Pharmacodynamics and arteriovenous difference of intravenous naloxone in healthy volunteers exposed to remifentanil

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Abstract

Purpose
Pharmacodynamic studies of naloxone require opioid agonism. Steady state condition may be achieved by remifentanil TCI (target controlled infusion). Opioid agonism can be measured by pupillometry. It is not known whether there are arteriovenous concentration differences for naloxone. The aim was thus to further develop a model for studying PK/PD aspects of naloxone and to explore whether a significant arteriovenous concentration difference for naloxone in humans was present.

Methods
Relevant authorities approved this study. Healthy volunteers (n=12) were given 1.0 mg intravenous (IV) naloxone after steady state opioid agonism was obtained by TCI of remifentanil (1.3 ng/ml). Opioid effect was measured by pupillometry. Arterial and venous samples were collected simultaneously before and for 2 h after naloxone administration for quantification of naloxone and remifentanil.

Results
Arterial remifentanil was in steady state at 12 min. One milligram IV naloxone reversed the effect of remifentanil to 93% of pre-opioid pupil-size within 4 min. The estimated duration of antagonism was 118 min. At that time the concentration of naloxone was 0.51 ng/ml. The time course of arterial and venous serum concentrations for naloxone was similar, although arterial AUC (area under the curve) was slightly lower (94%) than the venous AUC (p= 0.03). There were no serious adverse events.

Conclusion
Onset of reversal by IV naloxone was rapid and lasted 118 min. The minimum effective concentration was 0.5 ng/ml. Using TCI remifentanil to obtain a steady- state opioid agonism may be a useful tool to compare new naloxone products.

Keywords
pharmacodynamics, naloxone, remifentanil, pharmacokinetics, arteriovenous difference

Introduction

Naloxone reverses the physiological effects of opioids and is a cornerstone in the treatment of acute opioid overdoses characterised by respiratory depression, reduced consciousness and pin-point pupils.

For most drugs acting through the central nervous system, onset of action lags behind the build-up of blood concentrations. Factors such as time to cross the blood-brain barrier or to generate an effect after reaching the site of action within the brain may explain this phenomenon.

Pain relief, drug-liking or pupil size changes are commonly used to study the effects of opioids in man (1, 2). Pupil size is the superior predictor of response to naloxone in overdose victims (3). Pupillometry is an easy and non-invasive procedure, and is therefore often used to study the pharmacodynamics of opioids (4-6). Studies of the reversal (antagonism) of opioid effects are uncommon, not least for naloxone (7-10) as opioid agonism is required. Different approaches such as per oral administration of alfentanil or tramadol (9, 11) have been used to study opioid antagonism. A bolus of intravenous remifentanil has also been used to study the effects of the μ-opioid receptor antagonist samidorphan (12).

Opioids, such as heroin, fentanyl and remifentanil, may display a significant initial arteriovenous (A-V) concentration gradient (13-15). Therefore, arterial sampling is usually required to determine concentration-effect relationship for these drugs. Since arterial sampling is cumbersome and more invasive than IV, it would be important to know whether naloxone also displays an A-V concentration gradient.

Skulberg et al. (20) have recently shown that target-controlled infusion (TCI) of remifentanil provides good conditions for studying the pharmacodynamics of naloxone; however, steady state condition was not proven. Skulberg et al. also documented that pupillometry was superior to heat pain threshold for this purpose. Unfortunately, only venous naloxone concentrations were measured, prohibiting adequate PK/PD (pharmacokinetic/ pharmacodynamic) modelling. Moreover, the reversal action of the 0.8 mg intranasal (IN)/intramuscular (IM) naloxone administrations was far from maximal under the opioid exposure used. It was concluded that a remifentanil target of 1.3 ng/ml would be more favourable for further studies.

In the present study remifentanil was administered as TCI to rapidly achieve steady state corresponding to 1.3 ng/ml (16). An intravenous dose of 1.0 mg of naloxone was given to generate maximum reversal of the pupil size. Whether there was a significant arteriovenous gradient for naloxone was also investigated. The time course of arterial and venous remifentanil concentrations was also explored.

Materials and methods

This study was conducted according to the principles of the Declaration of Helsinki and The International Conference on Harmonisation (ICH), Good Clinical Practice (GCP). It was approved by The Regional Committees of Medical and Health Research Ethics in Norway (2014/2194), the Norwegian Medicines Agency (EudraCT 2014-005348-16) and registered in clinicaltrials.gov (NCT02405988) prior to the inclusion of the first participant. Participants

were insured through the Drug Liability Association, Norway, and they were compensated for their participation with 1000 NOK (110 Euro/ 115 USD).

Healthy men and women (ASA class I) aged 18–40 years were eligible. A full medical history and targeted examination including 12 lead ECG without pathologic abnormalities, BMI within 18.5–26.0 kg/m^2 and blood samples within normal reference values for haemoglobin, creatinine, ASAT, ALAT and gamma GT were required. Women in reproductive age had to have a negative serum HCG at inclusion and use safe contraception throughout the study period until their last visit. Breast-feeding women, participants taking any other regular medications including herbal, or any known drug allergies were excluded. All participants also had to pass the modified Allen's test (17) to determine collateral circulation of the hand.

Administration of remifentanil to volunteers requires particular care. 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 workplace and previous participation in trials where they received opioids or subjects who had a history of drug and/or alcohol abuse were excluded. Potential participants had to answer the CAGE-AID questionnaire (18), and no one answering yes to two or more questions was allowed to participate.

Twenty subjects were screened for inclusion, and seven participants did not meet the inclusion criteria. Thirteen subjects, all Caucasians were included; one of these was excluded before administration of study medications. Thus, 12 participants completed the treatment.

This was an exploratory, descriptive, open pharmacokinetic-pharmacodynamic study. Each subject participated in one study session that lasted 3 h. The subjects kept the supine position during the trial. The study was conducted at the Intensive Care Unit, with staff from the Clinical Research Facility, St. Olavs hospital, Trondheim University Hospital, Norway, from April 2015 to January 2016.

To investigate the pharmacodynamics of the opioid antidote naloxone, an opioid must be given. Remifentanil hydrochloride (Ultiva, GlaxoSmithKline, supplied by the St. Olavs hospital Pharmacy) was chosen as it has a terminal half-life of 3–10 min and lacks active metabolites (19). That makes it ideally suited to establish steady-state target conditions and thus create a stable opioid influence during the time of infusion. It was delivered with the Minto TCI model (20, 21), with a plasma target concentration of 1.3 ng/ml, using the Alaris PK Guardrail syringe pumps (CareFusion Cooperation, UK). A similar model was previously used in research in Norway (16), and also by the current research group (22).

The participants were required to fast, no solid food 6 h prior and no liquids 2 h prior to the start of remifentanil infusion (23). The participants received remifentanil for 12 min to obtain steady state target conditions, before 1.0 mg naloxone hydrochloride was given intravenously (IV) (Naloxone B. Braun 0.4 mg/ml, Braun, Melsungen, Germany). After naloxone administration, the remifentanil infusion continued for another 90 min. The participants therefore received remifentanil for a total of 102 min. During the procedure they were monitored by continuous oxygen saturation and three lead ECG and invasive blood pressure throughout remifentanil infusion. A trained anesthesiologist was present at all times during the administration of remifentanil.

Change in pupil size was used to determine the opioid effect and measured by a Neuroptics VIP 200 Pupillometer (Neuroptics, Irvine, CA, USA). The room had low and uniform ambient lighting at all study visits (controlled at the start and end of each study session). Pupils were given time to adapt prior to start of study (measurements – 20 to – 14). Pupillometry was measured at times – 20, – 17, – 14, – 3, – 1, 1, 4, 7, 9, 12, 14, 17, 19, 24, 29, 34, 39, 44, 49, 59, 69, 79, 89, 99, 109 and 119 min.

At the start of the trial all participants had local anaesthesia with lidocaine (10mg/ml) before an arterial cannula was placed in the radial artery of the participant for arterial blood sampling. Two IV cannulas were placed, one for venous blood sampling, and one for administration of naloxone and remifentanil. Naloxone samples were drawn at –12, 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90 and 120 min. Remifentanil samples were drawn at – 12, – 10, – 5, – 2, 30, 60 and 90 min. All samples were drawn simultaneously from the arterial and venous cannulas.

The samples for naloxone analysis were 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 frozen at – 20°C, and stored in an – 80°C freezer before the end of the day. The analytical procedure for quantification of naloxone with liquid chromatography-tandem mass spectrometry has been described in detail elsewhere (24). For the current analysis a partial pre-run validation was performed with a limit of quantitation (LOQ) of 0.02 ng/ml and the coefficient of variation (CV) and inaccuracy were found to be < 5.7 %, < 2.9 % (n = 8) respectively. For the quality controls (QC) (n = 9), CV and inaccuracy were found to be < 7.1 %, < 5.9 % (QC1, 0.05 ng/ml), < 2.0 %, < 1.1 % (QC2, 15.0 ng/ml) and < 2.1 %, < 3.7 % (QC3, 30.0 ng/ml). During in-run validation the calibration curves had a correlation coefficient (r^2) of 0.9990 in average (n = 6). CV and inaccuracy for the quality controls (n = 12) were < 6.4 %, < 4.4 % (QC1), < 3.8 %, < 3.6 % (QC2) and < 2.4 %, < 2.2 % (QC3).

Samples for remifentanil quantification were collected in Vacuette NH Sodium Heparin Blood Collection Tubes. The tubes were prefilled with 50% citric acid (weight/volume) solution to prevent hydrolysis of remifentanil through pH-control (25). After strictly mixing, the blood samples were immediately put on ice and frozen at – 20°C within 10 min and moved to – 80°C by the end of the day. Remifentanil was analysed by high performance liquid chromatography tandem mass spectrometry using fentanyl as the internal standard. Calibration range was 0.01–5 ng/ml (8 calibration standards). The limit of quantitation (LOQ) was 0.01 ng/ml. The method was fully validated according to Dadgar et al. (26) and Shah et al. (27). For details, see supplementary file 1, available online.

The primary endpoint in this study was to determine the serum-effect-site equilibration rate constant (k_e0) for naloxone in humans. Secondary endpoints were pharmacodynamic effect of naloxone on remifentanil and whether there was a significant arteriovenous difference for naloxone.

A formal sample size calculation was not performed as there was virtually no knowledge on the pharmacodynamics of IV naloxone available. Twelve subjects are commonly used in such studies, as it usually provides adequate data for inter individual variations of the pharmacokinetics of the study drug.

Serum concentration data was analysed by non-compartmental techniques using Win-Nonlin Standard version 7.0 (Pharsight Corporation, NJ, USA). Area under the curve; (AUClast

(linear trapezoidal rule) was calculated by computerised curve fitting. Data was described by arithmetic mean and 95% confidence intervals if not specified otherwise. SPSS version 23 (IBM, NY, USA) was employed for descriptive statistics.

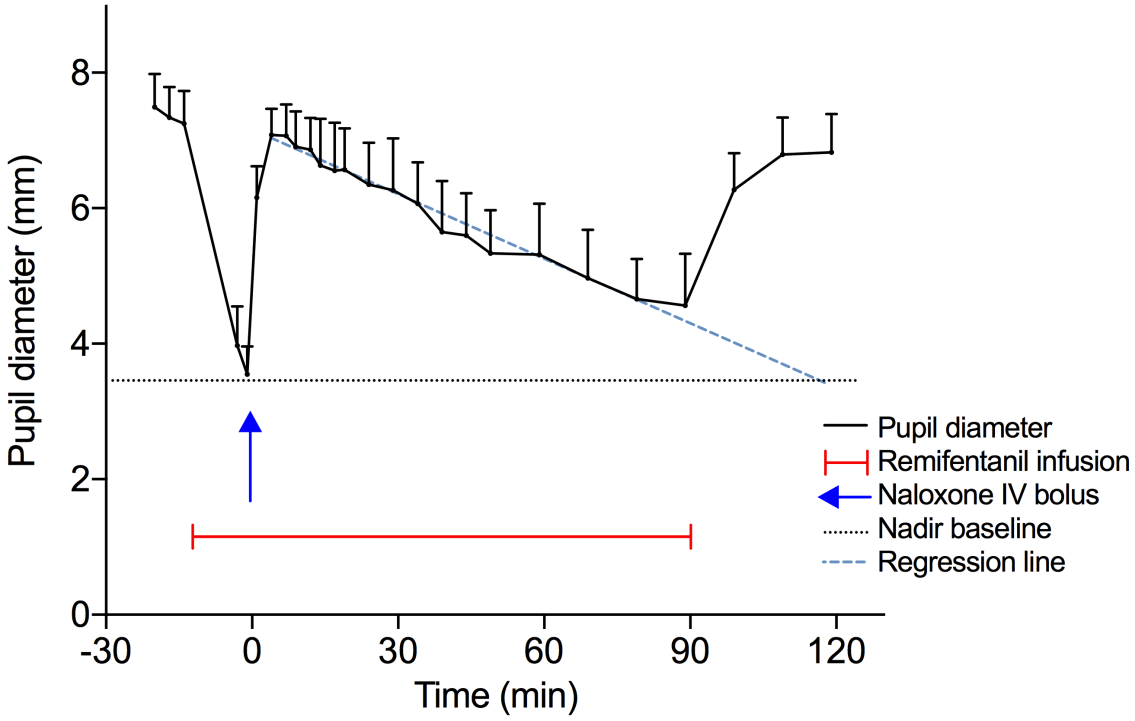
Comparison of arteriovenous differences was performed by paired samples *t*-test for all time points and corrected for multiple testing with Bonferroni's method. Comparison of changes in pupil size was performed by paired sample *t*-test. Concentration measurements below LOQ were not used in the analysis. Missing data was not imputed.

Results

Six men and six women completed the study. Their mean age was 23.0 (19–26 min-max), mean height 174.6 cm (163.0–187.3), mean weight 68.7 kg (55.4–88.9) and mean BMI 22.4 kg/m ^{2} (19.2–25.3). For one participant only arterial samples were available, due to failure of peripheral venous cannula. The arteriovenous comparison therefore comprised 11 subjects. The amount of remifentanil infused (mean (min–max)) in this study was 406 (372–449) µg.

The mean pupil diameter at the beginning of the study (average of three measurements) was 7.36 mm (7.11–7.61) (Fig. 1). The pupil size decreased to a nadir of 3.55 (3.14–3.96) mm 12 min after the start of remifentanil infusion.

Fig. 1
Pupil diameter during the course of the trial. Miosis was induced by remifentanil started at –12 min. It was rapidly reversed after a naloxone bolus that was given at t=0. n=12, mean (95% confidence interval). The blue broken line is the regression line (f(x) = –0.0292x+6.9924)) and is based on the period 19–89 min and crosses nadir baseline (black dotted line) at 118 min.



Miosis was rapidly reversed after naloxone was given at t=0 (12 min after start of remifentanil). Already at 1 min, the pupil diameter was 6.16 mm, and the maximum effect (Emax) of 7.08 (6.70–7.47) mm was reached at 4 min. The reversal of pupil diameter was 93%. Thereafter the pupil diameter gradually decreased to 4.57 (3.80–5.33) mm at remifentanil discontinuation (89 min after naloxone administration). This was significantly different from nadir. Thereafter the pupils rapidly returned to initial size 6.83 (6.26–7.39) mm. A regression line (f(x) = -0.0292x+6.9924)) based on the period 19-89 min crossed the nadir line at 118 min (Fig. 1). The mean venous concentration of naloxone at that time (120 min) was 0.51 ng/ml (Fig. 1).

As can be seen from Fig. 2, a counter clockwise hysteresis was observed. Please note that the plots for arterial and venous blood sampling are identical (Fig. 2).

Fig. 2
Hysteresis plot of pupil diameter and naloxone concentration during a stable remifentanil infusion. Red circles and a solid line represent arterial samples, and blue squares and broken line represent venous samples. n = 12, mean (95% confidence interval). The direction of time is illustrated by the arrow.

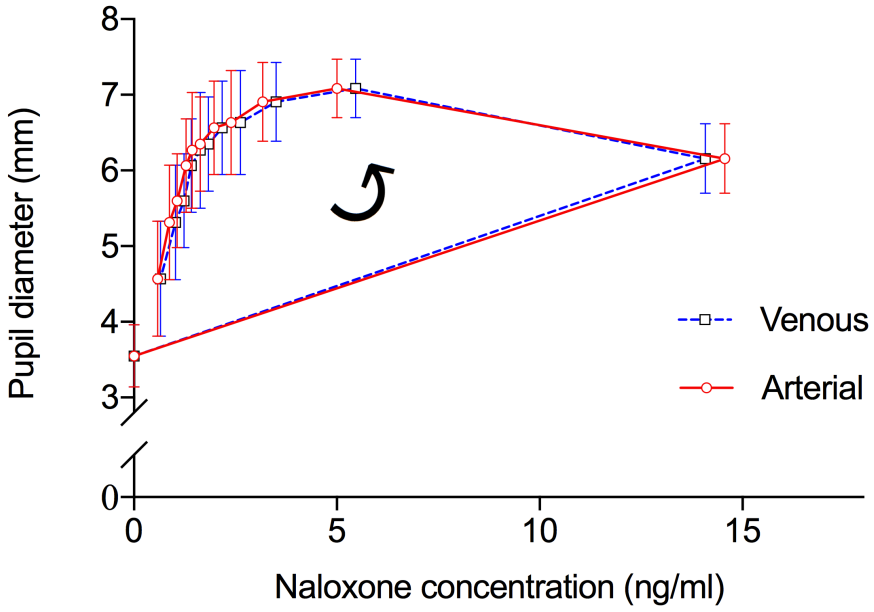
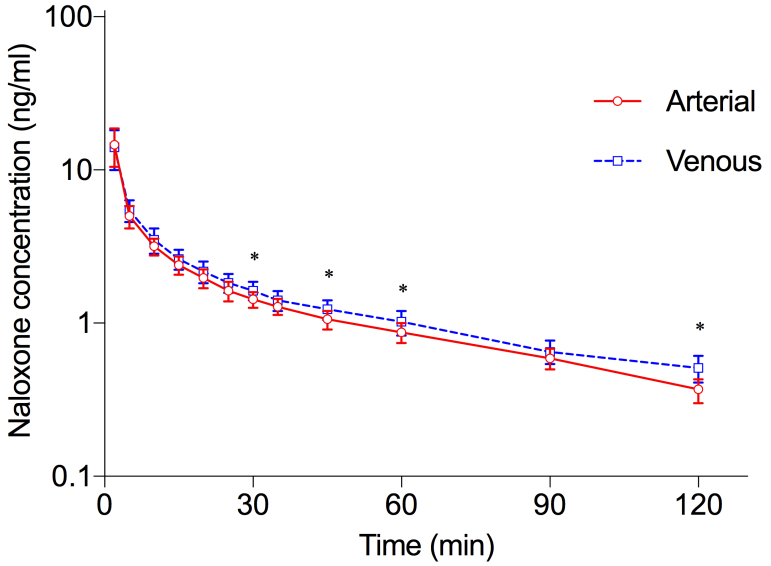


Figure 3 shows the time course of the arterial and venous serum concentrations of naloxone. The curves are similar and almost completely overlapping the first 30 min. After 30 min, there is a tendency for the venous samples to have a slightly higher concentration (Fig. 3).

Fig. 3
Time course of arterial and venous serum concentrations of naloxone after administration of 1.0 mg intravenous naloxone in human healthy volunteers (n = 11). Data is presented as mean (95% confidence interval). Red circles and solid line represent the arterial samples and blue squares and broken line represent the venous samples. Statistically significant differences are marked with an asterisk (*).



Cmax for arterial and venous sampling were 14.6 (10.5–18.6) and 14.1 (9.98–18.2), respectively. The venous Cmax were 97% of the arterial Cmax. The arterial AUClast was 205 (172–239) min*ng/ml, which was 94% of the venous AUClast of 219 (183–256) min*ng/ml (p= 0.03), and the correlation between this arterial and venous parameter was 0.941 (p<0.001). Paired sample t-test for each time point with Bonferroni correction showed a statistically significant arteriovenous difference (p<0.05) for the samples taken at 30, 45, 60 and 120 min, however, the mean differences were very small (Fig. 4).

Fig. 4

Time course of arterial and venous serum concentrations of remifentanil during a Minto target controlled infusion of remifentanil in human healthy volunteers (n = 11). The plasma target concentration was 1.3 ng/ml, and the achieved concentration was 1.15 ng/ml. Data is presented as mean (95% confidence interval). Red circles and solid line represent the arterial samples and blue squares and broken line represent the venous samples. The target concentration is indicated by the black dotted line.

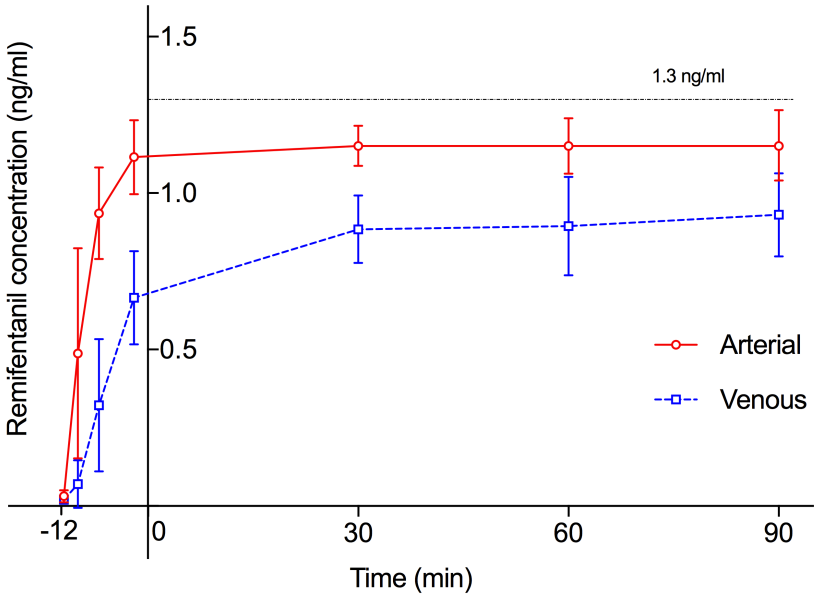


Figure 4 shows the time course of remifentanil concentrations in arterial and venous blood during the infusion. The arterial concentration reached a steady state of 1.15 ng/ml (12% lower than the expected 1.3 ng/ml) at 12 min. A clear arteriovenous time-dependent difference was observed with venous concentrations at 77–80% of the arterial concentrations.

Adverse events were reported using Common Terminology Criteria for Adverse Events version 4.0. There were no serious adverse events. Two subjects experienced mild nausea a few minutes after naloxone administration. The nausea resolved after a few minutes, without requiring any medication. In one subject, nausea returned towards the end of the study but resolved spontaneously. These two events were defined as having a possible relationship to the test drug. Two events were not related to the drug intervention.

Discussion

An intravenous dose of 1.0 mg naloxone reversed the remifentanil induced miosis within 4 min. Thereafter, the pupillary size gradually decreased but did not restore completely until remifentanil was discontinued. The estimated duration of action of naloxone was 118 min. There were no significant arteriovenous differences in serum concentration of naloxone. Remifentanil reached steady state after 12 min target-controlled infusion. No significant adverse effects occurred.

Except for the study of Skulberg et al. (20), previous pharmacodynamic studies of naloxone did not employ steady state opioid agonism (11,13,14). The advantage of steady state is that confounding processes such as absorption, distribution and elimination of the agonist were avoided. Quantification of arterial remifentanil concentrations confirmed that the TCI infusion reached steady state at 12 min and maintained it throughout the infusion period of 90 min as predicted. The observed steady state, however, was about 12% lower than predicted. This is not unreasonable, as the dose infused was far lower than in ordinary clinical practice, for which the infusion algorithms were developed. Finally, our observations of a clear, time-dependent arteriovenous concentration difference for remifentanil confirm previous reports (15).

This study differs from Skulberg et al. (20) by administering a larger dose of naloxone and by using the IV, rather than the IM route. While Skulberg et al. explored three targets of remifentanil (1.0, 1.3 and 2.5 ng/ml), only 1.3 ng/ml was used here. The TCI-concentration was chosen to obtain a better balance between agonist and antagonist than in the previous study. However, we did not succeed completely. While Skulberg et al. only observed small effects of the 0.8 mg IN dose and only half-maximal effect of the 0.8 mg IM dose, the 1.0 mg IV dose under 1.3 ng/ml remifentanil gave complete reversal within a few minutes. This gave us too few observations to enable a valid calculation of the primary outcome, the serum effect-site equilibration (k_e0) and its half-life ($t_{1/2}k_e0$) for naloxone. However, we could demonstrate that the dose gave opioid reversal lasting 118 min. This corresponds to studies in opioid dependent subjects showing a maximum level of withdrawal symptoms in 5 min after 1 mg naloxone IV (7), lasting between 90 and 180 min. Since the serum concentration of naloxone at 118 min in our study was 0.51 ng/ml, we suggest that the minimum effective serum concentration of naloxone is 0.5 ng/ml in healthy, opioid naïve volunteers. This is relevant, as approval of new nasal naloxone sprayers was based on the time course of blood concentrations of naloxone (28, 29).

Hysteresis plots of serum naloxone concentration versus pupil diameter showed a counter clockwise hysteresis loop (Fig. 2). There was a lag between increases in serum concentration and the observed effect on pupil size, but the plot has a steep climb before it returns along the x-axis, demonstrating that naloxone reached the effect site rapidly. This is similar to the plots seen for the opioid antagonist samidorphan and the agonist fentanyl (12, 30). Compared to those of Skulberg et al. (20), the expectation that IV dosing kicks in far more rapid and probably more profoundly than IM and IN were met. We cannot determine whether this is a distribution delay to the effect site, slow receptor kinetics or other mechanisms. However, it means that when we construct a model of the data, we need to put in an effect compartment as a link to a pharmacodynamic model.

This is the first report on arterial and venous naloxone concentrations. Significant arteriovenous difference in concentrations has previously been shown for opioids such as heroin, fentanyl and remifentanil (13-15). We therefore hypothesised that arterial naloxone concentrations might be higher than the venous concentrations in the initial face. Although some minor statistically significant differences were seen, these are likely not of biological significance. It therefore seems that venous serum concentrations of naloxone reflect the arterial ones. Venous concentrations may therefore be used for modelling purposes allowing us to combine the present data with those previously published by Skulberg et al. (22) for calculation of the $t_{1/2}k_e0$ under remifentanil agonism. This is of interest, as it has been shown that the $t_{1/2}k_e0$ for naloxone depends on the opioid in question with mean values of 11.2 and 5.42 min for morphine and morphine-6 glucuronide, respectively (31). Moreover, we will also enter data from previously unpublished and published (24) pharmacokinetic studies to generate robust data of the population kinetics of naloxone given IV, IM and IN.

Conclusion

Naloxone 1.0 mg IV quickly reversed the effect of a 1.3 ng/ml TCI remifentanil infusion at steady-state with an Emax at 4 min, measured by pupillometry. The estimated duration of effect was 118 min. Estimated minimum effective concentration of naloxone was 0.5 ng/ml.

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Conflict of interests

Norwegian University of Science and Technology (NTNU) and its subsidiary Technology Transfer Office (TTO) have a licencing agreement with Den norske Eterfabrikk (DnE) regarding a naloxone nasal spray formulation. 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. Farma Industry AS (sister company to dne pharma) has recently gained market approval in 12 european countries for this spray, containing 1.4 mg Naloxone-HCl. 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.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00228-018-2545-y) contains supplementary material, which is available to authorized users.

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