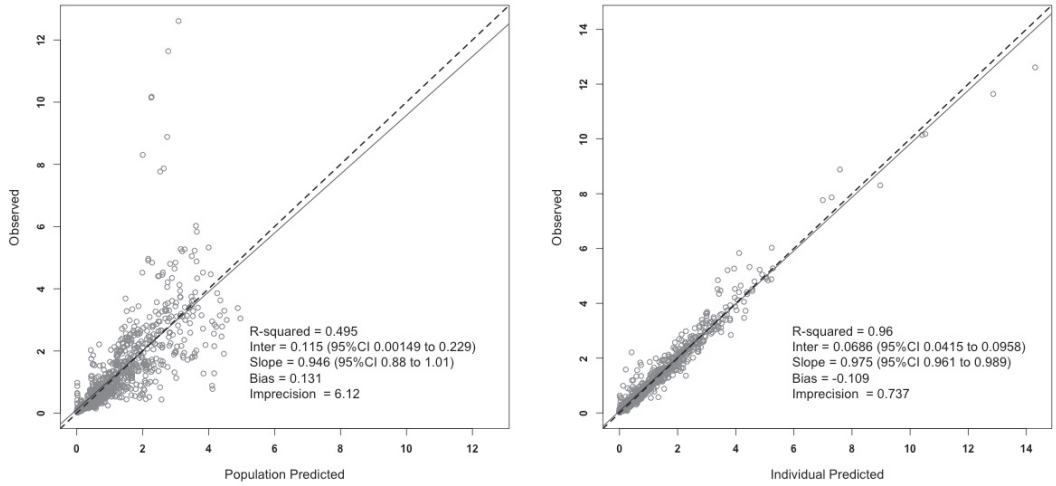

Figure S4. OP-plot intramuscular

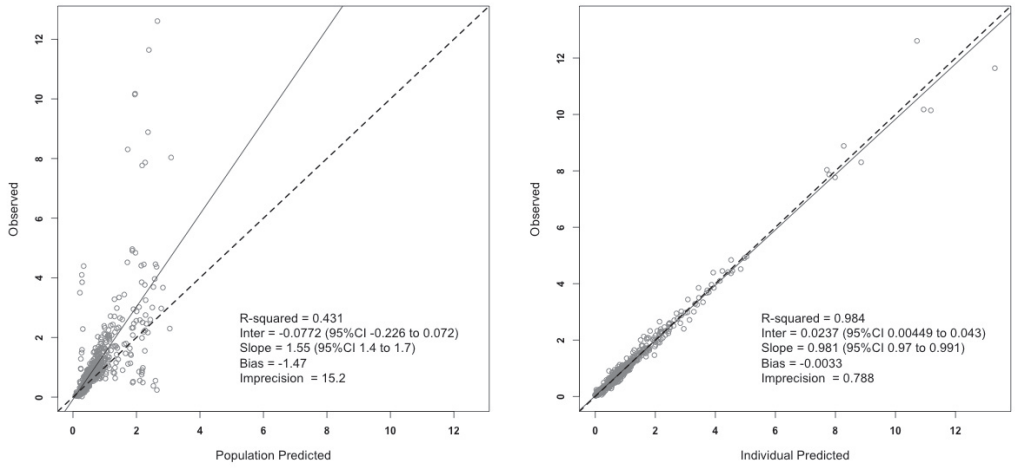

Table S5. Median validation statistics for internal and external validation of the final models

	Internal validation		External validation	
	IN	IM	IN	IM
PE (ng/mL)	-0.014	0.0029	-0.064	-0.052
Relative PE (%)	-1.03	0.45	-15	-7.1

4 Pharmacokinetic population model - simulations

The two population models were used to perform Monte Carlo simulations. A "standard person" weighing 70 kg served as a simulation template for 1000 naloxone time-concentration profiles calculated from parameters sampled from the model population joint density, including the full covariance matrix. Simulated naloxone concentrations were corrupted by noise using the same error polynomial as in the population model. Simulated parameter values were restricted to be physiologically plausible by applying the same boundaries as in the model. Simulation a) mentioned above was applied for estimating time to 0.5 ng/ml as well as times to 50% of C_{max}.