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Opioid-induced respiratory depression: implications & prevention

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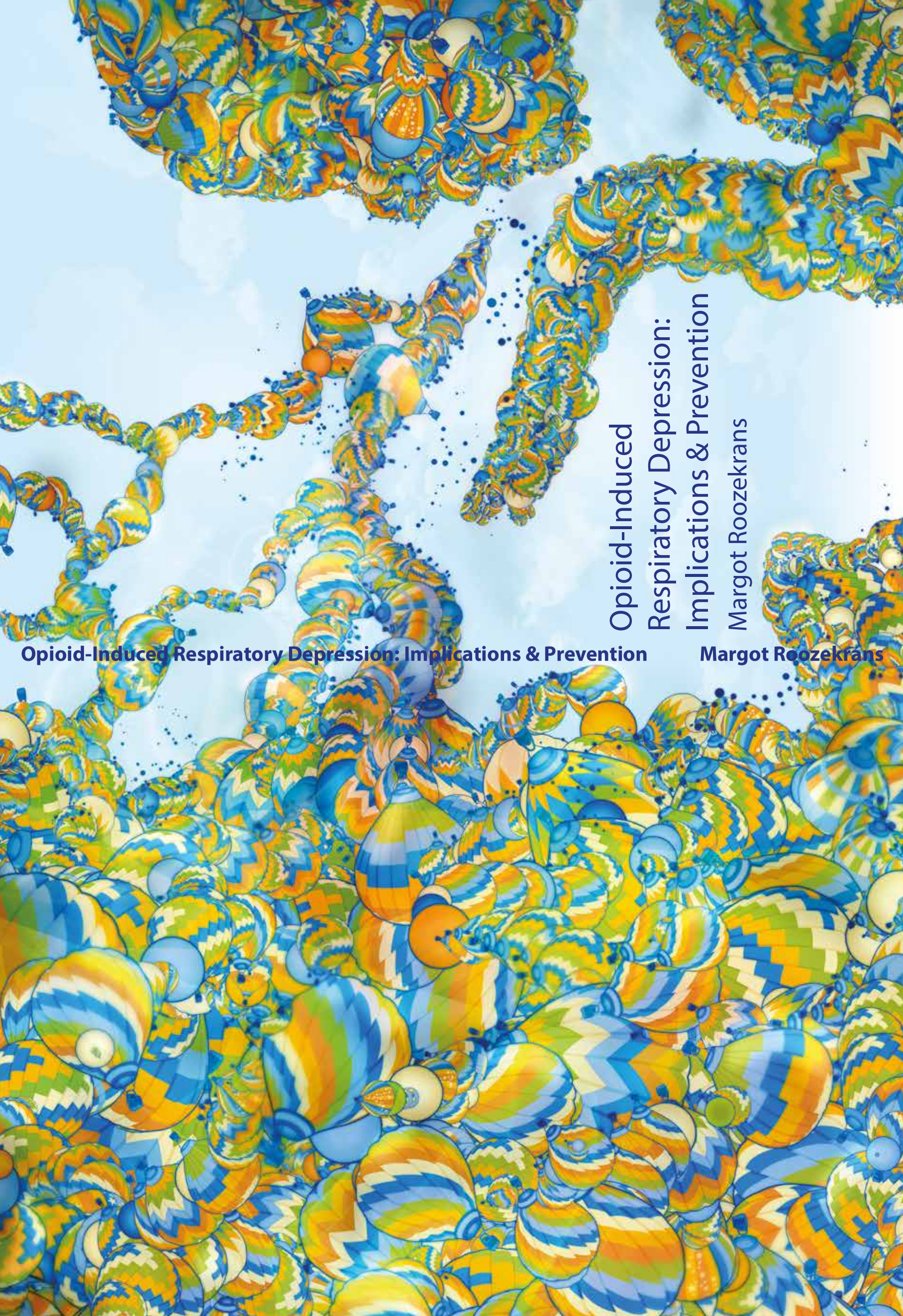

Opioid-Induced Respiratory Depression: Implications & Prevention **Margot Roozekrans**

Opioid-Induced Respiratory Depression:
Implications & Prevention

Margot Roozekrans

Opioid-Induced Respiratory Depression:
Implications & Prevention

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Chapter 1

Introduction

Ventilation enables the exchange of oxygen and carbon dioxide in the lungs. Since adequate ventilation is crucial to the survival of humans and all other mammalian species, a complex control system involving both feedback and feed forward reflexes is present in all mammals that ensures adequate ventilation and consequently stable intracellular levels of pCO_2 (pH) and pO_2. The control system makes use of specific chemoreceptors. Changes in arterial pCO_2 are sensed by central chemoreceptors in the medulla, while changes in arterial pO_2 are sensed by peripheral chemoreceptors that reside in the carotid bodies (at the bifurcation of the common carotid artery; Fig. 1). Stimuli such as hypoxia, hypercapnia and acidosis will increase minute ventilation, while hypocapnia and alkalosis will decrease minute ventilation. Respiratory centres in the brainstem integrate these stimuli and provide the neural drive to respiratory muscles (*i.e.* the diaphragm, intercostal muscles and muscles providing airway patency).[1] These respiratory centres can easily be affected by centrally acting drugs, such as opioids. Exogenous opioids activate the endogenous opioid receptor system causing the slowing and at high dose diminishing of rhythmic breathing activity.[1] Opioid-induced respiratory depression (OIRD) is possible since opioid receptors, especially mu-opioid receptors (MOR), are expressed on respiratory neurons in the brainstem[2]; the endogenous opioid system plays an important role in the fragile process of respiratory homeostasis.[3]

THE OPIOID EPIDEMIC

OIRD may affect not only patients treated with potent opioids in the hospital setting, but it may affect all patients receiving opioid therapy, such as for the treatment of chronic low back pain in the outpatient clinic or prescribed by their general practitioner. The prevalence of OIRD is commonly underreported.[3] Recent publications in the lay press suggest the presence of an opioid epidemic from prescription opioids. Since 1999 the number of patients in the United States (US) that gave died from prescription opioid abuse or misuse quadrupled.[4] It is estimated that more than 250,000 individuals have died since the year 2000 due to opioids. The combination of OIRD and a high abuse potential make opioid especially dangerous, currently causing more deaths than illegal opioids or car accidents in the US. Interestingly, while an opioid epidemic is present in the US, other countries seem to be less affected despite high levels of opioid use. For example, in the Netherlands about 1 million patients use a prescription opioid for chronic pain. Still, the number of admissions because of problems due to addiction or otherwise is stable at 700 for many years now.[5]

A BENEFIT-HARM COMPOSITE OF DRUG EFFECT

The intention to treat patients with moderate to severe acute and chronic pain is to reduce their symptoms and improve their quality of life. Still, opioids come with side effects of which OIRD is potentially life threatening. It is therefore useful to quantify the efficacy of an opioid (*i.e.* its benefits) combined with its side effect profile (*i.e.* the harm it induces). We want to treat the patient with a potent analgesic but simultaneously do not want to expose that same patient to serious adverse events. One way of understanding the complex behaviour of drugs that produce wanted effects and unwanted side effects is by integrating benefit and harm into a risk-benefit composite. The utility or safety function is an example of such a composite measure.[6] In the case of opioid analgesia and OIRD, the utility function (UF) converts two rather distinct dose-response curves into one useful function. The UF allows comparison between drugs and

within specific patient populations. The UF is defined as the probability of benefit minus the probability of an adverse event. For opioids, positive values indicate that the probability of analgesia exceeds the probability of an adverse event such as OIRD (and *vice versa*). Dahan et al. showed earlier that the UF of the potent opioid analgesic fentanyl differs between opioid responders and non-responders, suggestive that opioid analgesic efficacy is uncoupled from OIRD.[7]

TREATMENT OF OIRD WITH NON-OPIOID DRUGS

One way of treating significant OIRD is the administration of the non-selective opioid receptor antagonist naloxone. It inhibits all pharmacological effects of opioids, OIRD as well as analgesia. It is therefore clinically relevant to develop respiratory stimulants that are without effect on the opioid system and reverse OIRD without affecting analgesia.[3] Known respiratory stimulants that do not interact with the opioid system include CO_2, caffeine, aminophylline, atropine, doxapram, almitrine, the experimental drug GAL021 and AMPAkines.[8,9] These respiratory stimulants stimulate ventilation through non-opioidergic receptor systems. For example, ampakines stimulate AMPA receptors in the brainstem respiratory centers[10-12]; almitrine,

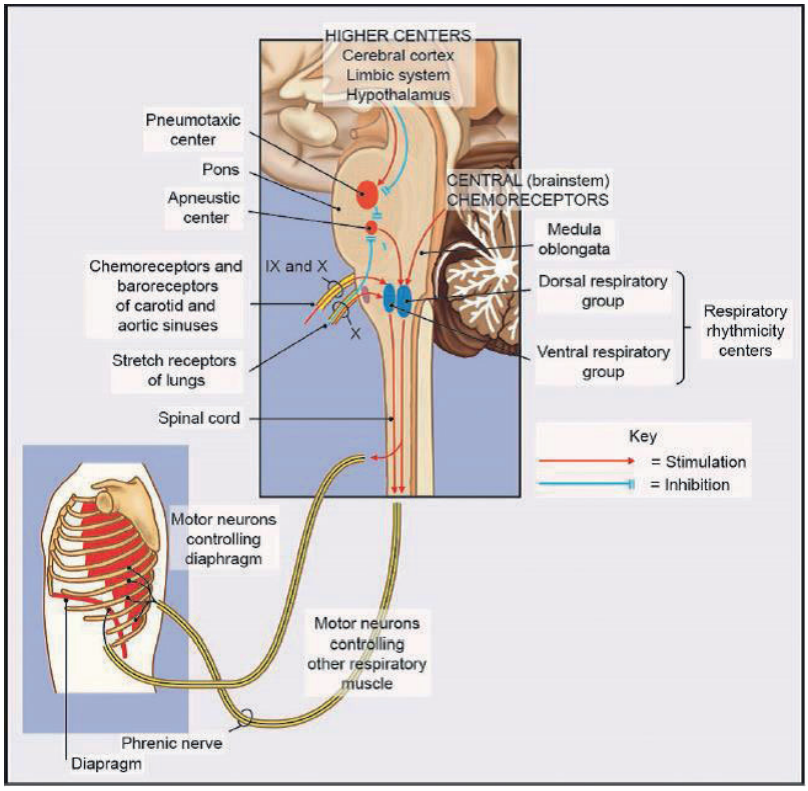

Figure 1. *Schematic representation of the ventilatory control system. It demonstrates ventilation is regulated by peripheral and central chemoreceptors, lungs and higher brain area centers that project into respiratory centres in the brain stem, which regulate the diaphragm and other respiratory muscles.*

doxapram and GAL021 inhibit background potassium channels expressed on type 1 cells of the carotid bodies (these agents mimic the effect of hypoxia).[3,13-15] The effect of all of these agents is highly dependent on dose and underlying conditions. For example, as discussed in this thesis, the effect of doxapram and GAL021 depend on the level of OIRD that requires reversal (Chapter 2 and 4). The greater the level of OIRD, the less the efficacy of reversal.

THESIS OUTLINE

The main aim of this thesis is to study the ability to reverse OIRD with respiratory stimulants that interact with the carotid bodies. Additionally, the effect of an experimental opioid analgesic on OIRD and the ability of naloxone to reverse its respiratory effects was studied.

In **Chapter 2** the effect of the analeptic doxapram on opioid pharmacokinetics (PK) and pharmacodynamics (PD) is investigated. This PK-PD/PK-PD study incorporates the effect of doxapram on cardiac output in the opioid's PK-PD model.

Chapter 3 evaluates the effect of the novel background potassium channel blocker, GAL021, on OIRD. Both poikilocapnic and isohypercapnic respiratory studies are performed. Additionally, GAL021's effect on hemodynamic parameters and antinociception is reported.

In **Chapter 4** the results obtained in Chapter 3 are re-analysed using a population PK-PD design.

In **Chapter 5** the utility function (UF) for the opioid alfentanil is constructed.

Chapter 6 evaluates the respiratory effect of an experimental opioid (RM101) and the possible reversal of OIRD by naloxone compared to placebo infusion.

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Chapter 2

Doxapram-mediated Increase in Cardiac Output Reduces Opioid Plasma Concentrations: A Pharmacokinetic/ Pharmacodynamic - Pharmacokinetic/ Pharmacodynamic Modeling Study in Healthy Volunteers

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INTRODUCTION

Doxapram is an analeptic drug that induces ventilatory stimulation and increases wakefulness, blood pressure and cardiac output (CO).[1,2] Early animal studies showed dose-dependent respiratory stimulation with increases in minute ventilation and phrenic nerve activity that disappeared after bilateral sectioning of the carotid sinus nerves.[3,4] Consequently, the respiratory effect of doxapram is thought to arise at the carotid bodies (CBs), although a (small) central effect at the brainstem cannot be excluded.[1,3] Recent work elucidated the molecular mechanism of action of doxapram at the CBs. Cotten et al. showed that doxapram inhibits the channel function of cloned TWIK-related acid-sensitive potassium channels (TASK1, TASK3 and the heterodimer TASK1/TASK3).[5] These "background" potassium channels are expressed on type 1 CB cells,[5-7] and are inhibited by low arterial oxygen concentrations, resulting in stimulation of breathing.[7] Given the similarity in mechanism of action, doxapram is considered a drug that mimics the effects of hypoxia at the CB.[1] In clinical practice doxapram is used as respiratory stimulant in patients with respiratory failure (especially in neonates) and in the perioperative setting.[1]

Several experimental and clinical studies show amelioration of opioid- and anesthesia-induced respiratory depression by doxapram.[8-11] None of these studies measured cardiac output and investigated a possible role for doxapram-induced changes in CO on pharmacokinetics (PK) and pharmacodynamics (PD) of the respiratory depressant drugs that require reversal. We hypothesize that since doxapram increases CO it may well affect PK (and consequently PD) through changes in distribution and elimination clearances of these drugs.[12-14] It is further important to realize that stimulation of respiration at the level of the carotid bodies has a ceiling effect on the relief of respiratory depression, when the respiratory depression is due to an effect at the level of the brainstem.[15,16] We recently showed this for GAL021, a potassium channel blocker that stimulates breathing via the CB but does not increase CO.[15,17] While at moderate levels of opioid-induced respiratory depression (OIRD; alfentanil plasma concentration 40-80 ng/mL) GAL021 produced pronounced respiratory stimulation, our model analysis predicted that at deeper levels of OIRD (alfentanil concentration > 100 ng/mL) the effect of GAL021 on respiration is small. Hence, we hypothesize that at deep levels of OIRD, doxapram will cause respiratory stimulation coupled to reduced analgesia, predominantly related to the decrease in plasma drug concentration.

In the current study, we investigated the effect of doxapram and placebo on alfentanil-induced antinociception in healthy volunteers. We measured plasma concentrations of alfentanil and doxapram, antinociception and cardiac output. We performed a PK-PD analysis of the effect of doxapram on cardiac output and linked these results to a PK-PD analysis of the effect of alfentanil on antinociception. To get an indication of the effect of doxapram on alfentanil-induced respiratory depression we measured end-tidal pCO_2 at predefined time intervals.

MATERIALS AND METHODS

METHODS

The study had a randomized, double blind, placebo-controlled crossover design and was performed between July and October 2012. The study protocol was approved by the local Human Ethics Committee of the Leiden University Medical Center and the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands). The study was

registered in the Dutch trial register under number NTR3500. Before participation, all subjects gave written informed consent. The study was performed according to the ethical principles for medical research involving human subjects (Declaration of Helsinki).

SUBJECTS

Healthy male volunteers, aged 18-45 years with a body mass index in between 18 and 30 kg/m^2, participated in this study. Subjects with a history of alcohol or drugs abuse, ECG abnormalities, smoking, psychiatric disease or lung disease were excluded from participation. All subjects were asked to refrain from any food for the 8 hours prior to the administration of alfentanil.

STUDY DESIGN

Two intravenous lines were inserted, one for administration of alfentanil (Rapifen, Janssen-Cilag, Tilburg, The Netherlands) and one for administration of doxapram (Dopram, Eumedica, Brussel, Belgium) or placebo. An arterial line was placed in the radial artery of the non-dominant arm to obtain samples for measurements of alfentanil and doxapram PK and to measure cardiac output. Throughout the experiment heart function (ECG, heart rate), blood pressure and oxygen saturation were measured continuously.

Experiments were performed on two occasions. On one occasion alfentanil was combined with doxapram, on the other with placebo. The order of occasions was randomized. Doxapram HCl (2 mg/mL) in 5% w/v glucose or placebo (5% w/v glucose) and alfentanil (0.5 mg/mL) were administered intravenously according to the following scheme:

(1) T = 0 min: alfentanil loading dose of 8 μg.kg^{-1}.min^{-1} for 2 minutes, followed by a maintenance dose of 0.6 μg.kg^{-1}.min^{-1} for 98 minutes (ALF-low).

(2) T = 30 min: doxapram/placebo loading dose of 40 μg.kg^{-1}.min^{-1} for 9 minutes, followed by a maintenance infusion of 8 μg.kg^{-1}.min^{-1} for 21 minutes (DOXA-low).

(3) T = 60 min: doxapram loading dose of 55.5 μg.kg^{-1}.min^{-1} for 20 minutes, followed by an infusion rate of 22 μg.kg^{-1}.min^{-1} for 52 minutes (DOXA-high).

(4) T = 100 min: alfentanil loading dose of 8 μg.kg^{-1}.min^{-1} for 2 minutes, followed by a maintenance infusion of 0.9 μg.kg^{-1}.min^{-1} for 30 minutes (ALF-high).

(5) T = 132 min: all infusions stopped.

RANDOMIZATION, ALLOCATION AND BLINDING

A randomization list was computer generated by an independent statistician who delivered the list to the pharmacy. The pharmacy prepared the study medication and dispensed one alfentanil syringe and one blinded syringe containing either doxapram or placebo on the morning of the experiment. Blinding was lifted after data analysis was completed.

MEASUREMENTS

End-Tidal pCO_2. Prior to any drug administration and at t = 55, 95 and 125 min, we measured the end-tidal pCO_2 of the subjects. To that end subjects breathed through a facemask from which the exhaled air was sampled for 3-5 min. The average change in end-tidal pCO_2 from baseline ($\Delta PETCO_2$) was used in the analysis.

Cardiac output (CO). A FloTrac/Vigileo system (Edwards Lifesciences Corp., Irvine, CA, USA) was attached to the arterial line for the beat-to-beat measurement of CO. Minute averages of the CO were used in the analysis.

Antinociception. Transcutaneous electrical stimuli were applied to the skin overlying the right tibial bone at about 10 cm above the malleolus via a computer interfaced constant current stimulator, which was locally designed and constructed. The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA per 1 s with a pulse duration of 0.2 ms at 20 Hz with a cutoff of 128 mA. The subjects were instructed to press a button on a control box when they first experienced pain (pain threshold) and when no further increase in stimulus intensity was acceptable to them (pain tolerance). After a training period, baseline values were obtained in triplicate. The averaged baseline value was used in the data analysis. Pain responses were obtained at t = 28, 40, 58, 78, 97, 108, 130, 141, 156, 171, 192 and 215 minutes.

Alfentanil concentrations in plasma. Arterial blood samples for plasma concentration of alfentanil were taken at T = -5 (baseline), 1, 2, 4, 5, 7, 8, 10, 19, 31, 40, 47, 59, 80, 88, 99, 101, 102, 104, 105, 107, 110, 119, 131, 140, 155, 170 min. Separation of plasma was within 15 min of blood sampling after centrifugation for 10 min at 3,500 min^{-1}. Plasma samples were immediately stored at -25°C until analysis. Concentrations were determined by capillary gas chromatography, as previously described,[18,19] with the following minor modifications: internal standard was added to 0.5 mL plasma and a Heidolph-mixer was used to extract the organic phase before analysis on a HP5890 series 2 gas chromatograph with an operating temperature of the detector at 325 °C. The residue was dissolved in 150 µL ethanol. The precision and accuracy were < 11 % in the quality controls and the concentration range of the standards was 11-355 ng/mL. In case of higher concentrations less sample was used and diluted with blank plasma. The correlation coefficients were > 0.997.

Doxapram concentrations in plasma. Arterial blood samples for plasma concentrations of doxapram were collected in K_2EDTA tubes at T = -5 (baseline), 35, 40, 47, 59, 70, 80, 88, 99, 110, 131, 140, 170 and 190 min. Plasma was analysed using a qualified liquid chromatography with tandem mass spectrometry method (LC-MS/MS). Calibration standards, quality controls, and incurred plasma samples were prepared by protein precipitation using acetonitrile. After centrifugation, the supernatant was used for LC-MS/MS analysis. All samples were injected onto a Waters Atlantis T3 C18 column (4.6 x 50 mm, 3 µm particle), eluted using gradient elution with a mobile phase consisting of mobile phase A (0.1% formic acid in water, v/v) and mobile phase B (0.1% formic acid in acetonitrile), and detected using an AB/Sciex API-4000 QTrap mass spectrometer. The ions were produced in the positive electrospray ionization mode and detected in multiple reaction monitoring mode using transitions at m/z 379.2 to 264.0 for doxapram and m/z 260.0 to 116.0 for the internal standard with retention times of 1.40 and 1.42 min, respectively. The calibration standard concentrations ranged from 2 to 5,000 ng/mL with quality controls at 5, 50, and 500 ng/mL. The weighted (1/x) quadratic calibration curves were utilized with a lower limit of quantification of 2.0 ng/mL and correlation coefficients of greater than 0.999. The method precision (%CV) and accuracy (%RE) met acceptable criteria (within 15%) for quality control sample concentrations.

DATA ANALYSIS

End-tidal pCO_2. The change in end-tidal pCO_2 relative to baseline was compared between treatments using a linear mixed effects model using SPSS v23.0 for Windows; p-values <0.05 were considered significant.

Pharmacokinetic-Pharmacodynamic (PK-PD) analysis. A PK-PD model was constructed in which the doxapram PK was linked to CO, which next was linked to alfentanil PK and subsequently to pain responses (a PK-PD/PK-PD analysis). The population analyses were performed in NONMEM version 7.3.0 (software for nonlinear mixed effects modeling; ICON Development Solutions, Hanover, MD). Model selection (*e.g.,* number of PK compartments) was based on the minimum objective function value (MOFV; χ^2 test), standard error of estimates and goodness of fit plots. For both PK and PD analysis, the model parameters were assumed to be log-normally distributed across the population. Residual error was assumed to have both an additive and a relative error for concentrations and only an additive error for cardiac output and analgesia. All values in the PK-PD analysis are median ± SE, unless otherwise stated. p-values < 0.01 were considered significant.

To fully understand the complex relationship between doxapram, alfentanil, CO and antinociception, the data were analyzed in multiple steps:

STEP 1. In Step 1 the pharmacokinetics of doxapram were characterized. Multiple compartment models were fitted to doxapram plasma concentration data.

STEP 2. In Step 2 the cardiac output changes induced by doxapram were modeled as follows:

$$CO(t) = CO_B \cdot [1 + 0.5 \cdot (CE_{DOX}(t)/C_{50DOX})^{\gamma_{DOX}}] \quad (1)$$

and CO changes induced by placebo as:

$$CO(t) = CO_B \quad (2)$$

where CO(t) is the predicted cardiac output at time t, CO_B is the baseline cardiac output, CE_{DOX} is the effect-site concentration of doxapram, C_{50DOX} is the doxapram concentration causing a 50% increase in cardiac output and γ_{DOX} a shape parameter. To eliminate a possible hysteresis between doxapram plasma concentration and CO, an effect compartment was postulated that equilibrates with the plasma compartment with a half-life $t_{1/2}k_{e0}$ (*i.e.*, the blood-effect-site equilibration half-life).

STEP 3. In Step 3 the alfentanil PK data were characterized. Multiple compartment models were fitted to the data.

STEP 4. In **Step 4A** the alfentanil PK data were characterized similarly to Step 3, but now with separate analyses for the data obtained with *versus* without concomitant doxapram infusion. In **Step 4B** cardiac output, predicted by the doxapram PK-PD model (Step 2), was incorporated in the alfentanil PK model as a time-varying covariate, with the assumption that changes in CO might affect the alfentanil elimination and distribution. The covariate was incorporated as follows:

$$CL(t) = CL0 \cdot (1 + \alpha_1 \cdot 0.5 \cdot [CE_{DOX}(t)/C_{50DOX}]) \quad (3)$$

$$Q_2(t) = Q_20 \cdot (1 + \alpha_2 \cdot 0.5 \cdot [CE_{DOX}(t)/C_{50DOX}]) \quad (4)$$

$$Q_3(t) = Q_30 \cdot (1 + \alpha_3 \cdot 0.5 \cdot [CE_{DOX}(t)/C_{50DOX}]) \quad (5)$$

where CL0, Q_20 and Q_30 are the alfentanil terminal and inter-compartmental clearances prior to any change in cardiac output, α_x the covariate coefficient and CE_{DOX} and C_{50DOX} as given in Step 2. We tested whether $\alpha_1 = \alpha_2 = \alpha_3$ and whether $\alpha_x = 0$.

STEP 5. In Step 5, a population PK-PD analysis of alfentanil-induced nociception data was performed using the alfentanil PK empirical Bayesian estimates (EBE's) derived from Step 4B:

$$S(t) = S_B \cdot [1 + 0.5 \cdot (CE_{ALF}(t)/C_{50ALF})] \quad (6)$$

where S(t) is the predicted current at time t, S_B the baseline pain threshold or tolerance, CE_{ALF} the effect-site concentration of alfentanil and C_{50ALF} is the alfentanil concentration causing a 50% increase in current causing pain threshold or tolerance. To eliminate a possible hysteresis between alfentanil plasma concentration and pain response, an effect compartment was postulated that equilibrates with the plasma compartment with a half-life $t_{1/2}k_{e0}$ (*i.e.*, the blood-effect-site equilibration half-life). Pain threshold and tolerance data were analyzed simultaneously.

SIMULATIONS

Finally, to get an indication of the clinical implications of the effect of doxapram on alfentanil-induced analgesia, we simulated the doxapram-analgesia relationship for doxapram concentrations up to 10,000 ng/mL at an arbitrary constant effect-site concentration of alfentanil.

	Estimate ± SEE	**ω^2 ± SEE**
Doxapram PK parameters estimates (step 1)		
V_1	9.0 ± 0.9	0.10 ± 0.05
V_2	32.5 ± 2.1	-
CL (L/min)	0.36 ± 0.02	0.02 ± 0.03
Q (L/min)	1.0 ± 0.17	0.29 ± 0.14
PK-PD analysis: doxapram → CO (step 2)		
CO_B	7.8 ± 0.3	0.02 ± 0.008
C_{50DOX} (ng/mL)	5,200 ± 1,720	670 ± 610
$t_{1/2}k_{eO}$ (min)	3.2 ± 2.0	-
γ_{DOX}	1 (fix)	-

Table 1. *Doxapram pharmacokinetic and pharmodynamic parameter estmates*
PK; pharmacokinetic, PD; pharmacodynamic, V_1 and V_2; the volumes of compartments 1 and 2, CL; clearance from compartment V_1, Q; clearance from compartment V_2, CO; cardiac output, CO_B; baseline cardiac output, C_{50DOX}; doxapram effect-site concentration causing a 50% increase in cardiac output and $t_{1/2}k_{eO}$; blood-effect-site equilibration half-life and γ; shape parameters.

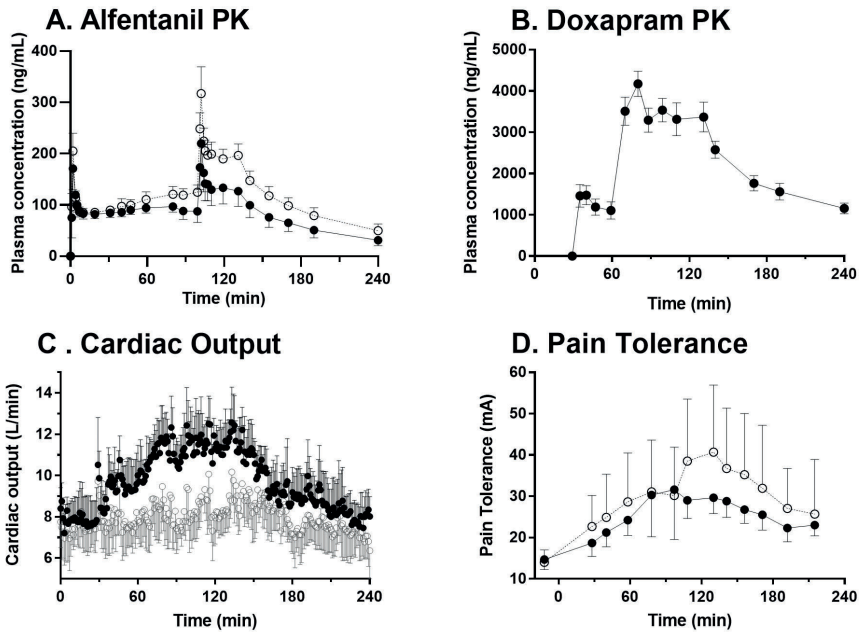

Figure 1. A*. Alfentanil concentrations during placebo and doxapram treatment.* ***B.*** *Doxapram plasma concentrations.* ***C.*** *Cardiac output during placebo and doxapram treatment. Each symbols is a 1-min average.* ***D.*** *Pain tolerance data during placebo and doxapram treatment. All data are mean values ± 95% confidence interval. Doxapram treatment is depicted by the closed symbols, placebo treatment by the open symbols.*

RESULTS

The data of nine subjects are included in the analyses. Two subjects decided not to participate after completing the first visit due to nausea and vomiting in one subject and excessive sedation in the other. Their data were discarded and two new subjects replaced them. The mean (range) age of the study population was 24.1 (20-27) years old, mean weight 85.4 (72-96) kg and mean BMI 25.7 (22-30) kg/m^2. In Figure 1 the mean PK and PD data are given. During doxapram infusions, the alfentanil plasma concentrations (Cp) were lower than during placebo infusions (Fig. 1): 116 ± 9 ng/mL (low dose doxapram) *vs.* 133 ± 12 ng/mL (placebo) and 120 ± 11 ng/mL (high dose doxapram) *vs.* 162 ± 16 ng/mL (placebo). Mean CO (± SD) was 7.6 ± 1.5 L/min during placebo administration, which increased to 9.2 ± 2.1 L/min and 11.1 ± 2.1 L/min during low- and high-dose dose doxapram infusion, respectively (Fig. 1). In both doxapram and placebo experiments the CO data were noisy in some subjects. However, since the noise occurred randomly (Figs. 1 and 2) we do not believe that this affected our study significantly.
Baseline end-tidal pCO_2 values were 38.2 ± 2.7 mmHg (mean ± SD) and 37.1 ± 3.1 mmHg on doxapram and placebo days, respectively. Doxapram had a respiratory stimulatory effect with reduced increases in end-tidal pCO_2 during alfentanil infusion (linear mixed effects model p = 0.017). End-tidal pCO_2 increases relative to baseline ($\Delta PETCO_2$) were at t = 55 min (ALF-low/DOXA-low): 7.0 ± 4.0 (placebo) and 4.3 ± 4.2 mmHg (doxapram, p > 0.05 *vs.* placebo); at 95 min (ALF-low/DOXA-high): 7.9 ± 5.7 mmHg (placebo) and 3.9 ± 5.3 mmHg (doxapram, p = 0.02); at

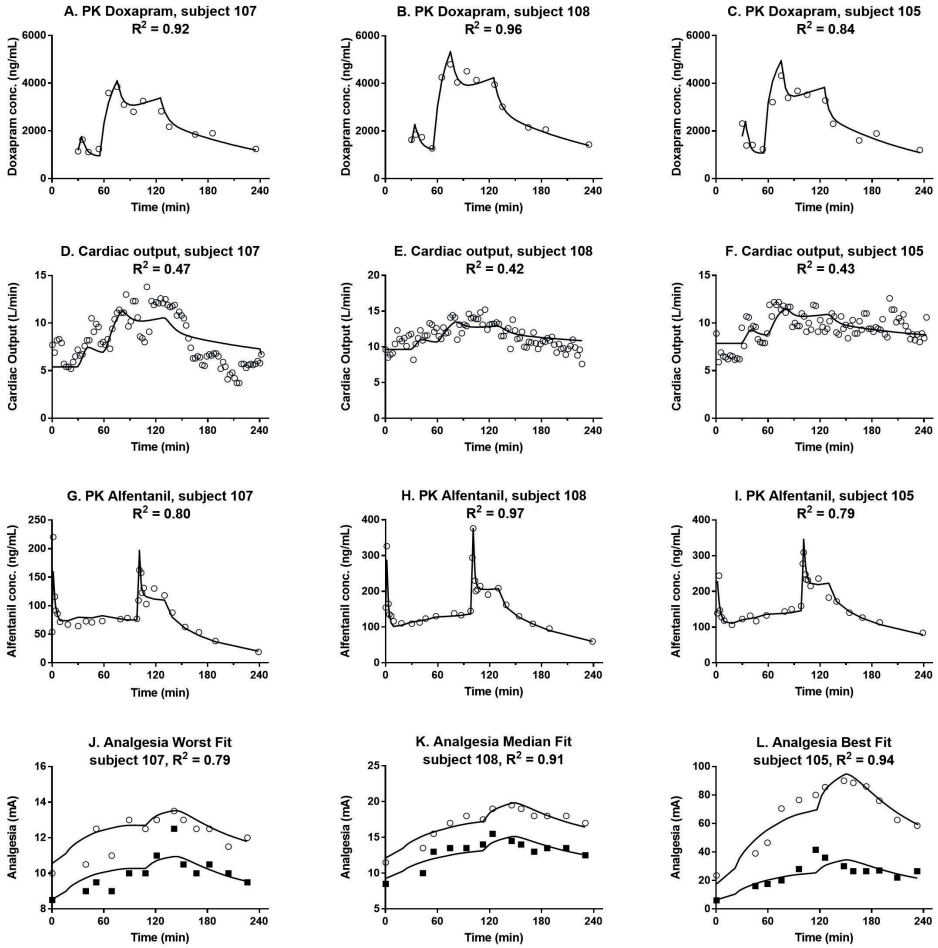

Figure 2. *Pharmacokinetic (PK) and pharmacodynamic (PD) data fits. The data fits are from subjects 105, 107 and 108 and are all from alfentanil/doxapram experiments. The choice of the 3 subjects was based on the goodness of fit of the analgesia data fits.* ***A-C:*** *Doxapram PK fits;* ***D-F:*** *Cardiac output fits;* ***G-I:*** *Alfentanil PK fits;* ***J-L:*** *Best, median and worst analgesia fits (pain tolerance closed symbols, pain threshold open symbols). Goodness of fit was based on the coefficient of determination (R^2). In each individual participant, pain tolerance and pain threshold were fitted simultaneously resulting in one R^2 value.*

t = 125 min (ALF-high/DOXA-high): 6.7 ± 4.8 mmHg (placebo) and 3.8 ± 4.8 mmHg (doxapram; p = 0.04).

PHARMACOKINETIC-PHARMACODYNAMIC ANALYSES

Steps 1 and 2 (PK-PD modeling of the effect of doxapram on cardiac output). The doxapram pharmacokinetic data were best described by a two-compartmental model. Pharmacokinetic and pharmacodynamic parameter estimates are given in Table 1. Inspection of the data shows that the models adequately describe the data. PK and PD data fits are given in Figure 2 (panels A-F, with corresponding R^2 values); goodness of fit plots in Figure 3 (A-D) showing absence of systematic errors for both PK and PD data. Doxapram increased CO by 50% at an effect-

	Estimate ± SEE	ω^2 ± SEE	v^2 ± SEE
Alfentanil PK parameters estimates (Step 4B)			
V_1 (L)	4.9 ± 0.6	0.05 ± 0.04	0.07 ± 0.03
V_2 (L)	8.7 ± 1.2	-	-
V_3 (L)	16.9 ± 1.6	0.03 ± 0.02	-
CL (L/min)	0.31 ± 0.03	0.07 ± 0.03	0.02 ± 0.008
Q_2 (L/min)	1.2 ± 0.09	-	0.10 ± 0.05
Q_3 (l/min)	0.32 ± 0.10	0.20 ± 0.07	-
α	1.05 ± 0.21		
PD analysis: alfentanil → pain tolerance (Step 5)			
SB THR (mA)	8.4 ± 1.2	0.09 ± 0.04	0.04 ± 0.03
SB TOL (mA)	13.5 ± 0.9	0.02 ± 0.02	0.02 ± 0.01
C_{50ALF} (ng/mL)	79.1 ± 27.6	1.01 ± 0.44	0.13 ± 0.20
$t_{1/2}k_{eO}$ (min)	13.3 ± 2.7	-	0.24 ± 0.18

Table 2. *Alfentanil pharmacokinetic and pharmacodynamic parameters estimates.*
PK; pharmacokinetic, PD; pharmacodynamic, V_1, V_2 and V_3; volumes of compartments 1, 2 and 3, CL; clearance from compartment V_1, Q_2 and Q_3; clearance from compartment V_2 and V_3, α; covariate coefficient (Eqs. 3-5), SB; baseline response for pain threshold (THR) and pain tolerance (TOL), C_{50ALF}; alfentanil effect-site concentration causing a 50% increase in pain response and $t_{1/2}k_{eO}$; blood-effect site equilibration half-life.

site concentration of 5,200 ± 1,720 (estimate ± SEE) ng/mL; the hysteresis between plasma concentration and effect ($t_{1/2}k_{eO}$) was 3.2 ± 2.0 min.

Steps 3 and 4 (alfentanil PK modeling). The alfentanil PK data were best described by a three-compartment model (data not show; MOFV 2956.575). Since a bivariate covariate (placebo/doxapram) significantly explained a large part of inter-occasion variability we compared alfentanil's PK under placebo and doxapram conditions. During doxapram treatment the alfentanil clearance (CL) was 30% higher than during placebo treatment (data not shown). We next constructed a model incorporating CO as a time-varying covariate on the elimination clearance CL and intercompartmental clearances Q_2 and Q_3, with covariate coefficients α_X (eqns. 3-5; Step 4B). Coefficients α were equal, *i.e.* $\alpha_1 = \alpha_2 = \alpha_3$, with a value > 0. Pharmacokinetic parameter estimates of Step 4B are given in Table 2. Best, median and worst data PK fits are given in Figure 2, panels G-I. Goodness of fit plots in Figure 3, panels E and F, showing that the data were well described by the model of Step 4B. The MOFV of the model was 2865.769.

Step 5 (final PK-PD model). The final PK-PD model using the PK EBE's of Step 4 described the data adequately with a MOFV of 1534.60 *vs.*1541.045 for a model with EBE's from an alfentanil PK model without the incorporation for CO as covariate (Step 3). Best, median and worst PD data fits are given in Figure 2, panels J-L; goodness of fit plots are given in Figure 3, panels G and H. Alfentanil's C_{50} was 79.1 ± 27.6 ng/mL and $t_{1/2}k_{eO}$ 13.3 ± 2.7 min.

Simulations. Results of the simulation is given in Figure 4, showing the non-linear decrease in analgesia with increasing effect-site concentrations of doxapram. At doxapram's C_{50} analgesia was reduced by 34%.

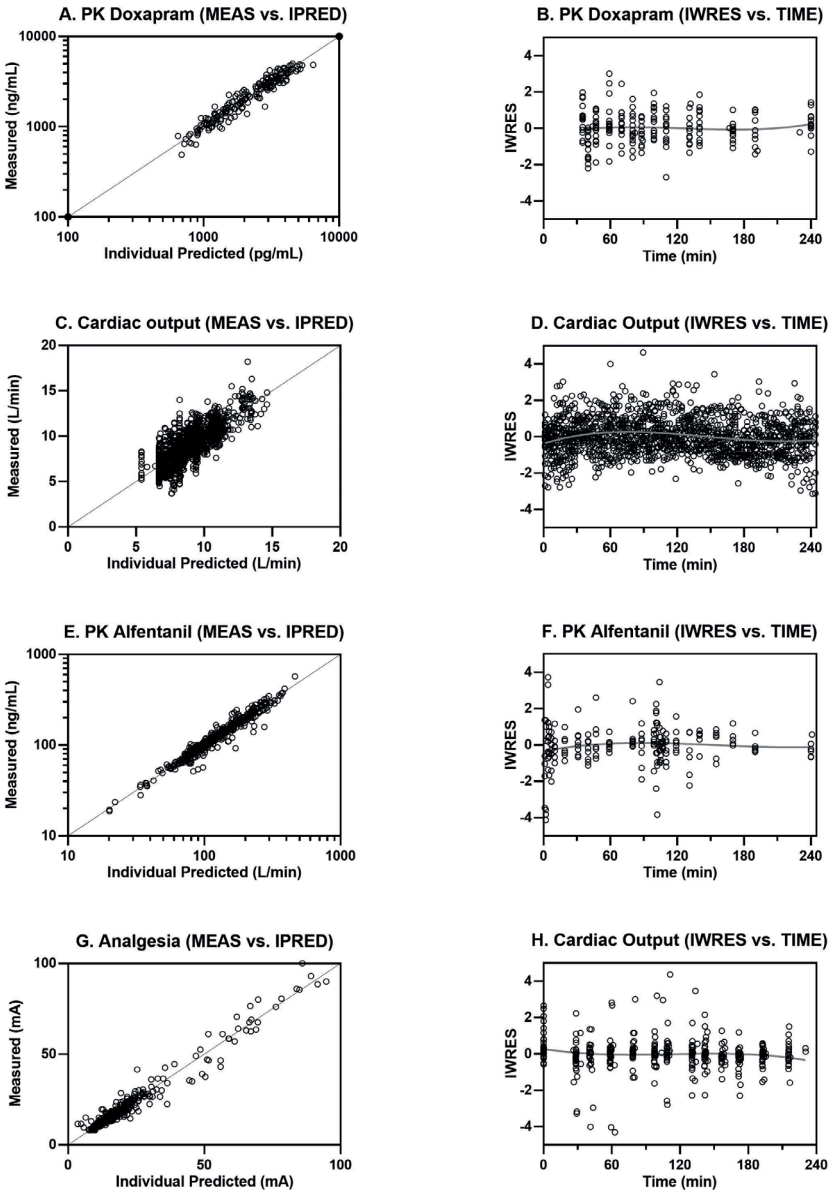

Figure 3. *Goodness of fit plots: doxapram pharmacokinetic (PK) analysis (**A and B**), cardiac output **(C and D)**, alfentanil pharmacokinetics **(E and F)** and analgesia **(G and H)**. A, C, E and G: measured versus individual predicted values. B, D, F and H: individual weighted (IWRES) residuals versus time.*

DISCUSSION

In this placebo-controlled study in healthy male volunteers, we investigated the effect of doxapram on alfentanil Cp, alfentanil-induced ventilatory depression and antinociception. Compared to placebo treatment, alfentanil Cp was reduced by 14% and 26% during low- and high-dose doxapram infusion, respectively. High-dose doxapram caused a reduction in antinociception by 25%. Our PK-PD analyses indicate that (1) the reduction in alfentanil concentration is well explained by the doxapram-induced increase in CO; the increased CO was associated with increased intercompartmental and elimination clearances, and (2) the reduction in alfentanil Cp explained the reduction in antinociception (see Fig. 5 for the full PK-PD/PK-PD model). Finally, a modest reduction of respiratory depression was observed during high-dose doxapram infusion with reduced increase in end-tidal pCO_2 by 3-4 mmHg compared to placebo.

DOXAPRAM'S EFFECT ON CARDIAC OUTPUT

During alfentanil administration, doxapram increased CO by 21% and 46% during low- and high-dose doxapram infusion, respectively. Since no systematic changes in CO were observed during the combination of placebo and alfentanil infusion (Fig. 1), an effect of alfentanil on CO seems of limited importance in our study. The mechanism through which doxapram influences CO remains unknown. One possibility is an effect of doxapram at its primary target site. *i.e.* the CBs. Activated peripheral chemoreceptors of the CBs drive sympathetic outflow, causing a systemic pressure response.[20] In hypertensive patients there is proof for enhanced pressure and ventilatory responses to hypoxia, while peripheral chemoreflex inhibition (by hyperoxia, CB ablation or denervation) reduces peripheral vascular resistance (see Paton et al. and references cited therein). It may therefore well be that during infusion of doxapram, the enhanced afferent input from the CBs to the brainstem produced an increased sympathetic outflow via activation of brainstem pressure centers (located in the rostral ventrolateral medulla and nucleus tractus solitarius) and consequently increases CO.[21] Further studies are needed to understand why activation of the CBs by doxapram results in a systemic pressure response while activation by GAL021 does not.[17]

EFFECT OF INCREASED CARDIAC OUTPUT ON ALFENTANIL PK AND ANTINOCICEPTION

A doxapram-induced difference in opioid pharmacokinetic profile (Fig. 1A) has not been studied before. So far, doxapram-provoked improvement of symptoms of respiratory failure

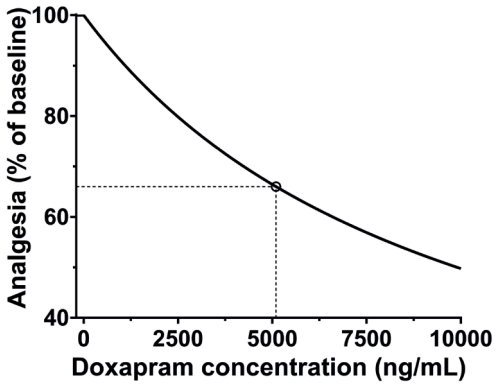

Figure 4. *Simulation of the relationship between doxapram effect-site concentration (x-axis) and alfentanil-induced analgesia at a fixed alfentanil effect-site concentration (y-axis). The open circle denotes the effect observed at doxapram's* C_{50}.

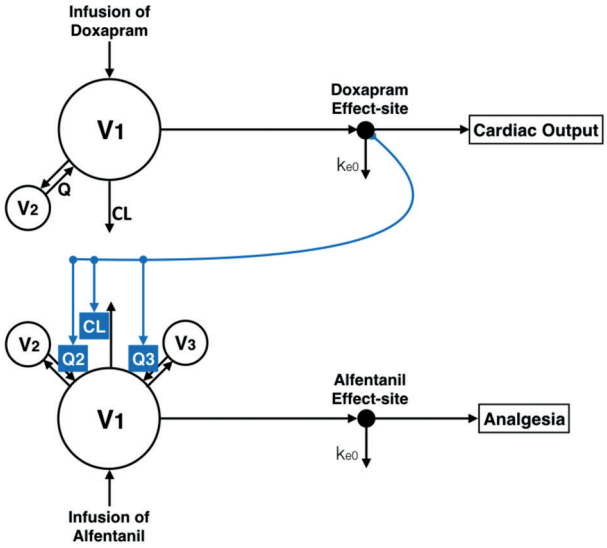

Figure 5. *The final PK-PD/PK-PD model. The top panel is the 2-compartment doxapram PK (pharmacokinetic model, which is linked via an effect-compartment to cardiac output. The bottom panel is the three-compartment alfentanil PK model, which is linked via an effect-compartment to analgesia. Doxapram's effect-site concentration (via its effect on cardiac output) increased the alfentanil clearances (CL, Q_2 and Q_3), causing a reduced alfentanil concentration in the effect-site and consequently a reduction of analgesia. V_X is the volume of compartment x (x = 1, 2 or 3), CL the clearance from compartment 1, Q_2 and Q_3 are the intercompartmental clearances, k_{e0} is the rate constant clearing drug out of the effect-site (with half-life $t_{1/2}k_{e0}$).*

has been exclusively attributed to doxapram's stimulatory effects at the level of the CBs. We showed an effect of doxapram on alfentanil Cp explaining part of doxapram's PD. Although we cannot exclude a pharmacokinetic interaction (alfentanil metabolizing CYP3A4 or CYP3A5 enzyme induction by doxapram), we explored whether the difference in CO is the main causative factor for the difference in alfentanil Cp between placebo and doxapram study days. Data from the literature indicate that alfentanil's distribution and elimination kinetics are CO dependent.[12,13] Henthorn et al.[12] showed in seven healthy volunteers that the tissue distribution of alfentanil (as measured by the intercompartmental clearances) was significantly correlated to CO. In halothane anesthetized pigs, Kuipers et al.[13] studied alfentanil PK during increases and decreases in CO by administration of either propranolol or dobutamine. They observed that changes in CO caused corresponding changes in distribution and elimination clearances. Both studies are in close agreement with our findings. In Step 4B of the analyses we showed that incorporating the effect-site doxapram concentration (Eqns. 3-5) significantly improved the alfentanil PK data fits. Since doxapram increased CO (Eqn.1), the increase in CO in fact explains the reduction in alfentanil PK and consequently the reduction in alfentanil-induced antinociception (Figs. 4 and 5). Most probably, the reduced alfentanil Cp is due to increased tissue uptake (related to the increase in Q_2 and Q_3) and liver elimination (CL). We need to realize that alfentanil is a drug with a low to moderate liver extraction (the elimination clearance of alfentanil is small relative to the hepatic blood flow),[13] with consequently a modest effect of CO on PK (14-26% reduction in Cp). Drugs with a higher hepatic extraction such as propofol may

show a greater effect on Cp by changes in CO.[22]

DOXAPRAM'S EFFECT ON RESPIRATION DURING HIGH-DOSE OPIOID INFUSION

Relative to placebo, we observed at high doxapram infusion a small but significant reduction in $\Delta PETCO_2$. Taking into account previous data, this small effect is fully explained by the reduction in alfentanil Cp.[17] This suggests that in our study doxapram's stimulatory effect on respiration *via* the CBs was negligible. Possibly at higher doxapram concentrations some stimulatory effect would have occurred. The current doxapram dose was 75% of the maximal recommended dose as specified by the manufacturer (see the summary of product characteristics). We opted not to give the maximum dose to minimize the occurrence of side effects. As stated earlier, the absence of a stimulatory effect of doxapram on ventilation may be related to a ceiling effect. In comparison to previous studies,[15,17] we used a relatively higher alfentanil-dosing scheme with maximum plasma concentrations of about 200 ng/mL. Our current data suggest that the CB stimulation by doxapram was unable to overcome the opioid-induced inhibition of brainstem rhythmic breathing activity. Our previous analyses suggest that more intense CB stimulation by applying higher doses of doxapram would have had only limited effect.[15]

CONCLUSIONS

In a population of healthy male volunteers, doxapram decreased alfentanil plasma concentrations with concomitant reduced alfentanil analgesia and respiratory depression. Pharmacokinetic modeling showed that the reduced plasma concentrations were related to an increase in distribution and elimination clearances that could be explained by a doxapram-induced increase in cardiac output. The reduction of respiratory depression was fully explained by reduced alfentanil plasma concentrations. The absence of carotid body-mediated ventilatory stimulation is probably related to reduced doxapram efficacy at deeper levels of respiratory depression. A general observation that can be made from our study is that higher opioid doses may be needed to obtain similar levels of analgesia when cardiac output increases, especially when opioids are given with moderate to high hepatic extraction.

Finally, in this experimental mechanistic study physiological influences on the PK and PD of a potent opioid with rapid onset/offset of effect and low clinical margin of safety were studied. We show that a PK-PD model for doxapram effectively drives the PK-PD model of alfentanil. Although in real life such combinations are probably less likely to occur, there are various drugs that are used more frequently in clinical practice that influence cardiac output and consequently may affect clearance of opioid analgesics as demonstrated here for doxapram and alfentanil.

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Chapter 3

Two Studies on Reversal of Opioid-Induced Respiratory Depression by BK-channel Blocker GAL021 in Human Volunteers

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J. Hay, J. McLeod, A. Dahan

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INTRODUCTION

Opioids are the cornerstone of treatment of moderate to severe acute and chronic pain. Opioids, however, come with serious side effects, of which respiratory depression is potentially lethal.[1] In the perioperative setting the estimated incidence of opioid-induced respiratory depression (OIRD) is 0.5 to 2%.[1] In chronic pain patients the incidence of OIRD is unknown.[2] Recent publications stress the fact that the number of fatalities from legally prescribed opioids for treatment of chronic pain are high.[2,3] This is predominantly attributed to an increased awareness of clinicians to diagnose and treat chronic pain and the apparent ease at which legally prescribed opioids change hands.[3] Taken the presented data, both in perioperative medicine and in the treatment of chronic pain the elimination of opioid-induced respiratory complications is important. Not only will it reduce morbidity and mortality but it will possibly result in improved pain treatment with less suffering from inadequate pain relief which often occurs due to the fear of opioid-induced respiratory depression.

Current clinical practice is to treat OIRD with the opioid antagonist naloxone, which, however, reverses OIRD as well as analgesia, and comes with other sometimes deleterious side effects.[1,4] A potent respiratory stimulant that effectively counteracts OIRD without any interaction with the opioid receptor system is lacking.[1] Various experimental drugs that enhance respiration are currently under investigation including serotonin-agonists, ampakines, phosphodiesterase inhibitors and potassium-channel blockers.[1,5] In the current study we investigated the efficacy of a new agent, GAL021 (Fig. 1), which inhibits calcium-activated potassium channels at the carotid bodies (*ie.* large conductance Ca^{2+}/voltage-activated K^+-channels, BK_{Ca}-channels, formerly known as Maxi-K-channels).[6] In rodents and monkeys, GAL021 dose-dependently increased ventilatory drive and antagonizes opioid (morphine/fentanyl)- and non-opioid (midazolam, isoflurane/propofol)-induced respiratory depression.[7-9]

We performed two studies to assess the effect of GAL021 on respiratory and non-respiratory end-points. The first study was a randomized-controlled trial that was designed as a *first-in-class* study to confirm the effects of GAL021 on established (opioid-induced) respiratory depression under isohypercapnic conditions. The main aim of the study was to assess whether the results confirm the mechanism of action of GAL021 in humans under conditions of a depressed ventilatory control system. To further explore the properties of GAL021 we performed an exploratory or learn study to assess the effects of GAL021 on ventilation under non-clamped conditions and on non-respiratory variables (hemodynamics, antinociception, sedation, adverse events). Our main hypothesis is that GAL021, given on top of established opioid-induced respiratory depression, is able to stimulate breathing without major effects on non-respiratory end-points.

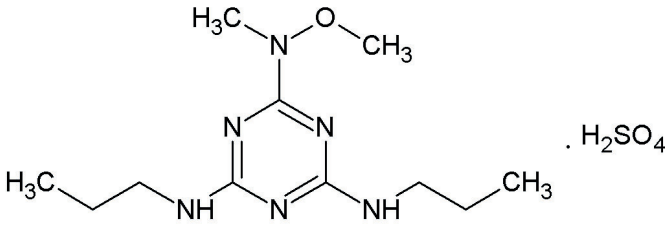

Figure 1. *The chemical structure of GAL021 dihydrosulphate.*

MATERIALS AND METHODS

Both studies (the proof-of-concept study (Study 1) and learn-study (Study 2)) had a randomized, double blind, placebo-controlled crossover design. The protocol was performed after approval was obtained from the Medical Ethics Committee of the Biomedical Research Ethics Review Foundation (BEBO, Assen, The Netherlands) and the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and was registered at www.trialregister.nl under number NTR3718. The studies were conducted from October 31, 2012 to February 11, 2013. An *a priori* power analysis was performed for Study 1 and yielded 12 subjects to detect a respiratory effect of GAL021 greater than placebo (see Section *Sample Size*). After completion of Study 1, the effect of GAL021 *vs.* placebo was studied in Study 2 on respiratory and non-respiratory variables, now against a background of poikilocapnia (*ie*. the subjects breathed room air). Study 2 was designed to study (i) the effect of GAL021 on alfentanil-induced respiratory depression under "real life" (*ie.* non-carbon dioxide clamp) conditions, and (ii) to get an impression of the effect of GAL021 on non-respiratory variables, including hemodynamics, pain responses and sedation. In this learn-study, the number of subjects was set at 8, considering the magnitude of effects observed in Study 1. The protocol allowed expansion of Study 2 to a maximum of 36 subjects in case further exploration was required. In Studies 1 and 2 adverse events were recorded.

SUBJECTS

Healthy men, aged 18-45 years and body mass index 18-30 kg/m^2, were recruited through an advertisement on a dedicated website. All subjects gave written and oral informed consent. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and hematology and an electrocardiogram to assess eligibility. Participants were healthy with no history of major medical or psychiatric disease, alcohol abuse, daily consumption of caffeine greater than 6 servings, smoking in the last year and any other investigational drug administered within three months prior to inclusion. Finally, participants had to fast for at least 6 hours prior to the administration of study drug.

STUDY DESIGN

Upon arrival in the laboratory all subjects received two intravenous access lines, one for administration of alfentanil and another for administration of GAL021 (Galleon Pharmaceuticals Corp., Horsham, PA, USA) or placebo (NaCl 0.9%). An arterial line was placed in the radial artery of the non-dominant arm for alfentanil blood sampling in Studies 1 and 2 (see Appendix 1), and measurement of blood pressure, cardiac output and arterial pCO_2 in Study 2. For safety monitoring, the ECG, blood pressure, heart rate and oxygen saturation were measured continuously.

Drugs. GAL-021 was prepared as a sterile product ready for dilution (colorless, pH 3.1). GAL-021 and placebo (normal saline) were diluted in Ringer lactate (final volume ≈ 250 ml) and administrated intravenously by infusion pump.

Alfentanil and GAL021 infusions in Studies 1 and 2. A stepped drug infusion regimen was applied as depicted in Figure 2. First, alfentanil was administered intravenously: a loading infusion of 1.33 µg.kg^{-1}.min^{-1} for 6 min, followed by a subsequent maintenance infusion of 0.3

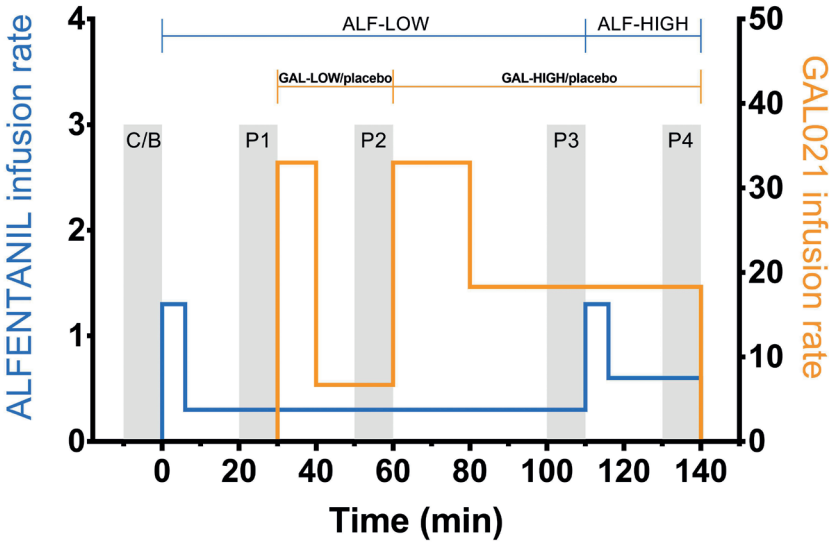

Figure 2. *Schematic representation of the design of Studies 1 and 2. The blue line is the infusion rate of alfentanil. In Study1, the infusion could be adapted depending on magnitude of the ventilatory (a doubling of loading and maintenance dose in case the target of respiratory depression (25-30%,) was not attained; a reduction of the maintenance dose by 50% in case of an overshoot in respiratory depression). The orange line is the infusion rate of GAL021 or placebo. In Study 1, respiration was measured under isohypercapnic conditions; in Study 2 poikilocapnic ventilatory and non-ventilatory variables were obtained. B represents baseline (no drug, no added inspired carbon dioxide), C represents the carbon dioxide clamp prior to any drug infusion, P1 represents low-dose alfentanil infusion prior to GAL021 or placebo infusion (ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low + GAL021-low), P3 the combination of low-dose alfentanil with high dose GAL021 or placebo (ALF-low + GAL021-high) and P4 the combination of high-dose alfentanil with high dose GAL021 or placebo (ALF-high + GAL021-high). Alfentanil and GAL021 infusion rate are in μg.kg^{-1}.min^{-1}.*

μg.kg^{-1}.min^{-1} given over 104 min, in order to achieve a 25-30% decrease in minute ventilation (ALF-low). If this level of respiratory depression was not reached during the first infusion, a second dose of 1.33 μg.kg^{-1}.min^{-1} was administered and the maintenance infusion was increased to 0.6 μg.kg^{-1}.min^{-1}; in case of an overshoot in respiratory depression during the loading infusion, the maintenance infusion was halved. After 30 min of steady-state ventilation, a concurrent intravenous infusion of GAL021 or placebo was started (GAL-low): a loading infusion of 33.3 μg.kg^{-1}.min^{-1} for 10 min, followed by a maintenance infusion of 6.67 μg.kg^{-1}.min^{-1} for 20 min (total infusion time of GAL-low 30 min). Next, the GAL021 infusion was increased (GAL-high) with a loading infusion of 33.3 μg.kg^{-1}.min^{-1} for 20 min, followed by a maintenance infusion of 18.3 μg.kg^{-1}.min^{-1} for 60 min (total infusion time of GAL-high 80 min). During the final thirty minutes of the GAL-high infusion, the infusion rate of alfentanil was increased (ALF-high) with a repeat loading as given in ALF-low (in case no adjustments were made this was 1.33 μg.kg^{-1}.min^{-1} for 6 min), followed by a maintenance dose twice that of ALF-low (in case no adjustments were made the maintenance infusion was 0.6 μg.kg^{-1}.min^{-1} given over 24 min). The target reduction in ventilation at ALF-high was 50-60%. Hereafter, both alfentanil and GAL021/ placebo infusions were ended.

Inhaled gas concentrations and ventilation measurements. During (breath-to-breath) ventilation measurement the subjects breathed through a facemask connected to a pneumotachograph system (#4813, Hans Rudolph, Shawnee, KS). The signal from the pneumotachograph was integrated to yield a volume signal. The inspired and expired oxygen and carbon dioxide partial pressures (pO_2 and pCO_2) were measured at the mouth with a capnograph (Datex Capnomac, Helsinki, Finland).
In Study 1, ventilation was measured at the background of isohypercapnia. To that end, varying concentrations of inhaled oxygen, carbon dioxide and nitrogen were delivered to the subjects *via* three computer-controlled mass flow controllers (Bronkhorst, Veenendaal, The Netherlands) ensuring the strict control of the end-tidal pO_2 and pCO_2 independent of the ventilatory response. See Refs.[10,11] for an elaborate explanation of the dynamic end-tidal forcing technique. The elevated end-tidal pCO_2 was such that the target pre-drug clamped minute ventilation was between 20 ± 2 L/min. The inspired oxygen concentration was also manipulated to keep the end-tidal pO_2 in the normoxic range (110 mmHg) throughout the study. In Study 2, the subjects breathed room air without any additional inspired carbon dioxide.

Study episodes. For analyses purposes 4 time points are defined in Study 1 and 4 in Study 2 (see also Fig. 2):
Study 1: Period C is defined as the 10-min period prior to any drug infusion but with carbon dioxide clamp, P1 is the 10-min period during low dose alfentanil infusion prior to any GAL021 or placebo infusion (ALF-low), P2 is the 10-min period where low dose alfentanil is combined with low dose GAL021 or placebo infusion (ALF-low + GAL-low), P3 is the 10-min period where low-dose alfentanil is combined with a high dose GAL021 or placebo infusion (ALF-low + GAL-high) and P4 is the 10-min period where high-dose alfentanil is combined high dose GAL021 or placebo (ALF-high + GAL-high).
Study 2: Period B is the 10-min period prior to any drug infusion. P1 to P4 are defined as in Study 1. No carbon dioxide clamp was applied in Study 2.

Design of Study 1. When ventilation had reached a steady state at the elevated end-tidal pCO_2, alfentanil infusion was started (see Section *Alfentanil and GAL021 Infusions* above). For analyses purposes, 10-min averages of inspired minute ventilation, tidal volume, respiratory rate, end-tidal pCO_2 and oxygen saturation were obtained at periods C, P1 to P4 (Fig. 2). Each subject participated twice in Study 1, once receiving alfentanil and GAL021, once receiving alfentanil and placebo. The washout-period between sessions was at least one week.

Design of Study 2. Eight subjects who previously participated in Study 1 were included in Study 2. Selection of the subjects was based on their availability and unrelated to the respiratory responses in Study 1. Subjects in Study 2 were tested twice, once receiving alfentanil and GAL021, once receiving alfentanil and placebo, with at least 1 week between sessions. In this study, the infusion schemes of alfentanil and GAL021/placebo were similar to that of Study 1. The subjects breathed room air throughout the study. The following procedures were performed to collect data at regular intervals (at B, P1 to P4; see Fig. 2):

(a) Ventilation was measured for 5-10 min (while breathing room air) using the facemask/pneumotachograph system.

(b) Hereafter a blood sample was obtained for blood gas analysis. Here we report on the arterial pCO_2. The sample was analyzed with an I-Stat 1 system (Abbott Point of Care, Abbott Park, IL) using CG8+ cartridges.

(c) Next, alfentanil-induced antinociception was measured using an electrical pain model. Two electrodes were placed on the skin over the shinbone of the right leg.[12] An electrical stimulus train was generated by a computer-interfaced current stimulator (Leiden University Medical Center, Leiden, The Netherlands). After starting the stimulator the current increased from 0 by 0.5 $mA.s^{-1}$ and the subject indicated, by pressing a button on the control panel, when pain was first observed (pain detection threshold) and by pressing another button when he could not tolerate the pain any further (pain tolerance). This ended the stimulus train. If a muscle response was triggered during this procedure, the electrodes were relocated until no further response was observed. This procedure was practiced at the beginning of the experimental session. Four baseline values were obtained prior to any drug infusion. These values were averaged and served as pre-drug control values. Here we present the pain threshold data.

(d) Just before respiratory measurements, the subjects were queried about the magnitude of sedation by means of a visual rating scale from 1 to 100 mm where 1 equals no sedation and 100 mm equals maximum sedation.[12]

(e) Throughout part 2 of the study the mean arterial pressure and cardiac output were measured using the FloTrac™/Vigileo™ system (Edwards Lifsesciences Corp., Irvine, CA, USA) connected to the arterial line. Minute averages were obtained from the device.

(f) Heart rate (Datex, Cardiocap) and oxygen saturation (Masimo SET pulse oximeter, Irvine, CA, USA) were collected throughout the study.

RANDOMIZATION AND ALLOCATION

This was a double-blind study. Randomization was performed by a study-independent statistician according to a computer-generated non-restricted randomization schedule and shared with the local pharmacy. Subjects were allocated in a 1:1 ratio. The pharmacy prepared the study drugs and dispensed them into identical syringes marked solely with the subject and visit number. The drugs were delivered to the research team on the morning of the experiment. The pharmacy further delivered alfentanil syringes in a solution of 0.5 mg/mL. Unblinding of the study was performed after data closure.

SAMPLE SIZE

Sample size determination was performed for minute ventilation at P3 and P4 and was based on data from a previous study that showed that changes in minute ventilation (over a 10-minute assessment period) had an intra-subject variance ranging from 6 to 9%.[13] Sample sizes of 8 and 12 yielded respectively 80% and 90% power to observe a statistically significant within-cohort difference ($\alpha = 0.05$, 1-sided). The sample size was set at 12 subjects for Study 1. In case of discontinuation, the subject was replaced by another and both experimental sessions were performed. The sample size of part 2 of the study was set arbitrarily at 8.

STATISTICAL ANALYSIS

The evaluable population consisted of all subjects who completed both crossover periods in both studies. Data are presented as mean (95% confidence interval) and point estimates of the difference between treatments (95% confidence interval). To get an indication of the ability of GAL021 to increase ventilation relative to placebo, the data of Study 1 were analyzed with a mixed model analysis of covariance with treatment segment, and treatment × segment as fixed factors and subject, subject × treatment and subject × segment as random factors and the value at segment P1 (ALF-low) as covariate. Analysis was performed for segments P2, P3 and P4 separately, with $p < 0.01$ considered significant (SAS, SAS Institute Inc., Cary, NC).

RESULTS

Study 1. Three subjects withdrew consent after completing one single experimental session for reasons of discomfort. Data of these three were not included in the analysis; three new subjects were enrolled and completed the study. Twelve subjects completed both experimental sessions. Median (range) age of the subjects was 21.5 (19-31) years, median weight 72.3 (62.9-84.3) kg and body mass index 22.3 (20.2-26.5) $kg.m^{-2}$. All subjects completed the study without major side effects (see paragraph *Adverse Events*).

On average, carbon dioxide was clamped at 48.8 (SD 0.2) mmHg and 49.2 (SD 0.04) mmHg in placebo and GAL021 experiments, respectively. Ventilation levels reached at Period C were 20.8 (19.4-22.3) L/min (placebo) and 19.8 (19.3-20.4) L/min (GAL021; Table 1). Three subjects required an additional loading infusion of alfentanil because of a limited effect of the initial loading infusion on ventilation as specified in the protocol. Six subjects received a reduced maintenance infusion because of an initial overshoot in ventilatory depression. The effects of alfentanil, GAL021 and placebo on ventilation, tidal volume, respiratory rate and oxygen saturation are given in Table 1. Most importantly, a separation between GAL021 and placebo on minute ventilation was observed at P3 (ALF-low + GAL-high) and P4 (ALF-high + GAL-high) by 6.1 (3.6-8.6) L/min and 3.6 (1.5-5.7) L/min, respectively (both $p < 0.01$ *vs.* placebo, Fig. 3A). The

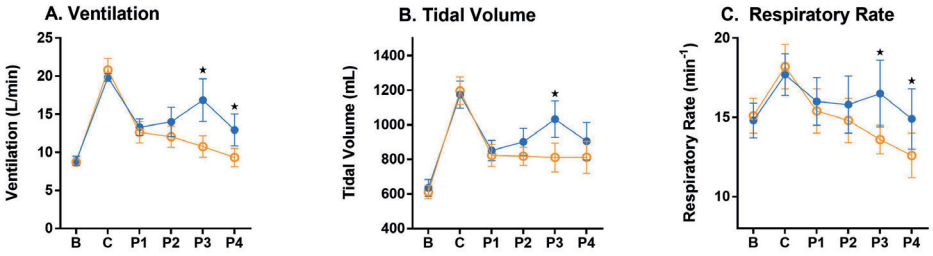

Figure 3. *Results of Study 1: Effect of GAL021 vs. placebo on minute ventilation* ***(A)****, tidal volume* ***(B)*** *and respiratory rate* ***(C)****. B represents baseline (no drug, no added inspired carbon dioxide), C represents the carbon dioxide clamp prior to any drug infusion, P1 represents low-dose alfentanil infusion prior to any GAL021 or placebo infusion (carbon dioxide-clamp + ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (carbon dioxide-clamp + ALF-low + GAL021-low), P3 the combination of low-dose alfentanil with high dose GAL021 or placebo (carbon dioxide-clamp + ALF-low + GAL021-high) and P4 the combination of high-dose alfentanil with high dose GAL021 or placebo (carbon dioxide-clamp + ALF-high + GAL021-high). Values are mean ± 95% confidence interval. * p < 0.01 vs. placebo.*

	Clamp	P1	P2	P3	P4
Ventilation (L/min)					
Placebo	20.82 (19.4-22.3)	12.63 (11.3-14.0)	12.05 (10.7-13.4)	10.77 (9.4-12.2)	9.32 (8.2-10.5)
GAL021	19.84 (19.3-20.4)	13.31 (12.2-14.4)	14.00 (12.1-15.9)	16.86 (14.1-19.6)	12.94 (10.8-15.1)
Mean difference	-0.98 (-2.2 to 0.2)	0.68 (-0.6 to 2.0)	1.95 (0.6-3.3)	6.09 (3.6-8.6)*	3.62 (1.5-5.7)*
Tidal volume (mL)					
Placebo	1196 (1116-1275)	823 (760-886)	818 (766-869)	811 (728-894)	812 (719-906)
GAL021	1174 (1095-1252)	851 (792-910)	901 (822-980)	1032 (927-1137)	905 (796-1014)
Mean difference	-22 (-83 to 39)	28 (-26 to 82)	83 (21-146)	221 (124-318)*	93 (-13 to 198)
Respiratory rate (min^{-1})					
Placebo	18.2 (16.8-19.6)	15.4 (14.0-16.8)	14.8 (13.4-16.3)	13.6 (12.6-14.5)	12.6 (11.2-13.9)
GAL021	17.7 (16.4-19.0)	16.0 (14.5-17.5)	15.8 (14.0-17.6)	16.5 (14.4-18.7)	14.9 (13.1-16.8)
Mean difference	-0.5 (-1.3 to 0.2)	0.6 (-0.3 to 1.5)	1.0 (0.0-1.9)	3.0 (1.2-4.8)*	2.4 (0.7-4.0)*
End-tidal pCO_2 (mmHg)					
Placebo	48.8 (47.4-50.2)	48.9 (47.6-50.3)	48.9 (47.6-50.3)	49.0 (47.7-50.2)	48.6 (47.3-49.9)
GAL021	49.2 (47.9-50.3)	49.2 (48.0-50.3)	49.2 (47.9-50.4)	49.3 (48.2-50.4)	49.2 (48.0-50.4)
Mean difference	0.4 (-0.8 to 1.6)	0.2 (-1.0 to 1.4)	0.9 (-0.9 to 1.4)	0.3 (-0.8 to 1.5)	0.6 (-0.4 to 1.5)
Oxygen saturation (%)					
Placebo	98.6 (97.9-99.3)	97.3 (96.6-98)	97.4 (96.6-98.1)	97.6 (96.4-98.7)	97.3 (96.3-98.3)
GAL021	98.9 (98.4-99.5)	97.5 (96.7-98.3)	97.7 (96.8-98.5)	98.1 (97.3-98.9)	97.0 (96.2-97.9)
Mean difference	0.3 (-0.1 to 0.8)	0.3 (-0.3 to 0.9)	0.3 (-0.3 to 0.9)	0.5 (-0.2 to 1.2)	-0.3 (-0.9 to 0.4)

Table 1. *Effect of GAL021 on respiratory variable obtained under clamped end-tidal pCO_2 conditions (Part 1 of the study). Values are mean (95% confidence interval); Mean difference = GAL021 – Placebo.*
** $p < 0.01$ versus placebo,*
Clamp is CO_2 clamp prior to any drug infusion (only in part 1 of the study), P1 is low-dose alfentanil infusion prior to any GAL021 or placebo infusion (ALF-low), P2 is combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low + GAL021-low), P3 is the combination of low-dose alfentanil with high dose GAL021 or placebo (ALF-low + GAL021-high), P4 is the combination of high-dose alfentanil with high dose GAL021 or placebo (ALF-high + GAL021-high).

effects on minute ventilation were due to effects on tidal volume (at P3, Fig. 3B) and respiratory rate (at P3 and P4, Fig. 3C). No effect of either alfentanil or GAL021/placebo was observed on oxygen saturation (Table 1). A scatter plot of the individual ventilation data is given in Figure 4.

Study 2. Eight subjects of Study 1 participated in Study 2 and completed both experimental sessions. All subjects completed the study without major side effects (see paragraph *Adverse Events*). Examination of (poikilocapnic) ventilation and arterial pCO_2 (Table 2 and Fig. 5) shows separation between GAL021 and placebo at P3 and P4 with mean differences in effect estimates of 0.6 (0.1-1) L/min and -3.4 (-6.2- -0.6) mmHg at P1 and 1.0 (0.5-1.6) L/min and -1.5 (-3.1 to 0.1) mmHg at P4, respectively. No treatment differences were observed for plasma alfentanil concentrations, blood pressure, cardiac output, pain threshold and sedation (Table 2). A scatter plot of the individual ventilation data is given in Figure 4.

Adverse events. Both alfentanil and GAL021 were well tolerated by the subjects and no interventions were required. Adverse events occurring in Studies 1 and 2 are given in Table 3. Apart from evident opioid-related side effects such as pruritus and nausea, specific differences were observed between Studies 1 and 2 (feeling warm/sweating, nausea, headache) and between treatments (pain at infusion site).

DISCUSSION

RESPIRATORY DEPRESSION

Opioid-induced respiratory depression remains an important concern taken its possible morbidity and potential fatal consequences. Indeed both in the acute and chronic opioid-use settings numerous (near)-fatalities have been reported.[1-3] Current clinical practice to reverse OIRD is by intravenous or intramuscular injection of the opioid receptor antagonist naloxone. While naloxone is an effective reversal agent it comes with disadvantages including reversal of analgesia, sympathicoexcitation and risk of renarcotization if not continuously administered (due to its short half-life of 15-30 min).[4] Furthermore, some opioids are difficult to reverse related to their high affinity for the opioid receptor (*eg*, buprenorphine).[13] Hence, there is a need for an agent that selectively stimulates breathing without any effect on other physiological systems. Such an agent should stimulate respiration without any interaction with the opioid receptor system. Various agents have been evaluated to that end, including modulators of potassium channels, serotonin receptor agonists, agents that enhance glutamatergic transmission and phosphodiesterase inhibitors.[1,5] Here we present data on a novel agent, GAL021. Earlier animal data demonstrated that GAL021 induces potent ventilatory stimulation during OIRD without affecting analgesia. In conscious rats and non-human primates (Cynomolgus monkeys) an infusion of GAL021 reversed morphine-induced respiratory depression producing a rapid and dose-dependent diminution of the evoked respiratory depression for the duration of the infusion.[7-9] Termination of GAL021 infusion led to the return of OIRD. Importantly, GAL021 did not diminish morphine-induced analgesia in rats as tested by the tail-flick assay.[7]

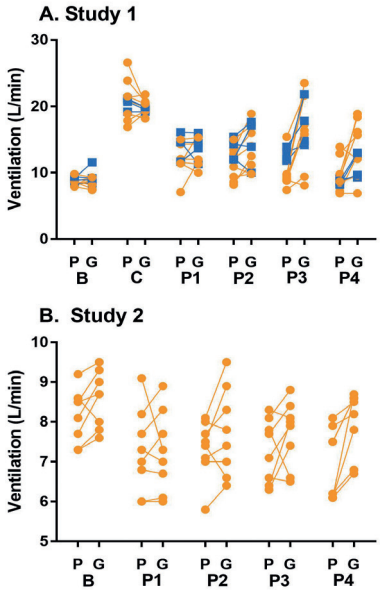

***Figure 4. A**. Scatter plot of the effect of treatment on ventilation of Study 1 at baseline (B), clamped ventilation (C) and time points P1 (carbon dioxide-clamp + ALF-low), P2 (carbon dioxide-clamp + ALF-low + GAL021-low), P3 (carbon dioxide-clamp + ALF-low + GAL021-high) and P4 (carbon dioxide-clamp + ALF-high + GAL021-high). In orange the subjects that participated in Studies 1 and 2. **B.** Scatter plot of the effect of treatment on ventilation of Study 2 at baseline (B) and time points P1 (carbon dioxide-clamp + ALF-low), P2 (carbon dioxide-clamp + ALF-low + GAL021-low), P3 (carbon dioxide-clamp + ALF-low + GAL021-high) and P4 (carbon dioxide-clamp + ALF-high + GAL021-high). In orange the subjects that participated in Studies 1 and 2.*

	Baseline	P1	P2	P3	P4
Ventilaton (L/min)					
Placebo	8.15 (7.7 - 8.6)	7.28 (6.6 - 8.0)	7.32 (6.8 - 7.8)	7.28 (6.7 - 7.8)	6.95 (6.3 - 7.6)
GAL021	8.58 (8.1 - 9.1)	7.23 (6.5 - 7.9)	7.74 (7.0 - 8.5)	7.84 (7.4 - 8.3)	7.98 (7.4 - 8.6)
Mean difference	0.28 (0.1 - 0.8)	-0.04 (-0.6 to 0.5)	0.42 (-0.1 to 0.9)	0.56 (0.1 - 1.0)	1.03 (0.5 - 1.6)
Arterial pCO_2 (mmHg)					
Placebo	43.4 (42.0 - 44.8)	47.4 (44.9 - 49.9)	47.7 (46.2 - 49.3)	48.6 (46.1 - 51.0)	51.7 (48.1 - 55.3)
GAL021	42.6 (40.6 - 44.5)	46.4 (42.3 - 50.5)	46.7 (43.3 - 50.0)	45.3 (41.7 - 48.9)	48.0 (43.1 - 52.9)
Mean difference	-0.4 (-2.2 to 1.4)	-0.9 (-4.3 to 2.5)	-1.2 (-3.1 to 0.7)	-3.4 (-6.2 to -0.6)	-1.5 (-3.1 to 0.1)
Arterial pO_2 (mmHg)					
Placebo	95.1 (92.1 - 98.1)	95.8 (91.7 - 99.8)	93.1 (90.3 - 96.0)	88.1 (82.3 - 93.9)	94.0 (87.1 - 100.9)
GAL021	94.7 (90.8 - 98.5)	93.8 (86.6 - 101.0)	94.6 (83.0 - 106.2)	96.0 (88.2 - 103.8)	94.2 (84.3 - 104.1)
Mean difference	0.0 (-5.3 to 5.3)	-1.0 (-7.1 to 5.1)	2.6 (-2.6 to 7.8)	9.2 (0.9 - 17.5)	8.2 (4.1 - 12.3)
Cardiac output (L/min)					
Placebo	7.7 (6.5 - 9.0)	6.1 (5.2 - 7.0)	6.0 (4.9 - 7.0)	6.3 (4.5 - 8.0)	6.3 (4.7 - 7.8)
GAL021	7.2 (5.6 - 8.8)	6.0 (4.6 - 7.4)	6.2 (4.4 - 8.1)	6.6 (5.2 - 8.1)	6.8 (5.3 - 8.4)
Mean difference	1.0 (-1.6 to 3.5)	0.8 (-1.2 to 2.8)	1.2 (-0.7 to 3.1)	1.5 (-0.8 to 3.8)	1.1 (-1.1 to 3.4)
Mean arterial pressure (mmHg)					
Placebo	95 (89 - 101)	87 (83 - 91)	86 (83 - 90)	87 (83 - 92)	86 (82 - 90)
GAL021	95 (89 - 101)	86 (78 - 95)	87 (79 - 96)	85 (80 - 90)	83 (76 - 91)
Mean difference	13 (-16 to 42)	13 (-17 to 42)	14 (-15 to 43)	13 (-17 to 42)	10 (-19 to 39)
Heart rate (min^{-1})					
Placebo	63 (56 - 70)	54 (50 - 58)	53 (48 - 58)	54 (49 - 59)	54 (49 - 58)
GAL021	61 (49 - 73)	53 (46 - 60)	53 (47 - 60)	57 (50 - 63)	61 (58 - 64)
Mean difference	7 (-12 to 25)	7 (-11 to 26)	8 (-10 to 26)	12 (-4 to 28)	14 (-1 to 29)
Pain threshold (% of baseline)					
Placebo	100	113 (101 - 125)	118 (100 - 136)	123 (107 - 139)	140 (114 - 165)
GAL021	100	124 (98 - 150)	131 (108 - 154)	142 (108 - 175)	160 (120 - 201)
Mean difference	-	11 (-20 to 42)	13 (-14 to 40)	19 (-18 to 55)	21 (-33 to 75)
Sedation VAS (mm)					
Placebo	13 (-5 - 32)	66 (46 - 86)	49 (20 - 77)	48 (24 - 71)	63 (42 - 85)
GAL021	11 (-6 - 22)	62 (44 - 80)	47 (25 - 69)	56 (32 - 80)	71 (49 - 92)
Mean difference	-2 (-5 to 0)	-4 (-13 to 6)	-2 (-21 to 18)	8 (-2 to 18)	7 (-9.0 to 24)

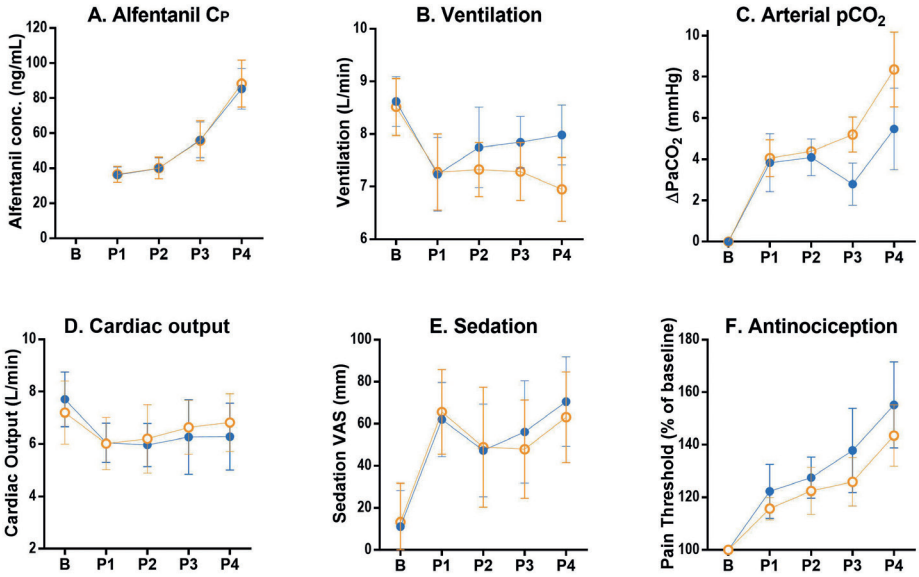

Figure 5. *Results of Study 2:* ***A.*** *Plasma alfentanil concentrations (CP)* ***B.*** *Poikilocapniac ventilation* ***C.*** *Arterial pCO_2.* ***D.*** *Cardiac output* ***E.*** *Sedation* ***F.*** *Antinociception.*

B represents baseline (no drug), P1 represents low-dose alfentanil infusion prior to any GAL021 or placebo infusion (ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low + GAL021-low), P3 the combination of low-dose alfentanil with high dose GAL021 or placebo (ALF-low + GAL021-high) and P4 the combination of high-dose alfentanil with high dose GAL021 or placebo (ALF-high + GAL021-high). Values are mean ± 95% confidence interval. Orange represent placebo infusion, blue represents GAL021 infusion

Our proof-of-concept study (Study 1) assessed the ability of GAL021 to increase ventilation during established alfentanil-induced respiratory depression in a group of healthy male volunteers. This study was performed at clamped and elevated end-tidal carbon dioxide levels. This was done to quantify ventilatory changes without the confounding stimulatory or inhibitory effects of changes in arterial pCO_2. Application of this technique allows the assessment of the true pharmacological effect of a drug on the ventilatory control system. The observations made in Study 1 on carbon dioxide-clamped ventilation are in close agreement with observations made in Study 2 on poikilocapnic ventilation and arterial pCO_2 (Figs. 5B and C, Table 2). Since these two variables are interconnected (an increase in arterial pCO_2 stimulates breathing and

Table 2. *Effect of GAL021 on poikilocapnic ventilation, artrial pCO_2 and nonrespiratory variables (study 2)*
Values are mean (95% confidence interval). Mean difference is GAL021 – Placebo.
Basline is baseline (no drug, no added inspired CO_2), P1 is low-dose alfentanil infusion prior to any GAL021 or placebo infusion (ALF-low), P2 is combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low + GAL021-low), P3 is the combination of low-dose alfentanil with high dose GAL021 or placebo (ALF-low + GAL021-high), P4 is the combination of high-dose alfentanil with high dose GAL021 or placebo (ALF-high + GAL021-high).
VAS is visual analogue scale.

hyperventilation reduces arterial pCO_2) their evaluation is best done jointly. Combined these variables show that under "real life" conditions, a separation between GAL021 and placebo on ventilation, during alfentanil-induced respiratory depression, is observed. The data from Study 1 indicate that ventilation increases due to changes in tidal volume and respiratory rate.

Rat studies suggest that GAL021 acts mainly through an effect at the carotid bodies. GAL021 dose-dependently increases carotid sinus activity, while its effects on ventilation were diminished upon carotid body denervation.[14] The carotid bodies, located at the bifurcation of the common carotid artery, contain the peripheral chemoreceptors, which are responsible for about 30% of the tonic ventilatory drive and respond to hypoxia with a brisk hyperventilatory response.[15] Type 1 carotid body cells (which are sensitive to hypoxia) express various potassium channels (including BK_{Ca}-channels). Upon blockade, BK_{Ca}-channels release neurotransmitters that activate the sinus nerve and consequently increase respiratory drive.[16,17] In *Slo*$^{-/-}$ mice that lack various subunits of the BK_{Ca}-channel, the effects of GAL021 were severely diminished (but not abolished).[18] Jointly these data suggest that GAL021 acts through blockade of the BK_{Ca}-channel of the type 1 carotid body cells but additional mechanisms at the carotid bodies or other sites are not excluded.

HEMODYNAMICS

BK_{Ca}-channels are expressed in vascular smooth muscles and may play a role in regulating cerebral and systemic vascular tone.[19] We previously observed that doxapram produces a sharp increase in cardiac output before any effect on ventilation was apparent.[20] Apart from its effects at TASK-channels, doxapram, like GAL021, interacts with the BK_{Ca}-channel,[21] and this channel may be the site of action of the cardiostimulatory effects of doxapram.[19] In the current study, however, GAL021 was without effects on mean arterial pressure, heart rate and cardiac output. This indicates a differential effect of GAL021 and doxapram on the cardiovascular system and suggests that the BK_{Ca}-channel is not the site of action of the cardiovascular stimulatory effects of doxapram.

ANALGESIA AND SEDATION

Since GAL021 primarily acts *via* a non-opioid mechanism and the BK_{Ca}-channel does not seem to be involved in nociceptive pathways, opioid-analgesia should theoretically not be compromised. Indeed, both in animal studies and in the current study opioid-analgesia was not affected by GAL021.[7] However, our study was not powered to detect possible effects from

	Pruritus	Feeling warm/ sweating	Light-headed-ness	Nausea	Head-ache	Pain at infusion site	Limb Weak-ness	Halluci-nations	Total
	Incidence in study 1 - Incidence in study 2								
Placebo	4 - 3	1 - 1	3 - 1	6 - 1	7 - 1	0 - 0	1 - 0	0 - 0	22 - 7
GAL021	3 - 4	6 - 3	0 - 0	3 - 2	4 - 0	6 - 2	2 - 0	1 - 0	25 - 11

Table 3. *Adverse events observed in studies 1 and 2*
The first number represents the number of subjects that experience the adverse event in study 1, the second in study 2.

GAL021 on analgesia. Although the average effect of analgesia during GAL021 infusion was higher than during placebo infusion (difference +10-20%, Fig. 4F) further studies using multiple pain models are required to assess the true effect of GAL021 on opioid-analgesia. Similarly, we found no effect of GAL021 on opioid-induced sedation. This is important as some other respiratory stimulants do increase sedation.[22] Like analgesia, the absence of effects of GAL021 on sedation needs additional investigation.

SAFETY

An important part of our studies was the assessment of the safety of GAL021. In a previous study, the safety of GAL021 has been addressed in 30 healthy volunteers without the presence of another drug.[23] Apart from the observation of a burning sensation at the GAL021 infusion site, adverse events were similar between GAL021 and placebo treated subjects. This is in agreement with our observation. The observation of injection site pain could be attributed to the low pH of the GAL021 infusate (pH ≈ 3.5). We additionally observed perspiration and hot flushes in 6 (of 12) subjects receiving GAL021, especially during hypercapnia (Table 3). This suggests a sympathetic effect of GAL021 under hypercapnic conditions. Interestingly, some adverse events occurred in Study 1 but not Study 2, such as nausea and headache. We attribute this to the hypercapnic conditions of Study 1.

DOXAPRAM AND AMPAKINES

Apart from GAL021, various non-opioid respiratory stimulants are clinically available or under investigation (reviewed in references 1 and 5). One of the first agents that was developed to induced respiratory stimulation is doxapram, available since 1962.[24] Doxapram inhibits background potassium channels (TASK1, TASK 3, TASK1/TASK3 heterodimer) as well as BK_{Ca}-channels expressed on type 1 carotid body cells.[21,25] A recent study in perioperative patients showed that a 1 mg/kg bolus dose of doxapram produces modest respiratory stimulation following total intravenous anesthesia.[26] We recently tested the effect of a continuous infusion of doxapram (total dose 2.7 mg/kg given over 94 min) on alfentanil-induced respiratory depression (plasma concentration 60-100 ng/mL), using a study design similar to the current study.[20] We observed no effect on ventilation under both isohypercapnic (*i.e.* at a clamped and elevated end-tidal pCO_2) or poikilocapnic conditions at two-thirds of the maximum recommended dose. Possibly higher doxapram dosages are required to induce reversal but its side effects profile (which includes panic attacks, sympathicoexcitation (causing hypertension secondary to elevations of cardiac output), sweating, nausea, convulsions) precluded higher infusion rates than used by us. Newly developed TASK-3 antagonists showed an improved efficacy profile compared to doxapram in the rat.[25] The effect in humans has not been tested as yet.

Alternative respiratory stimulants which include 5HT and dopamine receptor ligands, while effective in animals, are without significant effect in humans.[1,5] An exception is the ampakine CX717.[22] A recent study showed that oral CX717 increased the slope of the non-steady-state ventilatory response to hypercapnia during alfentanil-induced respiratory depression without affecting analgesia in healthy male volunteers (albeit at the expense of enhanced sedation). A caveat of that study is the use a non-steady-state approach (rather than a steady-state approach as used in this study) in measuring the ventilatory response to hypercapnia prohibiting the exact quantification of opioid-induced respiratory depression (see Refs.[27,28] for a discussion on this topic). Ampakines act through activation of AMPA (α-amino-3-hydroxy-5-methyl-4-

isoxazolepropionate) receptors.[1,5] Glutamatergic transmission through AMPA receptors within the brainstem respiratory centers (most importantly the preBötzinger complex) plays a crucial role in respiratory rhythmogenesis; AMPA receptor activation leads to an increase in respiratory frequency and not the desired increase in tidal volume.

CONCLUSIONS

Our studies demonstrated the stimulatory effects of the BK_{Ca}-channel blocker GAL021 on carbon dioxide-clamped ventilation during the condition of established opioid-induced respiratory depression. In an exploratory study, GAL021 also stimulated poikilocapnic ventilation during alfentanil administration, while it had no impact on sedation, antinociception, hemodynamic or safety parameters. While our data suggest that GAL021 is an attractive alternative to other respiratory stimulants taken its observed efficacy and favorable side effect profile, the current studies are not definitive. Our studies may be used to power future studies, which should address the ability of GAL021 to reverse OIRD at higher opioid concentrations and respiratory depression induced by other agents (*eg*. anesthetics and sedatives) and drug combinations (in clinical studies).[29] Furthermore, its effect on non-respiratory systems should be explored further.

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Chapter 4

Reversal of Opioid-Induced Respiratory Depression by BK-Channel Blocker GAL021: A Pharmacokinetic-Pharmacodynamic Modeling Study in Healthy Volunteers

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INTRODUCTION

Recent publications point to the many deaths that occur in the US and elsewhere from potent prescription opioids.[1-4] Death, related to cardiorespiratory arrest, is often due to an inadvertent overdose in a setting of opioid abuse and misuse. In the US, the number of people that died from prescription opioids has tripled from 1990 to 2010 to 16,000 deaths in 2010.[3,4] Taken the large availability of opioids (opioid analgesics are the cornerstone of modern treatment of moderate to severe pain and are taken daily by a large number of patients worldwide), eradication or reduction of OIRD is important as it will reduce the number of fatalities. One possibility to reverse or reduce the probability of OIRD is to co-administer respiratory stimulants that do not compromise opioid analgesic efficacy or have other deleterious side effects (such as enhancement of sedation).[1,5]Adding the opioid receptor antagonist naloxone in oral formulation is in this respect not useful, as it will undergo rapid elimination (naloxone has a high first-pass effect) and a parenteral formulation will reduce opioid analgesic efficacy.[6] We recently showed that the experimental blocker of the calcium-activated K^+-channel (BK_{Ca}-channel) GAL021 reverses OIRD in human volunteers without having effects on opioid analgesia, sedation or the cardiovascular system.[7] GAL021 targets the BK_{Ca}-channel expressed on type 1 cells of the chemoreceptors at the carotid bodies. These and other K^+-channels at the carotid bodies play an important role in the hyperventilatory response to hypoxia. Inhibition of type 1 cell K^+-channels by low oxygen levels or by specific drugs (*eg.* inhibition of BK_{Ca}-channels by GAL021) will cause depolarization of the type 1 carotid body cell, influx of Ca^{2+}-ions through activated voltage-dependent Ca^{2+}-channels, and the release of neurotransmitters that activate the carotid sinus nerve, a branch of the glossopharyngeal nerve.[8] This cascade of events eventually results in a brisk hyperventilatory response and in case of GAL021, may alleviate underlying respiratory depression. In contrast to naloxone, GALA021 has the potential to alleviate OIRD without affecting analgesia, in both oral and parenteral formulations.

In order to further understand the behavior of GAL021, we here present a population pharmacokinetic-pharmacodynamic (PK-PD) analysis of the effect of GAL021 on OIRD. The data were analyzed with three distinct PK-PD models, one assuming a multiplicative interaction between alfentanil and GAL021 without ceiling in the effect of GAL021 on ventilation (Model 1), one assuming a multiplicative interaction with ceiling in the effect of GAL021 on ventilation (Model 2) and one assuming an additive interaction between alfentanil and GAL021 (Model 3). In Fig. 1 the differences between the models are explained graphically.

This study is a prespecified subanalysis of the previously published randomized controlled Phase 1 trial on the influence of GAL021 on alfentanil-induced respiratory depression in male volunteers.[7] The PK-PD analysis allows us to address a number of important issues. It will give an estimate of the speed of onset/offset of GAL021 in its ability to reverse OIRD. And it will provide insight in the possibility that GAL021's respiratory stimulation may hit a ceiling in efficacy due to the fact that the brainstem respiratory circuitry remains affected by the opioid at central sites.[9]

MATERIALS AND METHODS

ETHICS

After approval of the protocol by the Medical Ethics Committee of the Biomedical Research Ethics Review Foundation (BEBO, Assen, the Netherlands) and the Central Committee on Research

Involving Human Subjects (CCMO, the Hague, the Netherlands), this study was performed at Leiden University Medical Center (Leiden, the Netherlands). The study was registered in the Dutch trial register (www.trialregister.nl) under number NTR3718. Prior to participation all subjects gave written informed consent. The study was performed according to the ethical principles for medical research involving human subjects (Declaration of Helsinki, amended in 2013). The current study describes the PK-PD analysis of GAL021 reversal of alfentanil-induced respiratory depression; a descriptive analysis of the data was published previously.[7]

SUBJECTS

Twelve healthy male volunteers (aged 18-45 years, BMI 18-30 kg/m^2) were recruited to participate in the study. Exclusion criteria were a history of major medical or psychiatric diseases, a history of alcohol or drug abuse, smoking in the last year, daily consumption of caffeine greater than 6 servings and any other investigational drug administration within three months prior to inclusion. Subjects had to fast at least 6 h prior to administration of the study drugs.

STUDY DESIGN

This phase 1 study had a randomized, placebo-controlled, double blind, crossover design. In the study breath-to-breath ventilation was measured under isohypercapnic conditions and during breathing experiments blood samples were obtained for determination of alfentanil and GAL021 concentrations. All subjects were tested twice, once during administration of alfentanil and GAL021 and once during administration of alfentanil and placebo. The washout period between sessions was at least 1 week. GAL021 and placebo were randomized using a computer generated randomization schedule provided by the sponsor and were allocated in a 1:1 ratio.

Ventilation measurements. During the experiments, subjects breathed through a facemask connected to a pneumotachograph (#4813, Hans Rudolph, Kansas City, MO), which allowed accurate measurement of breath-to-breath ventilation. The pneumotachograph was connected to a computer-controlled gas mixing system from which preset inspired gas mixtures were delivered. Using the dynamic end-tidal forcing technique,[7] the inspired O_2 and CO_2 concentrations were varied, enabling the end-tidal clamping of these two gasses. The value of the end-tidal pCO_2 concentration during the clamp was such that pre-drug administration ventilation levels were 20 L/min (SD 2 L/min); the end-tidal pO_2 concentration was kept constant at a normoxic value of 110 mmHg. The inspired and expired O_2 and CO_2 concentrations were measured at the mouth with a capnograph (Datex Capnomac, Helsinki, Finland). All measured variables were visualized on a computer screen in real time during the experiments and saved on disc for further analysis.

Study drug administration. The hospital pharmacy prepared all study drugs. GAL021 (Galleon Pharmaceuticals Corp., Horsham, PA, USA), a colorless product with a pH of 3.1, and placebo (normal saline) were diluted in Ringer lactate. Study medication and alfentanil 0.5 mg/mL (Rapifen™, Janssen-Cilag BV, Tilburg, The Netherlands) were prepared on the day of the study, identified by randomization and visit numbers in an infusion bag (study medication) or syringe (alfentanil). The sequence and infusion rates of the administration of the drugs are given in Fig. 2A and Table 1.

t = - 40 min	Elevation of end tidal pCO_2; drug infusion starts upon reaching steady/state ventilation.
Time point (see figure 2)	
1. t = - 30 min	A. Alfentanil loading infusion 1.33 $\mu g.kg^{-1}.min^{-1}$ for 6 min, followed by a continuous infusion of 0.3 $\mu g.kg^{-1}.min^{-1}$1 for 74 min (aim: 25-30% decrease in ventilation). B. If the reduction in ventilation was < 25%, a second dose of 1.33 $\mu g.kg^{-1}.min^{-1}$ followed by a continuous infusion of 0.6 $\mu g.kg^{-1}.min^{-1}$. C. In case ventilation decreased by > 30%, the maintenance infusion was halved to 0.15 $\mu g.kg^{-1}.min^{-1}$.
2. t = 0 min[a]	An intravenous GAL021/placebo loading dose of 33.3 $\mu g.kg^{-1}.min^{-1}$ for 10 min, followed by a continuous infusion of 6.67 $\mu g.kg^{-1}.min^{-1}$ for 20 min.
3. t = 30 min[a]	A GAL021/placebo loading dose of 33.3 $\mu g.kg^{-1}.min^{-1}$ for 20 min, followed by 18.3 $\mu g.kg^{-1}.min^{-1}$ for 60 min.
4. t = 80 min[a]	The alfentanil infusion was increased by repeating the loading dose as given in step 1 A-C, followed by a continuous infusion with twice the infusion rate as given in steps 1A-C (aim: 50-60% decrease in ventilation)
5. t = 110 min[a]	Alfentanil and GAL021/placebo infusions are stopped.

Table 1. *Alfentanil and GAL021 infusion protocols (see also Figure 2a).*

[a] *Times are approximation and depend on the duration of step 1.*

Blood sampling and analysis. All subjects had an arterial-line in the radial artery of the non-dominant arm. At multiple time points arterial blood samples were taken to determine plasma concentrations of alfentanil and GAL021. In total, 18 alfentanil samples were taken (one baseline sample and 17 samples after administration of alfentanil at times t = -29, -28, -26, -22, -11, 1, 17, 50, 82, 86, 90, 99, 111, 120, 140, 170, and 220 min) and 15 GAL021/placebo samples were taken (one baseline sample and 15 samples at t = 5, 10, 17, 29, 40, 58, 69, 90, 111, 120, 140, 170, and 220 min).

Blood samples, taken for pharmacokinetic analysis of alfentanil, were collected in K_2EDTA tubes and centrifuged at 4 °C for 15 min at 2,500 revolution/min, followed by separation of plasma. Plasma samples were immediately stored at -20°C until analysis. Alfentanil samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) as reported previously.[7] The GAL021 pharmacokinetic samples were collected in K_2EDTA tubes and centrifuged for 10 min at 3,200 revolution/min, followed by separation of plasma. The samples were placed on wet ice and frozen at -80°C within one hour. GAL021 samples were analysed by LC-MS/MS. Calibration standards, quality controls, and incurred plasma samples were prepared by protein precipitation using acetonitrile. After centrifugation, the supernatant was evaporated and then reconstituted in injection solvent for LC-MS/MS analysis. All samples were injected onto a Luna C18(2) column (2.0 x 50 mm, 3 µm particle) (Phenomenex, Torrance, CA), eluted using gradient elution with a mobile phase consisting of mobile phase A (acetonitrile/water, 20/80, v/v) and mobile phase B (acetonitrile) containing 0.1% trifluoroacetic acid, and detected using an AB/Sciex API-4000 mass spectrometer (ABI Sciex Qtrap, Toronto, Canada). The ions were produced in the positive electrospray ionization mode and detected in multiple reaction monitoring mode using transitions at 255.2 → 182.1 for GAL-021 and 269.3 → 190.2 for internal standard (GAL021-d14), with retention times of 1.51 and 1.46 minutes, respectively. The calibration standard concentrations ranged from 0.25 to 400 ng/mL with quality controls at 0.75, 50, and 300 ng/mL. The calibration curves were linear with a lower limit of quantification of 0.250 ng/mL and correlation coefficients > 0.995. The method precision (%CV) and accuracy

(%RE) were less than 10% for all of quality control sample concentrations.

PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) ANALYSIS

The population pharmacokinetics and pharmacodynamics of alfentanil and GAL021 were determined in two stages using NONMEM version 7.3.0 (software for nonlinear mixed effects modeling; ICON Development Solutions, Hanover, MD, United States).[10]

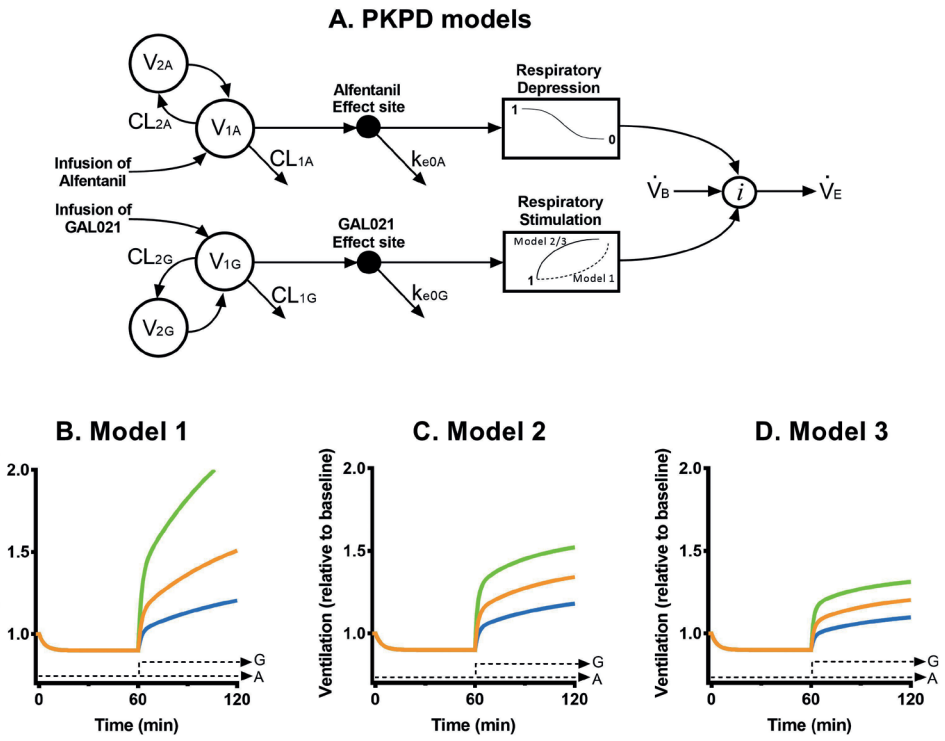

Figure 1. A. *Schematic representation of the pharmacokinetic (PK) nad pharmacodynamic (PD) models used to analyse the effects of alfentanil (A) and GAL021 (G) on ventilation. V_x is the volume of PK compartment x, CL_x clearance from PK compartment X, k_{eOA} the rate constant of equilibration of the effect compartment with the plasma compartment for alfentanil, k_{eOG} the rate constant of equilibration of the effect compartment with the plasma compartment for GAL021, VB baseline ventilation and VE minute ventilation. Depression of ventilation by alfentanil is described by a sigmoid E_{MAX} model. Stimulation of breathing by GAL021 is either modeled by a power model (Model 1) or a sigmoid E_{MAX} model (Models 2 and 3). Alfentanil and GAL021 interact on ventilation in either a multiplicative fashion (Models 1 and 2) or in an additive manner (Models 3) as depicted by i.* ***B-D.*** *Simulations to exemplify the behavior of the three models. A mild 10% depression of ventilation is simulated. At t = 60 minutes, three constant GAL021 infusions are started (without an initial bolus infusion): 0.035, 0.07 and 0.14 $mg.kg^{-1}.min^{-1}$ (blue, orange and green lines). Model 1 behaves with a dose-dependent increase in effect, whereas the effect of Models 2 and 3 hits a ceiling thereby restricting the ventilatory stimulation. The difference between Models 2 and 3 is that in Model 2 (as well as Model 1) the ventilatory effect is dependent on baseline ventilation, whereas for Model 3 there is a fixed increase in ventilation independent of baseline.*

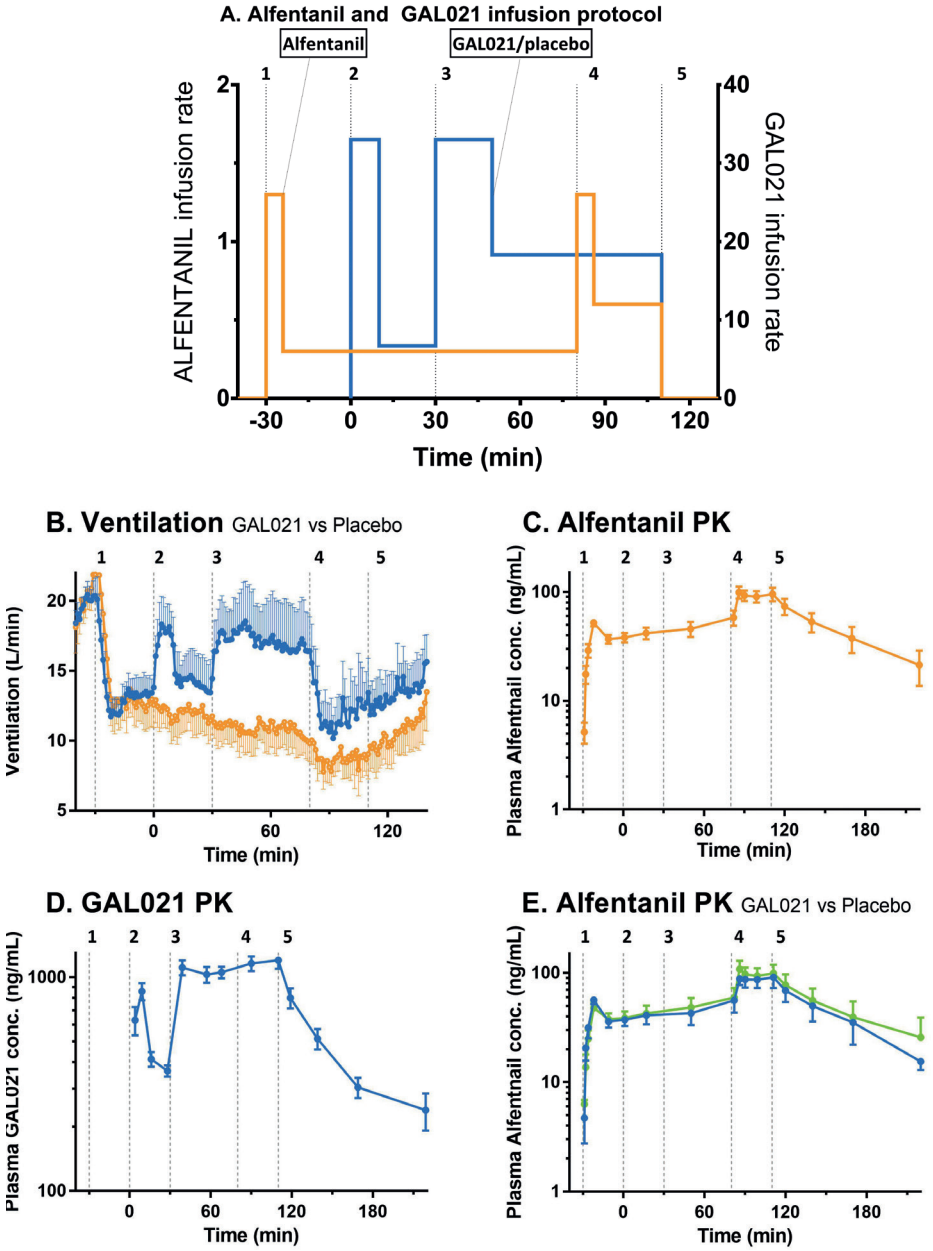

Figure 2. A. *Protocol of alfentanil and GAL021 infusions.* ***B.*** *Measured ventilation during infusion of alfentranil and GAL021 (blue) or placebo (orange).* ***C.*** *Mean plasma alfentanil concentrations (data of both GAL021 and placebo arems are included).* ***D.*** *Plasma GAL021 concentrations.* ***E.*** *Plasma alfentanil concentration in the GAL021 arm (blue symbols) and placebo arm (green symbols) of the study., The data are mean ± 95% confidence interval.*

Pharmacokinetic analysis. In the first stage, the PK data of the GAL021 and alfentanil were characterized separately. Multiple compartment models were fitted to the GAL021 and alfentanil plasma concentration data, with and without covariate weight. The number of compartments in the pharmacokinetic analysis was determined by the magnitude of the Minimum Objective Function Value (MOFV; χ^2-test).

Pharmacodynamic analysis. To eliminate possible hysteresis between the alfentanil and GAL021 plasma concentrations and putative effect-sites, two separate effect compartments for alfentanil and GAL021 were postulated with blood-effect-site equilibration half-lives $t_{1/2}k_{eOA}$ and $t_{1/2}k_{eOG}$, respectively. In the second stage, population pharmacodynamic model parameters were determined with fixed individual pharmacokinetic model parameters. Three distinct pharmacodynamic models were tested:

Model 1 assumes that alfentanil and GAL021 interact in a multiplicative fashion on ventilation (Fig. 1):[11]

$$VE(t) = VB \cdot EFA(t) \cdot EFG(t) \quad (1)$$

where VE is minute ventilation at time t, VB pre-drug baseline ventilation, EFA the effect of alfentanil on ventilation and EFG the effect of GAL021 on ventilation. For the effect of alfentanil on ventilation a sigmoid EMAX model was applied:[11,12]

$$EFA(t) = 1 - \left[CE_A(t)^{\gamma_A}/\left(CE_A(t)^{\gamma_A} + C_{50A}{}^{\gamma_A}\right)\right] \quad (2)$$

where CE_A is the alfentanil effect-site concentration, C_{50A} the alfentanil effect-site (or steady-state) concentration causing a 50% depression of ventilation and γ_A the shape or Hill parameter for alfentanil. For the effect of GAL021 on ventilation a power model was applied:[11,13]

$$EFG(t) = 1 + 0.25 \cdot \left(CE_G(t)/C_{25G}\right)^{\gamma_G} \quad (3)$$

where CE_G is the GAL021 effect-site concentration, C_{25G} the GAL021 effect-site (or steady-state) concentration causing 25% increase in ventilation and γ_G the shape or Hill parameter for GAL021.

Model 2 assumes that alfentanil and GAL021 interact in a multiplicative fashion on ventilation as described in equations 1 and 2, but the effect of GAL021 on ventilation an EMAX model was applied:[11,13]

$$EFG(t) = 1 + EMAX_G \cdot \left[\left(CE_G(t)/C_{25G}\right)^{\gamma_G}\right]/\left[4 \cdot EMAX_G - 1 + \left(CE_G(t)/C_{25G}\right)^{\gamma_G}\right] \quad (4)$$

where $EMAX_G$ is the maximum possible effect of GAL021 on ventilation.

Model 3 assumes that alfentanil and GAL021 interact in an additive fashion on ventilation:[11]

$$VE(t) = VB \cdot EFA(t) + EMAX_G \cdot \left[\left(CE_G(t)/C_{25G}\right)^{\gamma_G}\right]/\left[3 + \left(CE_G(t)/C_{25G}\right)^{\gamma_G}\right] \quad (5)$$

and for the effect of alfentanil on ventilation a sigmoid EMAX model was applied according

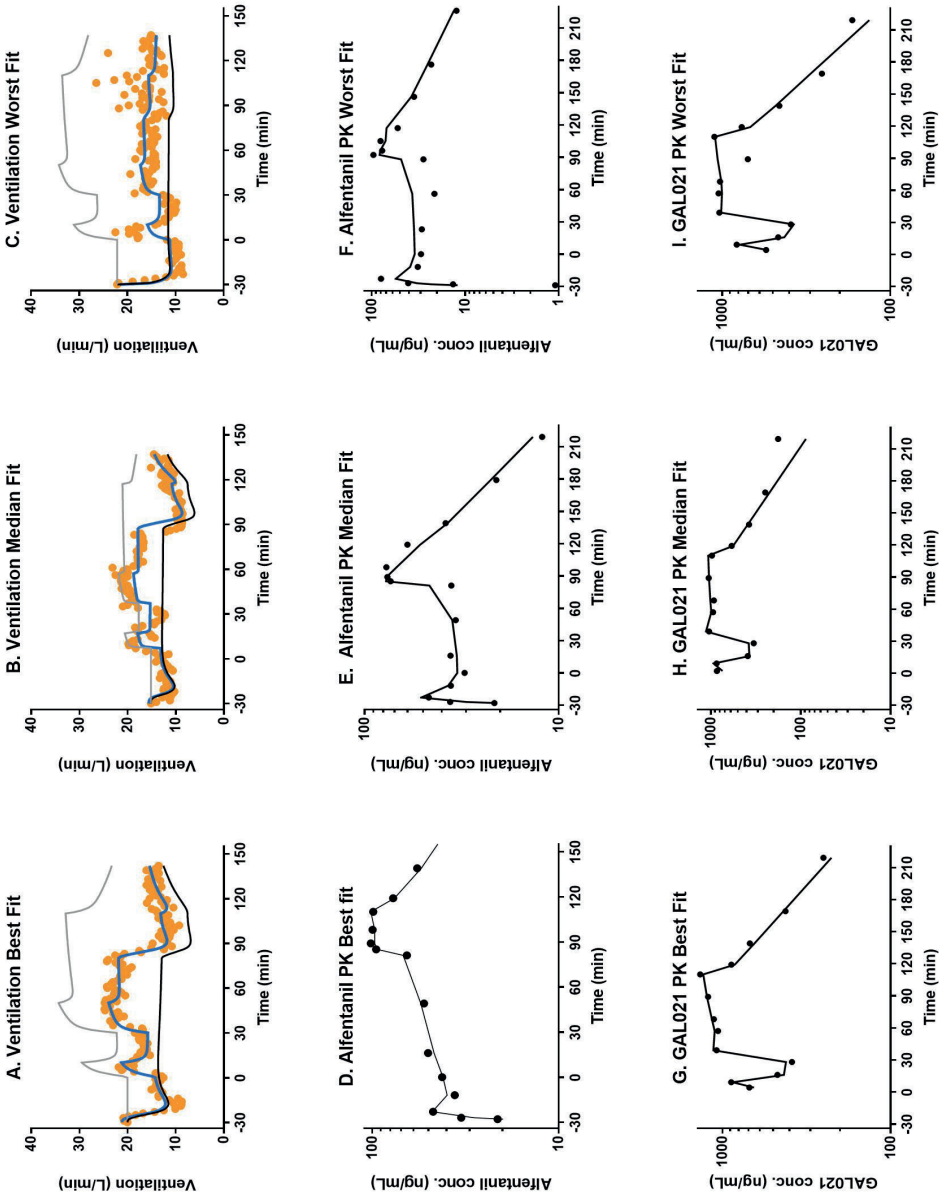

Figure 3. *Effect of GAL021 on ventilation. Best* ***(A)****, median* ***(B)*** *and worst* ***(C)*** *data fits. The orange dots are measured ventilation data points, the blue lines are individual predicted ventilation, the black thin lines are the alfentanil contribution to the predicted ventilation (EFA) and the gray lines are the GAL021 contribution to the predicted ventilation (EFG). Alfentanil/placebo ventilation data fits are not shown.* ***(D)****,* ***(E)*** *and* ***(F)*** *are best, median and worst alfentanil pharmacokinetic (PK) data fits, respectively.* ***(G)****,* ***(H)*** *and* ***(I)*** *are the best, median and worst GAL021 PK data fits respectively. Goodness of fit was based on the coefficient of determination.*

Drug	Parameter	Estimate ± SEE	ω² ± SEE
PK model parameters			
Alfentanil	V_1 (L)	8.49 ± 0.35	0.02 ± 0.007
	V_2 (L)	11.2 ± 0.55	[a]
	CL_1 (L/min)	0.30 ± 0.01	
	CL_2 (L/min)	0.59 ± 0.05	[a]
	σ_1	0.09 ± 0.02	
	σ_2	5.96 ± 0.07	
GAL021	V_1 (L)	6.72 ± 1.92	
	V_2 (L)	43.4 ± 1.55	[a]
	CL_1 (L/min)	1.02 ± 0.04	
	CL_2 (L/min)	2.20 ± 0.12	[a]
	σ_1	0.09 ± 0.01	
	σ_2	44.3 ± 6.02	
PD model parameters			
	VB (L/min)	21.5 ± 0.65	0.002 ± 0.010
	σ	1.97 ± 0.12	
Alfentanil	$t_{1/2}k_{eOA}$ (min)	2.72 ± 0.71	[a]
	C_{50A} (ng/mL)	54.4 ± 6.8	0.31 ± 0.15
	Y_A	1.0[b]	0.33 ± 0.10
GAL021	$t_{1/2}K_{eOG}$ (min)	0	[a]
	C_{25G} (ng/mL)	600 ± 8	[a]
	$EMAX_G$	1.16 ± 0.19	[a]
	Y_G	1.0[b]	0.32 ± 0.12

Table 2. *Pharmacokinetic and Model 2 pharmacodynamic parameter estimates*
Subscript A denotes alfentanil, subscript G GAL021. V; volume, CL; clearance, ω^2; variance of the model parameters across the population (in the log domain), σ_1; standard deviation of the proportional error (PK data), σ_2; standard deviation of the additive error (PK data), VB; baseline ventilation, σ; the standard deviation of the additive error (PD data), $t_{1/2}k_{eO}$; the blood effect-site-equilibration half-life, C_{50A}; the effect-site alfentanil concentration causing 50% respiratory depression, C_{25G}; the effect-site GAL021 concentration causing 25% increase in ventilation. $EMAX_G$; the maximum possible effect of GAL021 on ventilation. Y; a shape parameter. PK; pharmacokinetic, PD; pharmacodynamic, SEE; standard error of estimate.
[a] Not estimable, [b] not significantly different from 1.

to equation 2 and for the effect of GAL021 on ventilation a model with ceiling was applied according to equation 4.

Statistical analysis. For both PK and PD analyses, the model parameters were assumed to be log-normally distributed across the population. Residual error was assumed to have both an additive and a relative error for concentrations and only an additive error term for ventilation. Model discrimination was based on the magnitude of the Minimum Objective Function Value

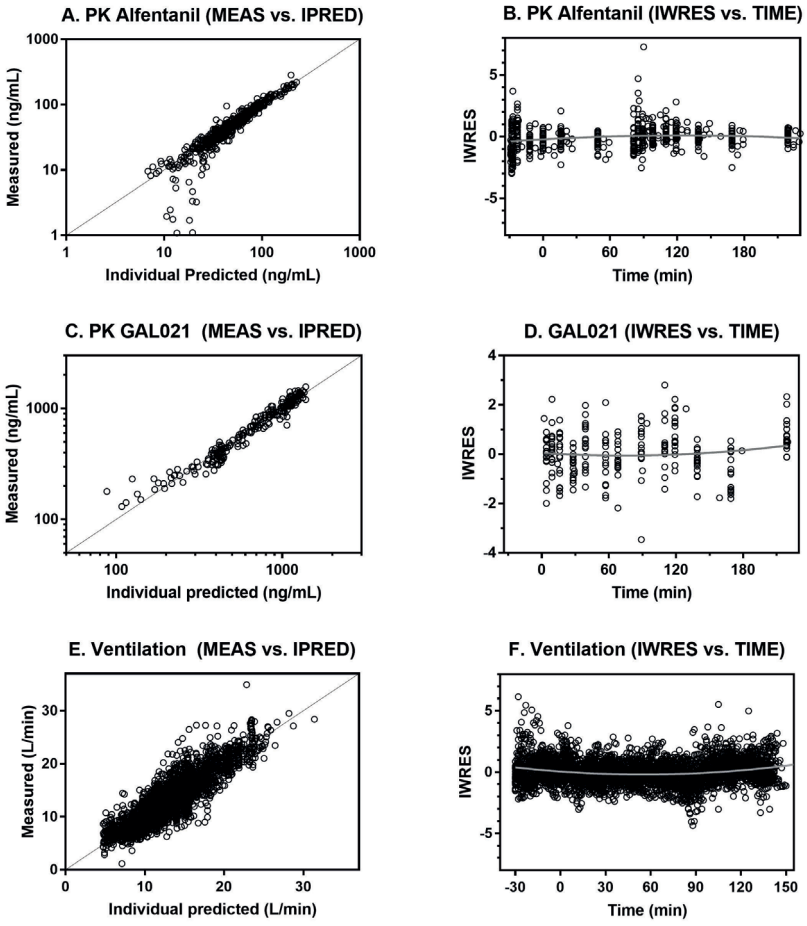

Figure 4. *Goodness-of-fits plots.* ***A*** *and* ***B*** *alfentanil pharmacokinetic (PK) analysis;* ***C*** *and* ***D*** *GAL021 PK analysis;* ***E*** *and* ***F*** *ventilation. MEAS is measured data, IPRED is the individual predicted values and IWRES is the individual weighted residuals. Through the IWRES vs time data a smoothin curve was fitted (gray lines).*

(MOFV; χ^2-test). p-values < 0.01 were considered significant. All values are median ± SE unless otherwise stated.

Standardized visual predictive check (SVPC). Standardized visual predictive checks were performed to evaluate the adequacy of the description of both fixed and random effects by simulating data using the model. The SVPC displays the fraction of the observations of each subject in the marginal distribution of the corresponding data simulations (concentration or ventilation) as a function of time, based on that subject's own drug administration schedule.[13]

RESULTS

A complete data set was obtained in twelve subjects. There were no unexpected side effects. The median age (range) of the subjects was 22.0 (19-34) years, the median weight 74.3 (60.7-84.3) kg and body mass index 22.1 (19.0-26.5) kg/m^2. The alfentanil and GAL021/placebo infusion protocol and mean ventilatory responses following alfentanil and GAL021/placebo administrations are shown in Figs. 2A and B. The difference in GAL021 *vs.* placebo responses indicates an appreciable reversal effect of GAL021 on alfentanil-induced respiratory depression.

PHARMACOKINETIC ANALYSIS

Mean alfentanil and GAL021 plasma concentrations are shown in Figs. 2C-E. Peak alfentanil concentrations reached during low- and high-dose infusion were 51.9 ± 1.8 (mean ± SD) and 99.2 ± 6.4 ng/ml, respectively. Peak GAL021 concentrations were 859 ± 40 and 365 ± 11 ng/mL during low-dose bolus and maintenance, and 1110 ± 46 and 1201 ± 54 ng/mL during high-dose bolus and maintenance, respectively. GAL021 had no significant effect on the alfentanil plasma concentrations (*t*-test: $p > 0.05$), but a small reduction in the plasma concentrations during GAL021 infusion cannot be excluded. The final PK models for both GAL021 and alfentanil consisted of a two-compartment model with one central (V_1) and one peripheral compartment (V_2). Covariate weight did not improve the GAL021 and alfentanil PK fits. The pharmacokinetic parameter estimates are displayed in Table 2. Best, median and worst alfentanil and PK data

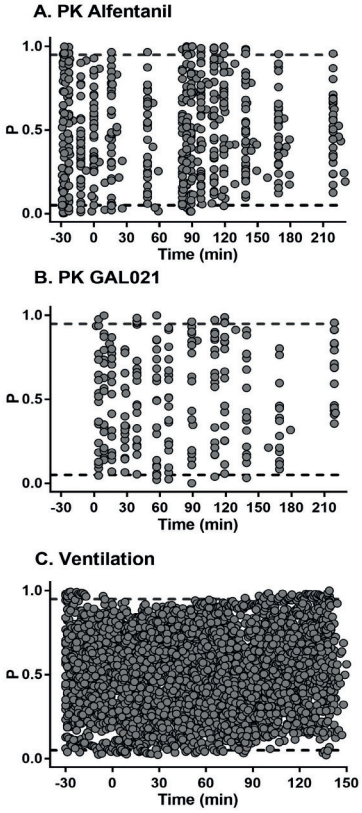

Figure 5. *Standardized visual predictive checks for alfentanil pharmacokinetic (PK) data* ***(A)****, the GAL021 PK data* ***(B)*** *and ventilation* ***(C)****. The broken lines represent the 2.5 and the 97.5% confidence limits. For all three measures, the 95% of the data lies within the 95% confidence intervals. P is probability.*

fits are given in Fig. 3. Goodness of fit plots are shown in Fig. 4. Inspection of the individual data fits and the goodness of fit plots indicate that the pharmacokinetic data are adequately described by the GAL021 and alfentanil PK models. A small misfit is, however, visible at the lower concentration ranges (occurring immediate after the start of infusion or following changes in infusion rate), where the predicted alfentanil concentrations are overestimated, predominantly just after the first administration, while GAL021 predicted concentrations are underestimated at the last sample times.

PHARMACODYNAMIC ANALYSIS

Alfentanil-induced respiratory depression ranged from 33% (low-dose) to 55% (high-dose) during administration of placebo; GAL021 effectively reversed these effects (Fig. 2B). Pharmacodynamic model selection was based on the Minimum Objective Function (MOF) value. MOF values favored the model in which GAL021 shows ceiling in its efficacy to reverse respiratory depression (Model 2) above the model that shows no ceiling (Model 1) with MOF values for Model 2 of 10364 versus Model 1 of 10379 ($p < 0.01$). The multiplicative model (Model 2) was superior in terms of MOF values to the additive Model 3 (MOF value 10405). The results of the analysis with Model 2 will be presented here.

Best, median and worst ventilation data fits (based on the coefficient of determination) are shown in Fig. 3 for the data derived from the GAL021 arm of the study (the placebo fits are not shown). In panels #A-C, the measured (closed symbols) and predicted V_E (blue lines) are given together with the two contributions to predicted effect, EF_A (alfentanil contribution, black lines) and EF_G (GAL021 contribution, grey lines). Inspection of the individual fits and the goodness of fit plots (Figs. 3 and 4) indicate that the model adequately describes the data. Estimated model parameter values (estimate ± SE) are given in Table 2. The effect of alfentanil on ventilation was rapid with a value for $t_{1/2}k_{eOA}$ of 2.7 ± 0.7 min and C_{50} of 54.4 ± 6.8 ng/mL. GAL021 produced a median increase in ventilation of 25%, which occurred at an effect-site or steady-state concentration of 600 ± 8 ng/mL. The value of GAL021's $t_{1/2}k_{eOG}$ was not significantly different from zero.

STANDARDIZED VISUAL PREDICTIVE CHECK (SVPC)

SVPCs are shown in Figure 5. With alfentanil and GAL021 inputs similar to those used in the current study, the accuracy of the PKPD model was acceptable with 95% of the data points within the 2.5 to 97.5 percentiles.

DISCUSSION

GAL021, a blocker of the BK_{Ca}-channel at the carotid bodies, effectively reverses OIRD induced by the potent μ-opioid alfentanil. Here we show that the interaction between GAL021 and alfentanil is best described by a pharmacodynamic model consisting of two parts that interact in a multiplicative fashion: a sigmoid E_{MAX} function that describes the inhibitory effects of the opioid on breathing and a function that describes the stimulatory effects of the K^+-channel blocker. The model predicts that GAL021 displays ceiling in its efficacy to reverse OIRD. We further observed that the GAL021 onset/offset times are rapid with a blood-effect site equilibration half-life not different from zero.

In current clinical practice, reversal of OIRD is by administration of naloxone. Naloxone is a

non-selective competitive antagonist at the μ-opioid receptor with fast receptor association and dissociation kinetics. Following intravenous administration, naloxone rapidly enters the brain compartment and causes a brisk reversal of opioid activity at the respiratory centers in the brainstem.[14] Albeit effective, naloxone has various disadvantages including reversal of analgesia, activation of the sympathetic system and risk of renarcotization.[1] Multiple respiratory stimulants have been developed that may serve as attractive alternatives to naloxone as they are devoid of effect at the opioid receptors.[5] This is advantageous for two reasons. Apart from not interfering with analgesia, such agents are not dependent on opioid-receptor kinetics in their ability to reverse OIRD. Some opioids (*eg.* buprenorphine and to a lesser extent morphine) have slow receptor kinetics, which reduces the probability and speed of their reversal in case of a serious OIRD and therefore require relatively high naloxone doses or a continuous naloxone infusion.[1,14]

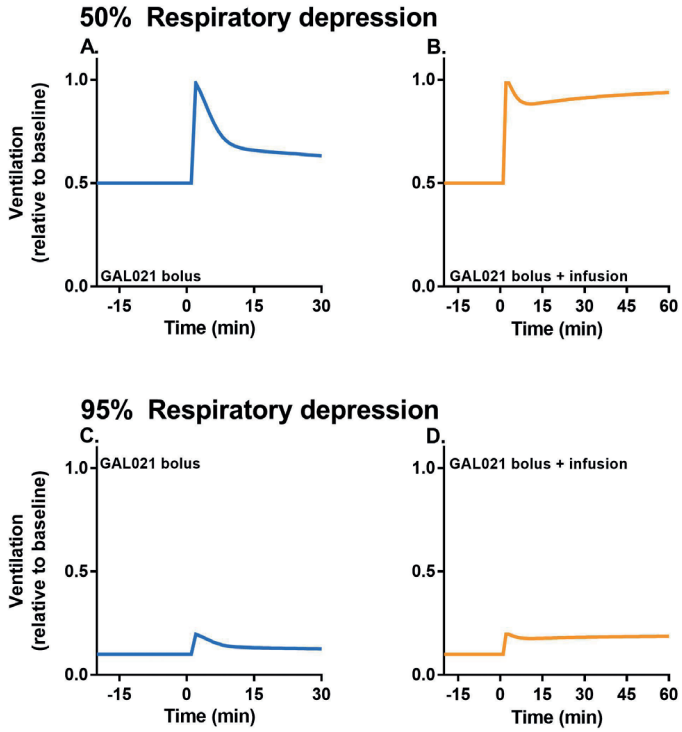

Figure 6. *Simulation of the effect of bolus and combined bolus and continuous GAL021 infusions on alfentanil-induced repsiratory depression. In these simulations, the effects of GAL021 on moderate (50%, **A** and **B**) and severe (90%, **C** and **D**) respiratory depression are simulated. At t = 0, the effect of a GAL021 bolus of 1.3 mg/kg (**A** and **C**) or a bolus of 1.3 mg/kg followed by a continuous infusion of 0.14μg.kg^{-1}.min^{-1} (**B** and **D**) are simulated. GAL021's bolus effects are rapid and require a continuous infusion of a persistent effect. The multiplicative nature of the interaction between alfentanil and GAL021 causes the difference in GAL021 ventilatory effect when reversing severe or moderate opioid-induced respiratory derepssion (OIRD).*

Most effective non-opioid agents developed to prevent or treat OIRD in humans include drugs that increase glutamatergic neurotransmission at brainstem respiratory centers (ampakines including CX717) and potassium channel blockers acting at the peripheral chemoreceptors of the carotid bodies (GAL021, doxapram, almitrine).[1,5,9,15] Drugs acting at the peripheral chemoreceptors through blockade of K^+-channels mimic the effect of hypoxia, which exclusively acts at the carotid bodies. The afferent information from the carotid bodies is translated into an increased ventilatory drive in brainstem respiratory centers. Since opioids depress the hypoxic drive at these same respiratory centers,[2,16] the efficacy of drugs acting via carotid body stimulation may be limited in effect and could possibly results in a ceiling in ability to reverse OIRD.[9] Indeed, the results from our modeling study suggest ceiling in reversal of OIRD by GAL021. Based on the MOF values, the multiplicative model with ceiling in GAL021 effect (Model 2) was deemed best and hence was assumed the most probable model to describe GAL021 reversal of OIRD. Further studies at higher GAL021 doses are needed to confirm the model-based prediction of ceiling.

Since GAL021 interacts in a multiplicative manner with alfentanil (*i.e.* that the amount of reversal is dependent on the magnitude of alfentanil-induced respiratory depression) the model predicts that at extreme levels of OIRD (for example opioid-induced apnea), the effect of GAL021 is limited (*i.e.* multiplication factor < 1). See also Fig. 6. If true, reversal must then be induced by other means or the patient should be ventilated. Model 1 was attempted initially as in a previous study of GAL021 in volunteers there were suggestions of a linear doses response relationship from 600 ng/mL on.[17] However, in that study no opioids were administered. This implies that the ventilatory sensitivity of GAL021 is altered in narcotized compared to non-narcotized subjects. This may be related to the central depressant effects of opioids that remain unaffected by GAL021 or due to the presence of a CO_2-clamp in the current study. Our analysis gives a clear indication of the site of action of GAL021. The blood-effect site equilibration half-life was not different from zero, which indicates that GAL021 acts at a site close to the arterial vascular bed (*i.e.* the sample site). The carotid bodies are located at the bifurcation of the common carotid arteries and are highly perfused with arterial blood through branches of the external carotid artery.[18] The concentration of GAL021 at the carotid body receptor site will therefore be close to that of arterial blood. These findings are in agreement with animal studies showing that in carotid body denervated rats and in BK-channel knockout mice the effect of GAL021 is greatly reduced.[19,20]

To better understand the properties of GAL021 as described by Model 2 we performed simulations on the effect of GAL021 infusion on alfentanil-induced moderate (50%, Figs 6A and B) and severe (90%, Figs. 6C and D) respiratory depression. In these simulations the alfentanil infusion is continued when GAL021 is infused, to mimic the effect of long-acting opioids such as fentanyl, buprenorphine and morphine. The onset of effect of a GAL021 bolus is rapid but respiratory stimulation dissipates within 5-10 min. To ensure a continuous effect, a bolus combined with an infusion is required. Given the multiplicative nature of the alfentanil GAL021 interaction, GAL021 has less effect at severe OIRD (compare panels A and C, B and D); higher infusion rates have then limited effect.
The rapid onset of action of GAL021 makes it an attractive alternative to naloxone in the treatment of OIRD. Further studies, however, are needed to assess whether GAL021 is able to reverse severe levels of OIRD such as apnea. Also naloxone is not always able to reverse

OIRD and in cases of severe OIRD artificial ventilation to overcome the period of OIRD is often the best option.[6,21] GAL021 has the potential to be used as long-term respiratory support following an opioid respiratory event, in the treatment of less severe respiratory depression, and as concomitant administration in a combination therapy, aimed to reduce the probability of OIRD without affecting analgesia. However, it is then important to know whether tolerance would develop to multiple doses of GAL021. Animal data show that there is no diminution in effect during long-term infusion (Galleon Pharmaceuticals Corp., data on file). GAL021 has 15-35% bioavailability (animal data on file); an oral compound, GAL160, is currently in preclinical development for the hospital and post discharge setting (up to 14-28 days). Naloxone remains useful as diagnostic as well as a therapeutic agent in severe OIRD.
Our PK analysis had some strong and weak points. A strong point is the use of arterial blood samples. Using arterial rather than venous samples has the advantage of obtaining more reliable PK and PD data fits.[22] A weakness of the study is the characterization of the disposition of alfentanil and GAL021 with two- rather than with three-compartment models.[23] This resulted in PK misfits: the goodness-of-fit plots show that at low concentrations the PK models overestimate the alfentanil concentrations and underestimate the GAL021 concentrations (Figs. 4A and C). For alfentanil, this occurred after the start of infusion and upon changes in infusion regimen, for GAL021 only at the last sample times. We relate the failure to characterize three-compartment PK models to the paucity in the PK data when concentrations are changing rapidly,[23] *i.e.* after administration of a loading dose (for alfentanil) and after discontinuation of an infusion (for GAL021). The parameter standard errors estimated with a three-compartment model were unacceptable (>100% coefficient of variation) for both drugs. We consider the small misfits in a minority of data not of major significance.

We evaluated the performance of the PK-PD model (Model 2) using a standardized visual predictive check (SVPC; Fig. 5). Visual predictive checks address the adequacy of fixed and random effects. Due to the variation in dosing among subjects in our study, the conventional VPC is not useful. We therefore applied the SVPC, which provides similar information as a VPC, but displays observations and predictions on a standardized scale.[24] As observed in Fig. 6, the SVPCs of the PK and PD data show that the simulated data are evenly distributed across the percentile of observations over time (P in Fig. 6, ranging from 0 to 1). This indicates that the model is an adequate descriptor of the data.

In conclusion, we performed a population PK-PD analysis of the reversal of alfentanil-induced depression of breathing by the potassium-channel blocker GAL021. GAL021 interacted in a multiplicative fashion with the opioid. Specific model predictions such as the presence of ceiling in GAL021's efficacy to reverse respiratory depression and the inability of GAL021 to reverse deep levels of respiratory depression need confirmation in future studies.

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Chapter 5

Benefit versus toxicity of opioid analgesia: novel utility functions of analgesia and respiratory depression

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INTRODUCTION

Opioid analgesics, especially those opioids that are full agonists at the μ-opioid-receptor, have a high likeability, rendering them high-risk drugs of abuse, misuse and eventually addiction.[1,2] Equally important, μ-opioid receptors are abundantly expressed on brainstem respiratory neurons and activation of these receptors is associated with bradypnea, hypoventilation and apnea.[3] This is especially true when the opioid is overdosed or used in combination with other centrally acting depressant drugs such as alcohol, benzodiazepines or other illicit substances.[4] It is currently well understood that the combination of misuse/abuse and respiratory depression is a serious threat especially to a society in which opioid prescriptions for non-cancer-related pain reached epidemic proportions.[5,6] For example, in 2013 in the Netherlands, 1 million individuals (on a population of 17 million) received a prescription for opioid treatment, a doubling since 2004.[7] There are no numbers of opioid deaths in the Netherlands but in the US prescription opioids have been implicated in 165,000 deaths since 1999.[6] Also in the acute setting such as postoperative care, opioid analgesics are associated with cardiorespiratory arrest and mortality.[3,8-10]

Despite these alarming numbers, opioid analgesics remain the cornerstone of modern medicine in the treatment of moderate to severe acute and chronic pain. The main reason is their high efficacy and current lack of adequate alternatives. Presently, novel opioid drugs are being developed with specific modes of action (*e.g.,* acting at multiple receptor systems that offset respiratory depression or so called "biased ligands" that circumvent the opioid respiratory pathway) that possibly have advantages over full μ-opioid-receptor agonists with less risk of respiratory depression.[11,12] There are various methods that allow assessment of opioid safety. For example, data obtained from post-marketing studies give some indication of safety in large patient populations. However, such studies are available only after development and registration. We propose the development and use of utility or safety functions to determine opioid toxicity (*e.g.*, respiratory depression but it may be any negative outcome such as sedation, dizziness, etc.) in light of opioid benefit (analgesia).[13,14] These utility functions may be created and reviewed early on in development (in phase 1 or 2 trials) and allow an objective and reliable characterization of the opioid benefit and risk to determine an optimal dosing regimen.[14] Additionally, utility functions may be used to compare drugs and establish which drug has a better benefit-risk behavior over the other in specific patient populations.[15]

In this study, we further developed the concept of the utility function by calculating probabilities of benefit and risk and calculate the probabilities of four distinct conditions: the probability of presence of benefit (adequate analgesia) with or without toxicity (respiratory depression, respectively) and the probability of inadequacy of benefit (inadequate analgesia) with or without toxicity (respiratory depression). We calculated these probabilities for the μ-opioid receptor agonist alfentanil by analysis of data derived from three different previously published data sets. Probabilities were calculated as function of concentration and as function of time following a bolus infusion.

MATERIALS AND METHODS

STUDY DESIGN

The construction of the alfentanil utility function is based on three different study protocols where the effects of alfentanil on ventilation and/or analgesia were analyzed. Part of the results of these three studies have been published previously.[16-18] In two studies (studies 1 and 2) both alfentanil-induced respiratory depression and antinociception were measured in the same subjects,[17,18] in one study (study 3) just alfentanil-induced antinociception was measured.[16] In studies 1 and 2 breath-to-breath ventilation data were collected at isohypercapnia using the "end-tidal forcing" technique.[17,18] The end-tidal pCO_2 was increased producing a ventilation level of 20 ± 2 L/min after which alfentanil was infused; during infusion the end-tidal pCO_2 was maintained at baseline level. The technique is described elsewhere in detail.[19] In brief, subjects breathed through a facemask attached to a pneumotachograph and pressure transducer system (Hans Rudolph Inc., Kansas City, MO, USA) and to three mass flow controllers (Bronkhorst High Tech, Veenendaal, The Netherlands) for the delivery of oxygen, carbon dioxide and nitrogen. All three controllers received input using custom made RESREG/ACQ software. Inhaled and exhaled oxygen and carbon dioxide partial pressures were measured at the mouth using a capnograph (Datex Capnomac, Helsinki, Finland). In studies 1-3, antinociception (or analgesia) was measured using an electrical pain model.[16-18] Two electrodes were placed on the skin over the shinbone of the right leg, 2 cm apart and 10 cm above the lateral malleolus. A custom made computer-interfaced current stimulator (Leiden University Medical Center, Leiden, the Netherlands) generated a current, which increased with 0.5 mA/s and started at 0 mA. The subject had to indicate on a control box when pain threshold (first pain was observed) and pain tolerance (pain could not be tolerated any further) were reached. Pressing the button for pain tolerance ended the stimulus train.

Design of study 1 (a study on the influence of respiratory stimulant GAL021 on alfentanil-induced respiratory depression and antinociception).[17] Subjects were dosed twice on separate visits, once with GAL021 and once with placebo; only the placebo data are included in the current analysis. Alfentanil was administered using a stepped drug infusion design. After reaching isohypercapnic steady-state ventilation, an initial intravenous infusion of 1.33 $\mu g.kg^{-1}.min^{-1}$ for 6 min was given (loading dose), followed by a continuous infusion of 0.3 $\mu g.kg^{-1}.min^{-1}$ for 104 minutes. This was done to achieve a 25-30% decrease in ventilation. If the reduction in ventilation was less than 25%, a second dose of 1.33 $\mu g.kg^{-1}.min^{-1}$ was given and the subsequent continuous infusion was increased to 0.6 $\mu g.kg^{-1}.min^{-1}$. If the loading dose caused ventilation to decrease by more than 30%, the continuous infusion was reduced to 0.15 $\mu g.kg^{-1}.min^{-1}$. Next (110 min after the start of infusion), another loading dose of 1.33 $\mu g.kg^{-1}.min^{-1}$ alfentanil was given for 6 min followed by a continuous infusion, which was twice the earlier infusion dose. After another 30 min, the alfentanil infusion ended. Total infusion time was 140-146 min. Arterial blood samples were taken a t = 0 (baseline), 1, 2, 4, 8, 19, 31, 47, 80, 112, 116, 120, 129, 141, 170, 200 and 250 min; t = 0 is start of alfentanil infusion.

Design of study 2 (a study on the influence of respiratory stimulant doxapram on alfentanil-induced respiratory depression and antinociception).[18] All subjects were dosed twice on separate visits, once with doxapram and once with placebo; only the placebo data are included in the current analysis. The study protocol was in accordance with study 1, except for differences

in infusion regimen and sample times. In this protocol, the loading dose was 8 $\mu g.kg^{-1}.min^{-1}$ for 2 min, followed by a maintenance infusion of 0.6 $\mu g.kg^{-1}.min^{-1}$ for 98 min. Hereafter, the loading dose was repeated and maintenance infusion was increased to 0.9 $\mu g.kg^{-1}.min^{-1}$ for 30 min. Total infusion time was 132 min. Arterial blood samples were taken at t = 0 (baseline), 1, 2, 4, 5, 7, 8, 10, 19, 31, 40, 47, 59, 80, 88, 99, 101, 102, 104, 105, 107, 110, 119, 131, 140, 155 and 170 min.

Design of study 3 (a pharmacokinetic-pharmacodynamic modeling study on alfentanil-induced antinociception).[16] All subjects were dosed twice on separate visits, once with alfentanil and once with placebo; only the alfentanil data are included in the current analysis. Subjects received a target-controlled-infusion of alfentanil with plasma concentration targets of 50 ng/mL for 10 min, followed by 100 ng/mL for another 10 min and 150 ng/mL for a final 10 min. Antinociception was measured during and for 270 min following infusion. Arterial blood samples were obtained at t = 0 (baseline), 3, 5, 9, 13, 15, 19, 23, 25, 29, 31, 33, 35, 38, 43, 53, 60, 75, 90, 120, 150, 180, 240 and 300 min.

PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS

The population pharmacokinetics (PK) and pharmacodynamics (PD) of alfentanil were determined in two stages using NONMEM version 7.3.0 (software for non-linear mixed effects modeling; ICON plc, Gaithersburg, MD).[20] In the first stage, the PK data were analyzed with a three-compartment model based on previous analyses.[16-18] To eliminate a possible hysteresis between alfentanil plasma concentration and effect site, an effect compartment was postulated with blood-effect site equilibration half-life $t_{1/2}k_{eO}$. In the second stage, population PD model parameters were determined with fixed individual PK model parameters as determined in the first stage.

Ventilation data were modeled by:[13,17]

$$V(t) = V_B \cdot [1 + (C_E(t)/C_{50V})^{Y_v}]^{-1} \quad (1)$$

where the V(t) is the ventilation at time t, V_B baseline ventilation, $C_E(t)$ is the effect-site concentration at time t, C_{50V} the effect-site concentration producing a 50% decrease in ventilation and y_v the shape or Hill parameter for ventilation.

Transcutaneous electrical pain responses were modeled by:[13,16]

$$P(t) = P_B \cdot [1 + 0.5 \cdot (C_E(t)/C_{50A})^{YA}] \quad (2)$$

Where the P(t) is the pain stimulus intensity at which a pain tolerance response occurs at time t, P_B is the pre-drug or baseline stimulus intensity at which pain tolerance response occurs, $C_E(t)$ is the effect-site concentration at time t, C_{50A} the effect-site concentration producing a 50% increase in stimulus intensity and y_A the shape or Hill parameter for analgesia.
Model parameters were assumed to be log-normally distributed. Residual error was assumed to contain an additive and a relative error for concentration and an additive error for effect parameters.

Utility Functions. The utility functions (U) were calculated as previously described.[13] In brief,

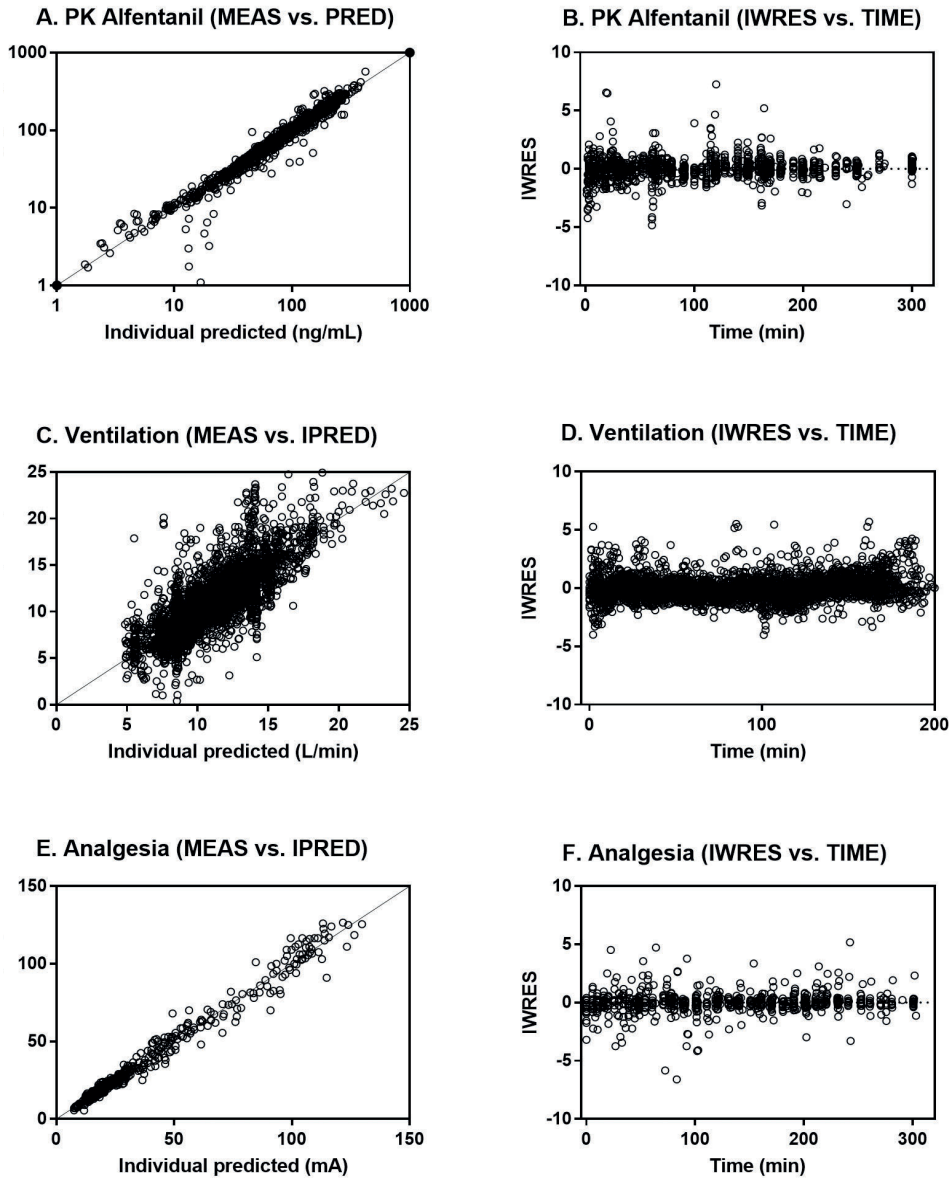

Figure 1. *Goodness of fit plots of the pharmacokinetic and pharmacodynamic analysis.* ***A.*** *Alfentanil concentration: measured versus individual predicted values.* ***B.*** *Alfentanil concentration: individual weighted residuals (IWRES) versus time.* ***C.*** *Ventilation: measured versus individual predicted values.* ***D.*** *Ventilation: individual weighted residuals (IWRES) versus time.* ***E.*** *Analgesia: measured versus individual predicted values.* ***F.*** *Analgesia: individual weighted residuals (IWRES) versus time.*

one thousand pharmacodynamic profiles as functions of concentration, U(CE), and time, U(t), were simulated according to the medians and inter-individual variabilities (ω^2) as listed in Table 1. The number of times severe respiratory depression and adequate analgesia occurred were divided by N, to obtain estimates of the probabilities of their occurrence. A 50% reduction in minute ventilation was taken as a threshold for severe respiratory depression, *i.e.* P(R > 0.5), and both a 25% and a 50% increase in tolerated electrical current were taken as threshold for analgesia, *i.e.* P(A > 0.25) and P(A > 0.5), respectively. The utilities U1 were defined as:

$$U1(A > 0.25) = P(A > 0.25) - P(R > 0.5) \quad (3)$$

and

$$U1(A > 0.5) = P(A > 0.5) - P(R > 0.5) \quad (4)$$

A disadvantage of this definition is that the U1 cannot be interpreted as a probability, because its values range between -1 and 1. The utility may be positive although the probability of adequate analgesia is small. We therefore calculated utility U2 giving the probability of adequate analgesia without severe respiratory depression (a desirable condition), with the probability of adequate analgesia defined as P(A > 0.25) or P(A > 0.5):

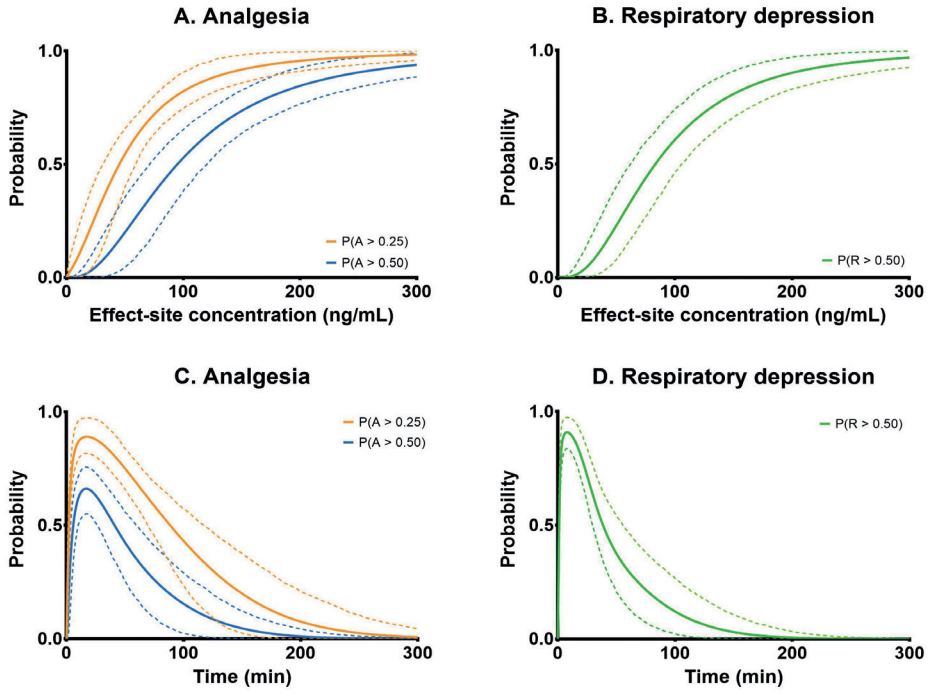

Figure 2. *Probability of analgesia and respiratory depression.* ***A.*** *Probability of analgesia versus effect-site concentration for P(A(CE) > 0.25) (orange lines) and P(A(CE) > 0.5) (blue lines).* ***B.*** *Probability of respiratory depression versus effect-site concentration for P(R(CE) > 0.5).* ***C.*** *Probability of analgesia versus time following a bolus infusion of 50 µg alfentanil for P(A(t) > 0.25) (orange lines) and P(A(t) > 0.5) (blue lines).* ***D.*** *Probability of respiratory depression versus time following a bolus infusion of 50 µg alfentanil for P(R(t) > 0.5). The broken lines are the 95% confidence intervals.*

$$U_2(A > 0.25) = P(A > 0.25 \text{ AND } R \leq 0.5) \quad (5)$$

and

$$U_2(A > 0.5) = P(A > 0.5 \text{ AND } R \leq 0.5) \quad (6)$$

Finally we defined the utility U_3 that gives the probability of severe respiratory depression and inadequate analgesia (the least desirable condition) for $P(A \leq 0.25)$ and $P(A \leq 0.5)$:

$$U_3(A \leq 0.25) = P(R > 0.5 \text{ AND } A \leq 0.25) \quad (7)$$

and

$$U_3(A \leq 0.5) = P(R > 0.5 \text{ AND } A \leq 0.5) \quad (8)$$

To assess the uncertainty in these measures, the above procedure was repeated for 1,000 bootstrap-derived replicated estimates of the model parameters (medians and expected value η covariances). Because the full bootstrap is not feasible with respect to computer time, we used the simplified nonparametric bootstrap method as implemented in NONMEM.[21] In short, rather than sampling from individual data to create bootstrap data sets and fitting each data set, the simplified bootstrap samples from the empirical Bayesian estimates of the interindividual variability terms (η) from the fit of the original data. This yields estimates of the interindividual variabiltity in the model parameters for each simplified bootstrap iteration, so that the uncertainty in the interindividual variability estimates - and hence the uncertainty in the utility functions - can be assessed. PK-PD profiles were calculated in the software environment R rather than in NONMEM to gain speed (with analytical expressions for concentrations after a bolus dose).

RESULTS

In the analysis we included PK data sets from 48 subjects, ventilation PD data from 19 subjects and analgesia data from 32 subjects. All subjects were Caucasian with a mean age (range) of 23.7 (19-31) years, mean weight of 78.8 (70.5-99.8) kg and mean body mass index 24.0 (20.2-29.5) kg/m^2.

PHARMACOKINETIC ANALYSIS

The PK model consisted of three compartments; one central compartment (V_1) and two peripheral compartments (V_2 and V_3). The PK parameters estimates are shown in Table 1. Two error terms were incorporated in the model, an additive (σ_1) and a relative (σ_2). Goodness of fits plots are shown in Figure 1, panels A and B. Inspection of the data demonstrates that the model adequately describes the data. There is a small misfit in the alfentanil PK data in the lower concentration ranges as described previously (data from study 1).[17]

PHARMACODYNAMIC ANALYSIS

The goodness of fits plots, shown in Figure 1 panels C to F, show that the PD models adequately describes the ventilation and analgesia data. PD parameter estimates are given in Table 1. The blood-effect-site equilibration half-life of alfentanil-induced ventilatory depression was relatively small (estimate = 3.99 ± 1.6 min); the C_{50V} or potency parameter estimate = 81.6 ± 20.5 ng/mL. Compared to ventilation, the blood-effect-site equilibration half-life for analgesic effect was greater by a factor of 3 ($t_{1/2}k_{eO}$ for analgesia = 11.7 ± 5.2 min), while potency parameter C_{50A}

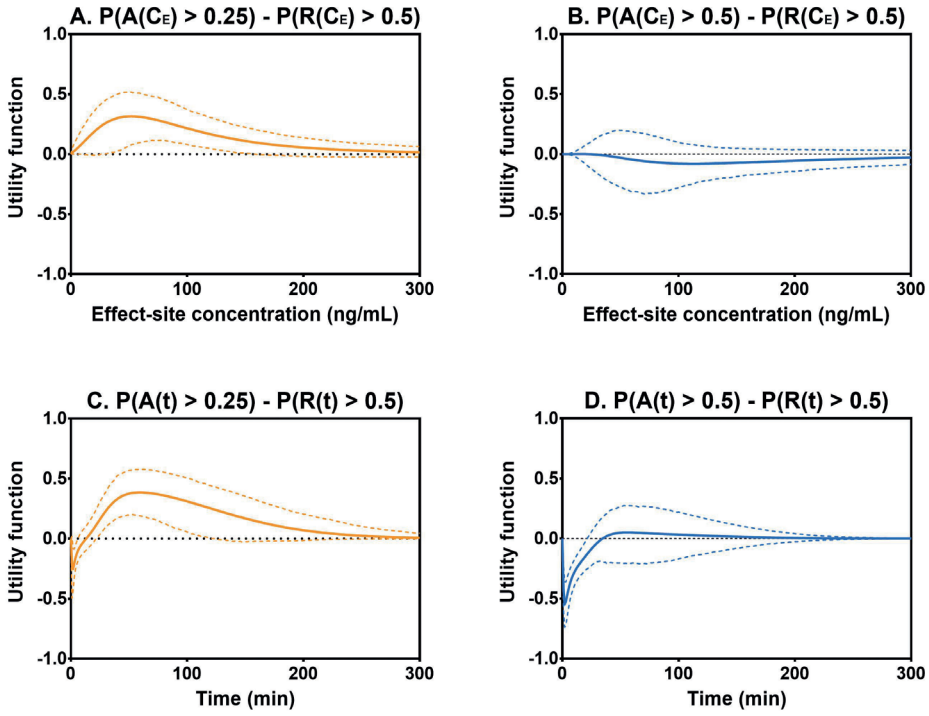

Figure 3. *Utility functions.* ***A.*** *Probability of at least 25% analgesia minus the probability of at least 50% respiratory depression versus effect-site concentration.* ***B.*** *Probability of at least 50% analgesia minus the probability of at least 50% respiratory depression versus effect-site concentration.* ***C.*** *Probability of at least 25% analgesia minus the probability of at least 50% respiratory depression versus time following a bolus infusion of 50 µg alfentanil.* ***D.*** *Probability of at least 50% analgesia minus the probability of at least 50% respiratory depression versus time following a bolus infusion of 50 µg alfentanil. The continuous lines represent the utility function, the broken lines the 95% confidence intervals.*

was of the same order of magnitude (C_{50} for analgesia = 97.9 ± 19.4 ng/mL).

UTILITY FUNCTIONS

The sigmoidal relationships between effect probability and effect-site concentrations are given in Fig. 2A for P(A(C_E) > 0.25) and P(A(C_E) > 0.5) and for P(R(C_E) > 0.5) in Fig. 2B. The relationships are given ± the 95% confidence intervals (CI). The probability of effect in the time domain following a bolus administration of 50 µg/kg alfentanil is given in Fig. 2C for P(A(t) > 0.25) and P(A(t) > 0.5) and in Fig. 2D for P(R(t) > 0.5). As expected the probabilities of at least 25% analgesia exceed that of at least 50% analgesia in both concentration and time domains. Directly following the bolus infusion (Figs. 2C and D) the probability of analgesia > 25% and respiratory depression > 50% both approach 1. Due the rapidly decreasing effect-site concentration the probabilities drop towards zero, albeit at a greater speed for respiratory depression than for analgesia (P(A(t) > 0.25): not different from zero at t > 200 min; P(R > 0.5): not different from zero at t > 130 min).

U1. The calculated utility functions ± 95% confidence intervals as function of effect-site

concentration are shown in Figs. 3A and B for $P(A(C_E) > 0.25) - P(R(C_E) > 0.5)$ and $P(A(C_E) > 0.5) - P(R(C_E) > 0.5)$. The utility function $P(A(C_E) > 0.25) - P(R(C_E) > 0.5)$ is positive over the effect-site concentration range of 0-300 ng/mL but only significantly different from zero from 26-158 ng/mL (Fig. 3A). This indicates that over the effect-site concentration range of 26 to 168 ng/mL $P(A > 0.25) > P(R > 0.5)$. The maximum effect ($U1(C_E) = 0.31$, 95% CI 0.08-0.52) occurred at an effect-site concentration of 52 ng/mL. The utility function $P(A(C_E) > 0.5) - P(R(C_E) > 0.5)$ was not different from zero over the effect-site concentration range of 0-300 ng/mL (Fig. 3B), indicative of a balance between the probability for analgesia and respiratory depression, *i.e.* $P(A(C_E) > 0.5) = P(R(C_E) > 0.5)$.

The utility in the time domain, given for a bolus dose of 50 µg/kg (Figs. 3C and D), is biphasic for both $P(A(t) > 0.25)$ and $P(A(t) > 0.5)$. For $P(A(t) > 0.25) - P(R(t) > 0.5)$ the function is negative from t = 2 to 6 min (peak effect -0.25, 95% CI -0.043 to -0.08, at t = 2 min) followed by values significantly greater than zero from t = 22-124 min (Fig. 3C). Thereafter the function is not different from zero, indicative of similar probabilities for analgesia and respiratory depression. For $P(A(t) > 0.5) - P(R(t) > 0.5)$ the function is negative from t = 1 to 21 min (peak effect -0.54, 95% CI -0.74 to -0.36, at t = 2 min), after which the function is not different from zero (Fig. 3D).

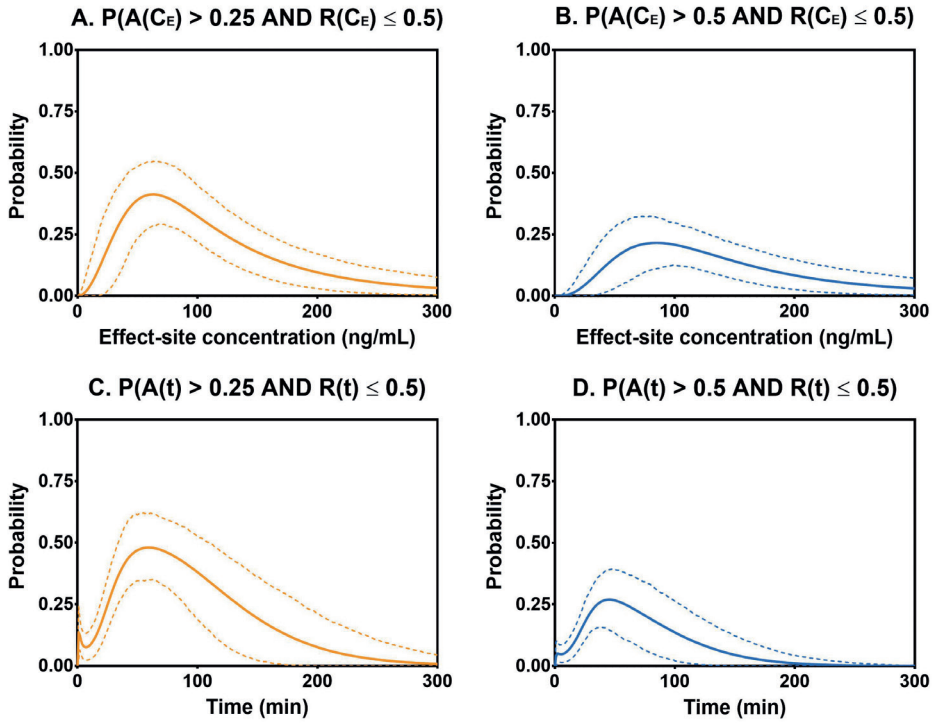

Figure 4. A. *Probability of at least 25% analgesia AND no more than 50% respiratory depression versus effect-site concentration.* ***B.*** *Probability of at least 50% analgesia AND no more than 50% respiratory depression versus effect-site concentration.* ***C.*** *Probability of at least 25% analgesia AND no more than 50% respiratory depression versus time following a bolus infusion of 50 µg alfentanil.* ***D.*** *Probability of at least 50% analgesia AND no more than 50% respiratory depression versus time following a bolus infusion of 50 µg alfentanil. The continuous lines are the probabilities, the broken lines the 95% confidence intervals.*

Parameter	Estimate ± SEE	ω^2 ± SEE	v^2 ± SEE
Pharmacokinetic parameter estimates			
V_1 (L)	5.73 ± 0.82	0.08 ± 0.03	0.04 ± 0.02
V_2 (L)	3.02 ± 1.41	0.58 ± 0.42	0.03 ± 0.02
V_3 (L)	12.1 ± 0.56	0.05 ± 0.02	*
CL (L/min)	0.29 ± 0.01	0.11 ± 0.04	0.01 ± 0.004
Q_2 (L/min)	1.55 ± 0.18	*	0.08 ± 0.03
Q_3 (L/min)	0.43 ± 0.05	0.04 ± 0.02	*
σ_1	2.85 ± 0.85		
σ_2	0.14 ± 0.01		
Pharmacodynamic parameter estimates			
Ventilation			
VB	19.5 ± 1.18	0.0719 ±0.0237	
C_{50V} (ng/mL)	81.6 ± 20.5	0.896 ± 0.305	
Y_V	1 (FIX)	0.746 ± 0.616	
$t_{1/2}k_{eOV}$ (min)	3.99 ± 1.63	*	
σ_V	2.26 ± 0.189		
Analgesia			
PB	16.0 ± 1.12	0.176 ±0.0589	
C_{50P} (ng/mL)	97.9 ± 19.4	1.08 ± 0.428	
Y_P	1 (FIX)	0.193 ± 0.079	
$t_{1/2}k_{eOP}$ (min)	11.7 ± 5.22	0.219 ± 0.155	
σ_P	3.60 ± 0.655		

Table 1. *Pharmacokinetic and pharmacodynamic parameter estimates*
*SEE; standard error of the estimate, ω^2; variance of the model parameter across the population, v^2; variance of between-occasion variability, V_1, V_2, V_3; volume of compartments 1, 2, and 3, CL; clearance of compartment V_1; Q_2 and Q_3; inter-compartmental clearances between compartments V_2 - V_1 and V_3 - V_1, σ_1; standard deviation of absolute residual variability, σ_2; standard deviation of relative variability, VB; baseline ventilation, C_{50V}; effect-site concentration causing 50% reduction of ventilation, Y_V; a shape parameter for ventilation, $t_{1/2}k_{eOV}$; the blood-effect-site equilibration half-life for ventilation, σ_V; standard deviation of the absolute residual variability for ventilation, PB; baseline pain toleranc, C_{50P}; the effect-site concentration causing a 50% increase in stimulus intensity, Y_A; a shape parameter for analgesia, σ_P; standard deviation of the absolute residual variability for analgesia; * not estimable.*

U2. The desirable outcome of opioid treatment of pain is analgesia with limited respiratory depression. We give the utility functions $P(A(C_E) > 0.25$ AND $R(C_E) \leq 0.5)$ and $P(A(C_E) > 0.5$ AND $R(C_E) \leq 0.5)$ in Fig. 4. In the concentration domain the probability of at least 25% analgesia but less than 50% respiratory depression reaches a maximum of 0.41 (95% CI 0.29-0.55) at an effect-site concentration of 68 ng/mL (Fig. 4A), while for at least 50% analgesia and less than 50% respiratory depression the maximum of 0.21 (95% CI 0.12-0.31) is reached at an effect-site

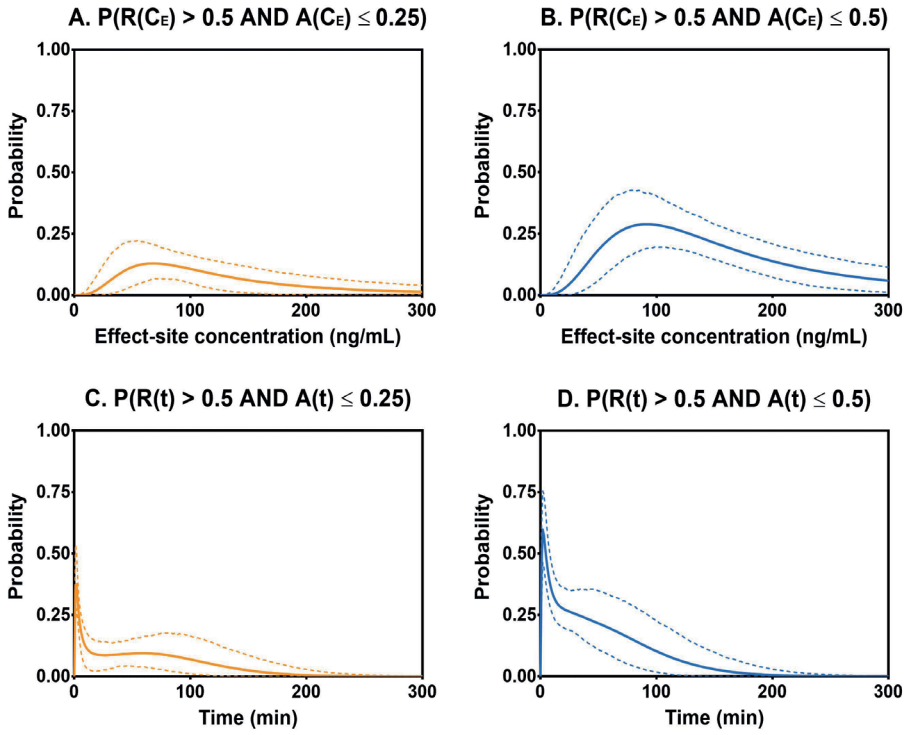

Figure 5. A. *Probability of at least 50% respiratory depression AND no more than 25% analgesia versus effect-site concentration.* ***B.*** *Probability of at least 50% respiratory depression AND no more than 50% analgesia versus effect-site concentration.* ***C.*** *Probability of at least 50% respiratory depression AND no more than 25% analgesia versus time following a bolus infusion of 50 µg alfentanil.* ***D.*** *Probability of at least 50% respiratory depression AND no more than 50% analgesia versus time following a bolus infusion of 50 µg alfentanil. The continuous lines are the probabilities, the broken lines the 95% confidence intervals.*

concentration of 93 ng/mL (Fig. 4B). The utilities in the time domain (after a bolus of 50 µg/kg alfentanil) are given in Fig. 4C and D.

U3. The least desirable outcome of opioid therapy is a severe respiratory depression, P(R > 0.5) with inadequate analgesia, P(A(C_E) ≤ 0.25) or P(A(C_E) < 0.5). We give the utility functions P(R(C_E) > 0.5 AND A(C_E) ≤ 0.25) and P(R(C_E) > 0.5 AND A(C_E) ≤ 0.5) in Fig. 5. In the concentration domain the probability of at least 50% respiratory depression with no more than 25% analgesia (Fig. 5A) is small with a peak at an effect-site concentration of 69 ng/mL (probability 0.13, 95% CI 0.7-0.21). The probability of at least 50% respiratory depression with no more than 50% analgesia (Fig. 5B) is greater, the greatest probability (0.29, 95% CI 0.20-0.41) was observed at an effect-site concentration of 98 ng/mL. The utilities in the time domain (after a bolus of 50 µg/kg alfentanil) are given in Fig. 5C and D.

Utility surface. In Figures 6A-6D, we plotted the continuum of probabilities of presence or absence of alfentanil analgesia in combination with presence or absence of serious respiratory depression, *i.e.* the utility surface. In panel A and B of Figure 6, we plotted the probabilities

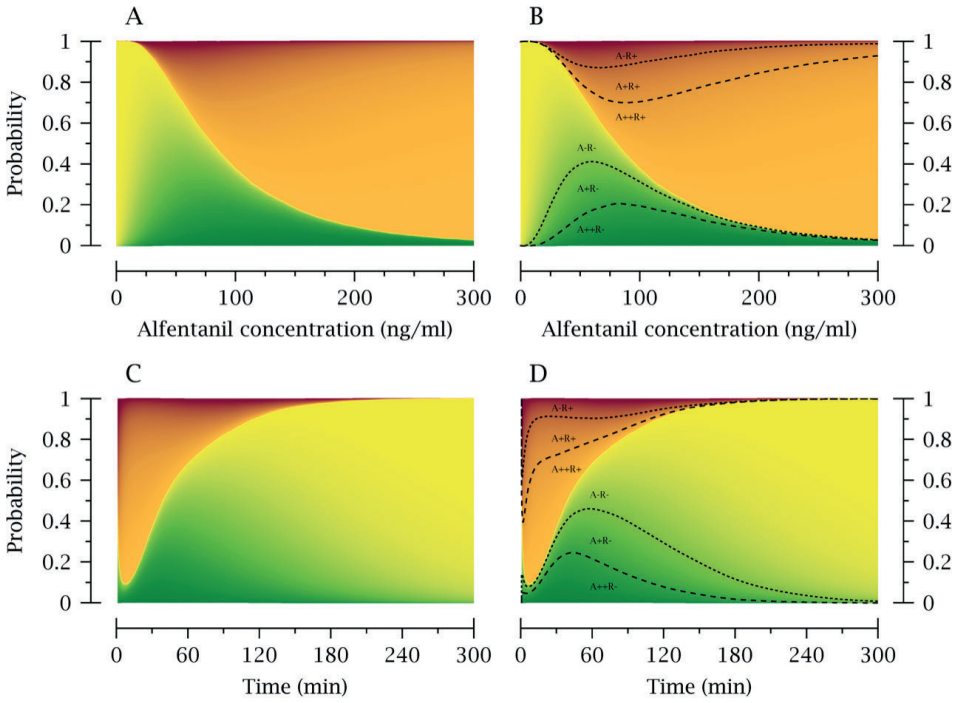

Figure 6. Alfentanil response surface: continuum of probabilities of alfentanil-induced analgesia and respiratory depression. ***A and B*** *Probability vs. alfentanil effect-site concentration.* ***C and D*** *Probability vs. time following a 50 µg/kg alfentanil bolus at time t = 0. The colour shading from green to yellow and red to orange represents the context dependency of the utility functions on the postulated threshold for analgesia. The lines in panels B and D are the curves of utility functions U2 and U3 (compare with Figs. 4 and 5). The surfaces are defined by A and R with A++ at least 50% analgesia, A+ at least 25% analgesia, A- less than 25% analgesia, R+ at least 50% respiratory depression and R- less than 50% respiratory depression.*

against effect-site concentration, in panel C and D the probabilities against time following a 50 µg/kg alfentanil bolus infusion (or 3.5 mg for a 70 kg patient) at time t = 0.
The utility surface analysis results in multiple conditions presented by colored surfaces and iso-utility lines (panels B and D); the iso-utility lines are the curves of the utility functions defined by U2 and U3 (compare with curves in Figs. 4 and 5), while the surfaces are defined by A and R with A++ at least 50% analgesia, A+ at least 25% analgesia, A- less than 25% analgesia, R+ at least 50% respiratory depression and R- less than 50% respiratory depression. This results in the following six conditions:
- Green surface: analgesia without respiratory depression (A++R- and A+R-);
- Red surface: no analgesia with respiratory depression (A-R+ and A+R+);
- Yellow surface: no analgesia and no respiratory depression (A-R-);
- Orange surface: analgesia with respiratory depression (A++R+).
The smooth transitions between colors are transients in between the given states.
It is clear from Figures 4 and 6 (panels A and B) that the optimum analgesia probability without serious respiratory depression (green surface in Fig. 6) is reached at an effect-site concentration

of 60 ng/mL, thereafter the probability of analgesia is reduced and eventually exceeded by the probability of serious respiratory depression (orange surface). Figures 6C and D shows that following a single injection (50 µg/kg) maximum analgesia with serious respiratory depression (orange surface) peaks at 10 min, an optimum in analgesia probability without serious respiratory peak at 60 min after which both analgesia and respiratory depression dissipate.

DISCUSSION

In the treatment of pain and nociceptive responses we aim to provide optimal analgesic effect with preferably no (or very little) side effects. Still, the most common group of drugs used in pain medicine and anesthesia to relief moderate to severe pain and blunt nociceptive responses, *i.e.* the opioid analgesics, produces a myriad of side effects. Since "benefit" (analgesia) and "harm" (side effects) coincide but often with dose or concentration-effect relationships that are distinct, *i.e.* not parallel, it is often difficult to reliably combine multiple end-points into one number or function.[14] Sheiner and Melmon introduced a concept derived from economic decision theory

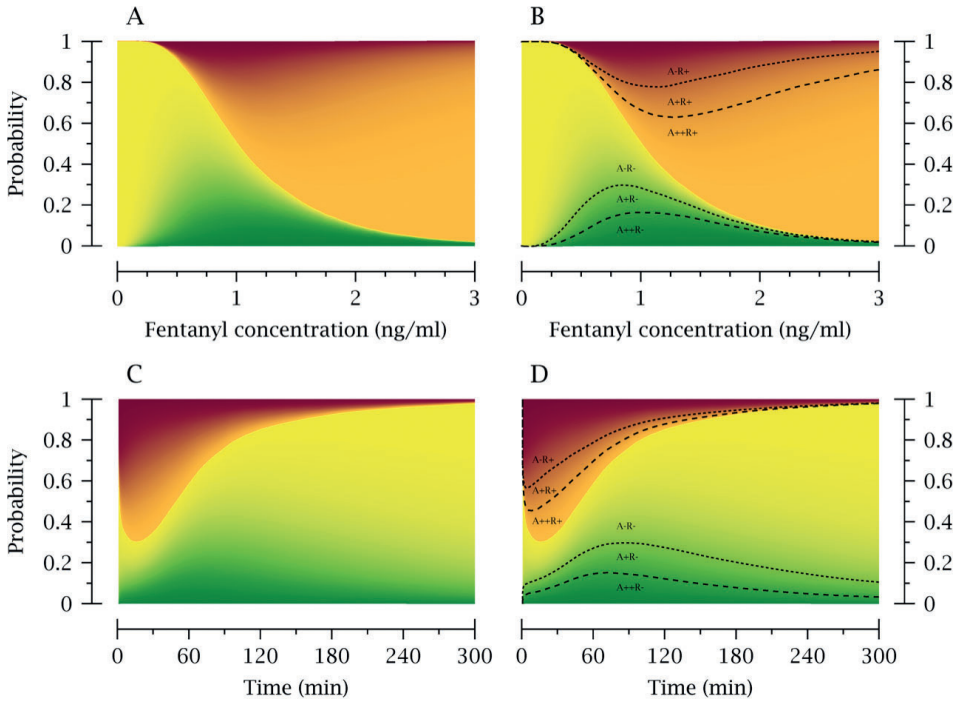

Figure 7. Fentanyl response surface: continuum of probabilities of fentanil-induced analgesia and respiratory depression. ***A and B*** *Probability vs. alfentanil effect-site concentration.* ***C and D*** *Probability vs. time following a 50 µg/kg alfentanil bolus at time t = 0. The colour shading from green to yellow and red to orange represents the context dependency of the utility functions on the postulated threshold for analgesia. The lines in panels B and D are the curves of utility functions U2 and U3. The surfaces are defined by A and R with A++ at least 50% analgesia, A+ at least 25% analgesia, A- less than 25% analgesia, R+ at least 50% respiratory depression and R- less than 50% respiratory depression.*

that allows combining of different end-points into one number, the utility, which they defined as the benefit of a drug minus the harm it produces.[22] They applied their concept to describe the benefit and harm of antihypertensive therapy. The concept was later used by Cullberg to define the outcome of anti-thrombin therapy.[23] In earlier studies, we applied the concept of the utility function to characterize a serious and potentially lethal opioid-side effect, respiratory depression, relative to the obtained analgesic efficacy. We tested various opioids including fentanyl, oxycodone and the relatively new opioids tapentadol and cebranopadol.[11,13,15,24] Our main aim was to create a set of utility functions that allows comparisons among drugs. We argued that a drug with positive utilities over the clinical concentration range (*i.e.,* with a higher probability for analgesia than respiratory depression) is preferable over a drug with a negative utility (*i.e.,* with a higher probability for respiratory depression than analgesia). In their editorial, Kharasch and Rosow argued that while the benefit-risk measure that we developed (*i.e.* the utility function) "appears to be precise and reproducible [and] also seems to be an excellent method for combining high-quality estimates of population pharmacokinetic-pharmacodynamic modeling", it requires refinement and validation that it predicts relevant clinical outcomes.[14] In the current study we further developed the utility function. We estimate 95% confidence limits around the function based on bootstrap sampling of the nonparametric distribution of the model parameters. Furthermore, we calculate the probabilities of a series of distinct conditions: the probability of adequate analgesia with or without severe respiratory depression (A++R-, A+R-, A++R+ and A+R+; Figs. 6 and 7) and the probability of inadequate analgesia with or without severe respiratory depression (A-R+, A-R-, Figs. 6 and 7). We argue that the use of these adapted utility functions is preferable above a utility function that is the result of the subtraction of one probability from the other, that is constrained in magnitude between values -1 to +1 and that gives no indication of the probability of distinct outcomes, such as desired (A++R-) and undesired outcomes (A-R+) and all possibilities in between.

In the current study we applied the adapted utility functions to the μ-opioid analgesic alfentanil. We combined the results of three previous pharmacokinetic-pharmacodynamic into one analysis. The analyses were performed in multiple steps. A population PK analysis led to individual PK parameters estimates that were inputted into the PD models of analgesia and respiratory depression. We subsequently calculated the probabilities of $P(A > 0.25)$, $P(A > 0.5)$ and $P(R > 0.5)$ in both concentration and time domains (Fig. 2) by performing 1000 simulations for both end-points. Subsequently, the original (U_1) and adapted utility functions (U_2 and U_3) were determined (Figs. 3-5). As stated previously, the utility function is context sensitive, in which the context is the numerical response threshold.[13] For example, for P(A) we applied two thresholds: $A > 0.25$ and $A > 0.5$; the difference in thresholds reflects the difference in analgesic effect, *i.e.* a 25% and 50% increase in tolerated electrical current. We regard the 25% threshold as the clinically more realistic end-point for chronic pain therapy (the effect-site concentration producing a 25% increase in stimulus intensity is in the range of concentrations observed in postoperative pain therapy),[25] while the 50% threshold may be more relevant in anesthesia practice.[26] Further studies will need to confirm whether our extrapolation of experimental data to clinical data is valid.

The results of our analyses are summarized in the utility surface of Fig. 6, which combines multiple conditions into one graph: analgesia without respiratory depression (green surface), respiratory depression without analgesia (red surface), neither respiratory depression nor analgesia (yellow surface) and analgesia combined with respiratory depression (orange surface). If we compare the two extremes (red *vs.* green surfaces), it is obvious that the probability of

the desired effect (green surface) exceeds the probability of the most unwanted effect (red surface). At an effect-site concentration of 68 ng/mL the probabilities differ significantly (odds ratio = 4.0; Fig. 6A and B); similarly, 1 hour after a bolus dose of 50 µg/kg, the probabilities differ (odds ratio 4.0; Fig. 6C and D).
It is important to realize that while the probabilities of the most desired condition (adequate analgesia without respiratory depression) and the least desired condition (inadequate analgesia with respiratory depression) are both maximal at about the same effect-site concentration, their probabilities are different. The probability of the most desired condition is much higher than the probability of the least desired condition, which would be expected of a clinically useful drug. Even if the probabilities were about the same, or reversed, the utility function would retain its utility as classifying it as a drug with lower clinical utility. The concentrations where the probabilities of the conditions are maximal depend on the thresholds chosen, as can be seen in Figure 6, and the potencies for the desired and side effects. The point is that the clinical utility of drugs may be compared, under the condition that the chosen thresholds are the same.

As we stated above, we consider the utility function suitable for a comparisons among drugs. As an example we compared the utility surfaces of alfentanil and fentanyl. Both are fenylpiperidines mu-opioid analgesics, are used as intravenous anesthetics. In Fig. 7, we plotted the fentanyl utility surface based on our previous population pharmacokinetic pharmacodynamic analysis.[13] Figs. 7A and B (utility *vs*. fentanyl C_E) may be compared to Figs. 6A and B (utility *vs.* alfentanil CE). These response surfaces of the two opioids are similar and implies that fentanyl is more potent by a factor of 70 to 80 (in terms of effect-site concentration). This potency difference derived from the utility surface is similar to the potency ratio of 75 observed for the effect-site concentration that causing one-half of the EEG-slowing (6.9 ng/mL for fentanyl and 520 ng/mL for alfentanil).[26] These similarities give further validity to our approach. The surface similarity indicates that for the same probability of analgesia, a similar probability of respiratory depression is observed for these two opioids. While we believe that the comparison between these two opioids is valid (both drugs were tested in a similar population of healthy volunteers using identical experimental set-ups), we, however, need to realize that our data are derived from young volunteers without pain or co-morbidities, and further studies should address the utility of opioid treatment in patients. For example, our approach is suitable to compare the utility of analgesic medication between patient sub-populations, such as patients with and without sleep apnea syndrome, patients with and without chronic pain, opioid-naïve patients and chronic opioid users. Additionally, our approach enables construction of utility functions of drug efficacy *versus* slowly emerging complications of long-term drug therapy, such as development of tolerance, hyperalgesia, cognitive dysfunction or the late occurrence of tissue damage.

In conclusion, we further refined the utility function as (surrogate) measure of opioid benefit *vs.* harm. We defined four distinct states of analgesia and respiratory depression that reflect four clinical conditions that are either desirable (analgesia without respiratory depression) or highly undesirable (respiratory depression without analgesia) and two intermediate (undesirable) states (neither analgesia nor respiratory depression and analgesia combined with respiratory depression). Our utility function may be used to compare the respiratory effects of analgesics and may be a useful tool in the development of novel (opioid) analgesics.

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Chapter 6

Respiratory effects and naloxone reversal of the novel opioid analgesic RM101

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Submitted

INTRODUCTION

According to the WHO pain ladder, weak opioids can be used for treatment of moderate pain while potent opioids are useful in the treatment of moderate to severe pain.[1-3] Common adverse events of opioids include dizziness, nausea/vomiting, constipation and respiratory depression (respiratory depression occurs due to activation of the μ-opioid receptor (MOR) in the respiratory centers of the brainstem). Additionally, opioids are drugs of abuse and especially the combination of abuse/misuse and respiratory depression is potentially life-threatening. Indeed, since 1999 the number of overdose deaths due to prescription opioids quadrupled in the US alone, with more than 250,000 deaths since 2004. Among the 47,055 drug overdose deaths that occurred in the United States in 2014, 28,647 (60.9%) involved an opioid.[3,4] Consequently, there is the need for an immediate solution to this devastating problem. One solution would be the development of an opioid without respiratory depression but with retained effective analgesia. For example, opioids that interact with multiple opioid receptors (MOR, κ-opioid receptor (KOR), δ-opioid receptor (DOR) or the nociceptin/orphanin FQ (NOP) receptor) might produce less respiratory depression due to less reliance on the MOR for effective analgesia and a respiratory stimulatory effect from activation of the DOR or NOP receptor.[5] Examples of such opioids are buprenorphine (MOR/KOR activation) and cebranopodol (MOR/NOP receptor activation). Both opioids show ceiling *(i.e.* plateau phase in maximal effect) in respiratory depression.[6-8]
In the current study, we performed a population pharmacokinetic-pharmacodynamic (PK-PD) modelling study in healthy volunteers to determine the respiratory effects of yet another novel opioid analgesic, acting at the three classical opioid receptors, RM101. Varying doses of this opioid were administered to objectify its depressant effects on isohypercapnic ventilation. Additionally, the ability of naloxone to reverse the respiratory effects of RM101 was studied.

MATERIALS AND METHODS

SUBJECTS

Healthy male subjects, aged 18-45 years with a body weight of 60 to 100 kg and a body mass index (BMI) of 18 to 30 kg/m^2, were allowed to participate in the study. Prior to enrolment, the local Human Ethics Committee of the Leiden University Medical Centre approved the protocols and written informed consent was obtained from the participants. They all underwent a medical screening (medical history, physical examination, 12-leads ECG, blood chemistry, hematology and virology) before randomization. Exclusion criteria were presence or history of a major medical condition, history of alcohol abuse or illicit drug use, use of medication (vitamins, herbal and mineral supplements included), smoking of more than 20 cigarettes per day, positive urine drug screen or alcohol breath test on the morning of the experiment, abnormalities in the blood chemistry/hematology/virology, QTc interval > 450 msec and known drug allergies. All participants had to refrain from food and drinks for at least 6 hours prior to dosing until 8 hours post-dosing.

STUDY DESIGN

We performed a randomised, placebo-controlled, double blind, dose-escalating respiratory study in 28 subjects, followed by a single-dose randomised study to determine the ability of naloxone to reverse the effect of RM101 in 8 subjects.

Respiratory study. Subjects were divided in dose-escalating cohorts: 0.2 µg/kg (cohort 1), 0.25 µg/kg (cohort 2), 0.3 µg/kg (cohort 3) and 0.4 µg/kg (cohort 4). Each cohort consisted of 6 subjects receiving RM101 and one subject who received a placebo infusion. Study drug administration occurred in 10 min. If two subjects within one cohort hit one or more stopping rules the cohort was discontinued. Stopping rules were: apnea > 90 sec, end-tidal pCO_2 > 67.5 mmHg, increase in QTc time > 60 msec above baseline values or QTc time > 500 msec.

Reversal study. Subjects were dosed with 0.25 µg/kg RM101 in 10 minutes. Five minutes after RM101 infusion, naloxone 0.4 mg intravenous was administered in 90 seconds. Depending on the effect, naloxone was repeated at 4 minute intervals, as necessary up to a maximum of 4 doses (1.6 mg in 12 minutes). Reversal was deemed effective when ventilation returned to baseline ventilation ± 20%.

Drugs and randomization. All drugs (RM101, naloxone, placebo) were prepared by the local pharmacy on the day of the study and delivered in identical syringes that contained a clear unidentifiable liquid. The syringe was coded with subject and visit numbers. RM101 was dissolved in citrate buffer (pH 5); placebo contained the buffer solution. Randomization was performed by the study sponsor using a validated system that automates the random assignment of treatment groups to randomization numbers.

Respiratory measurements. Subjects breathed through a facemask (fitted over nose and mouth). The airway gas flow was measured by a pneumotachograph (#4813; Hans Rudolph, Kansas City, MO) connected to a pressure transducer, which yields a volume signal. The pneumotachograph was heated (37°C) throughout the study period. This signal was calibrated with a 1-l calibration syringe (Hans Rudolph). The pneumotachograph was connected to a T-piece; one arm of the T-piece received a gas mixture at a flow rate of 45 l/min from a gas mixing system, consisting of three mass-flow controllers (Bronkhorst High-Tec, Veenendaal, The Netherlands) via which the flow of oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2) could be set individually at any desired level. A computer provided control signals to the mass-flow controllers allowing adjustment of the inspired gas mixture to force the end-tidal gas concentrations of oxygen and carbon dioxide to follow a specific pattern in time. Gas concentrations were measured with a gas analyzer (Datex Multicap, Helsinki, Finland); arterial hemoglobin oxygen saturation was measured via a finger probe with a Masimo pulse oximeter (Irvine, CA).
Respiration was measured at a clamped increased end-tidal carbon dioxide tension (pCO_2) using the "Dynamic End-Tidal Forcing" technique. In each subject, the end-tidal pCO_2 was increased in steps of 2–3 mmHg until isohypercapnic ventilation reached a value 20–24 L/min. The end-tidal pCO_2 was kept constant at this level throughout the study (baseline end-tidal pCO_2 level). The end-tidal oxygen concentration (pO_2) was kept constant at 110 mmHg throughout the study. After a steady state in isohypercapnic ventilation was obtained, the medication was infused in 10 minutes, and breath-to-breath ventilatory measurements were continued during infusion and obtained for the next 60 min. Thereafter, isohypercapnic 5-min measurements were obtained at 30-min intervals until t = 8 h after the administration, or until return within 10% of isohypercapnic baseline ventilation for two consecutive measurements for the respiratory study and 20% in the reversal study. The following variables were collected on a breath-to-breath basis on a computer disc for further analysis: inspiratory minute ventilation, end-tidal pCO_2, end-tidal pO_2, and oxygen saturation.

Blood sampling. All subjects received an arterial line in the radial artery of the non-dominant arm for blood sampling. In total 19 arterial samples were taken at t = 0, 2, 5, 8, 10, 12, 15, 18, 20, 25, 30, 40, 60, 90, 120, 180, 240, 300 and 480 min after the start of infusion of RM101/placebo at t = 0. In the reversal study, the arterial samples were used to determine plasma concentrations of naloxone at t = 0, 18, 20, 25, 30, 40, 60, 90, 120, 180, 240, 300 and 480 min. Plasma was separated within 30 minutes after collection and stored at -20 °C until analysis. Plasma samples were analysed using validated LC-MS/MS methods, with lower limits of quantification of 5 and 5 pg/ml respectively.

PHARMACOKINETIC-PHARMACODYNAMIC ANALYSES

The pharmacokinetic (PK)-pharmacodynamic (PD) data were analysed with the mixed-effects modelling software package NONMEM VII (ICON Development Solutions, North Wales, MD) using a population approach. We performed a two-stage approach. From the first stage (PK analysis), for each individual, empirical Bayesian estimates of the PK parameters were obtained and were applied in the second stage (PD analysis).

Pharmacokinetic analysis. To obtain the best pharmacokinetic model, two and three compartment models were fitted to the pharmacokinetic data. The number of compartments in the model was determined by the magnitude of decrease in the minimum objective function value (MOFV; χ^2-test; $P < 0.01$ was considered significant). Weight and dose were considered as covariates. The best model will be reported.

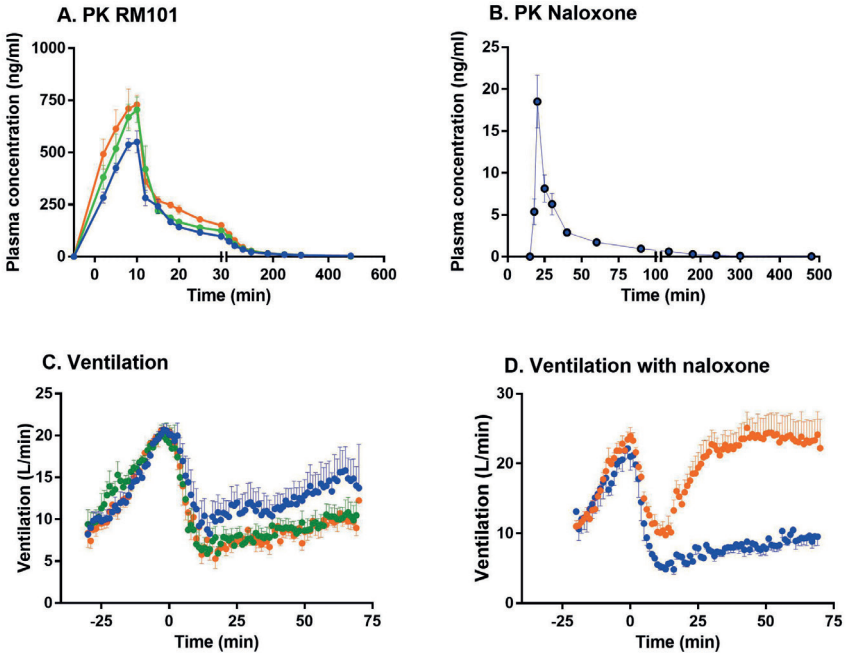

Figure 1. A. *Mean plasma RM101 concentrations.* ***B.*** *Mean plasma naloxone concentrations.* ***C.*** *Measured ventilation during infusion of RM101 in different dosages.* ***D.*** *Measured ventilation during infusion of RM101 with naloxone (orange) or placebo (blue). In* ***A*** *and* ***C*** *RM101 0.2 µg/kg; blue symbols, RM101 0.25 µg/kg; green symbols, RM101 0.3 µg/kg; orange symbols.*

Pharmacodynamic analysis. Ventilation data of each subject was averaged over 1-min periods. We assume that:

$$\text{Ventilation}(t) = VN(t) + G(t) \cdot (P(t) - PB) \quad (1)$$

Here, VN is normocapnic baseline ventilation, G is CO_2 sensitivity, PB is baseline end-tidal pCO_2, and P (t) is the measured end-tidal pCO_2 at (t), delayed with half-life $t_{1/2}k_{eOP}$. It is assumed that RM101 affects both VN and G, using a sigmoid EMAX models:

$$VN(t) = VN / (1 + CE(t) / C_{50}^{\gamma_{VN}}) \quad (2)$$

$$G(t) = G / (1 + CE(t) / C_{50}^{\gamma_G}) \quad (3)$$

Where CE is the effect-site concentration at (t), C_{50VN} the RM101 effect-site concentration causing a 50% depression of ventilation, C_{50G} the RM101 effect-site concentration causing a 50% decrease in CO_2 sensitivity and γ the shape or Hill parameter for respectively baseline ventilation (γ_{VN}) or CO_2 sensitivity (γ_G). To eliminate a hysteresis between the estimated drug concentrations and effect, an effect compartment was postulated. This effect compartment equilibrates with the plasma compartment with half-life $t_{1/2}k_{eOVN}$ for VN and $t_{1/2}k_{eOG}$ for G, respectively.
The effect of naloxone was assumed to be on parameter C50VN and C50G, assuming a linear relationship between dose and effect:

$$C50' = C_{50VN} \cdot (1 + C_{NLX} / C_{100NLX}) \quad (4)$$

$$C50' = C_{50G} \cdot (1 + C_{NLX} / C_{100NLX}) \quad (5)$$

Where C50' is the C_{50VN}, respectively C_{50G} after administration of naloxone, C_{NLX} is the effect-site concentration naloxone and C_{NLX} is the naloxone concentration in which the C50 of RM101 doubles.
Model parameters were assumed to be log-normally distributed. Residual error was assumed to have both an additive and a relative error for concentrations and only an additive error for all effect parameters. Model discrimination was based on the magnitude of the MOF value (χ^2-test). *P*-values < 0.01 were considered significant.

RESULTS

The data of 19 subjects are included in the respiratory analysis. Twenty-five subjects gave informed consent; three subjects were excluded due to either previous drug abuse or medical history. Of the 22 subjects, 3 subjects were randomized to placebo infusion and not included in the respiratory analysis. In cohort 3, one subject reached a stopping rule; he desaturated due to apnea (SpO_2 < 85%). In cohort 4, only one subject was dosed due to the fact that this one subject reached two stopping rules; apnea > 90 sec and his QTc time increased > 60 msec. Hereafter, this respiratory study was terminated. In the reversal study, 14 subjects were randomized. After 5 experiments (3 naloxone, 2 placebo), the RM101 dose was decreased to 0.2 µg/kg due to excessive vomiting after administration of naloxone. Eleven subjects (3 subjects

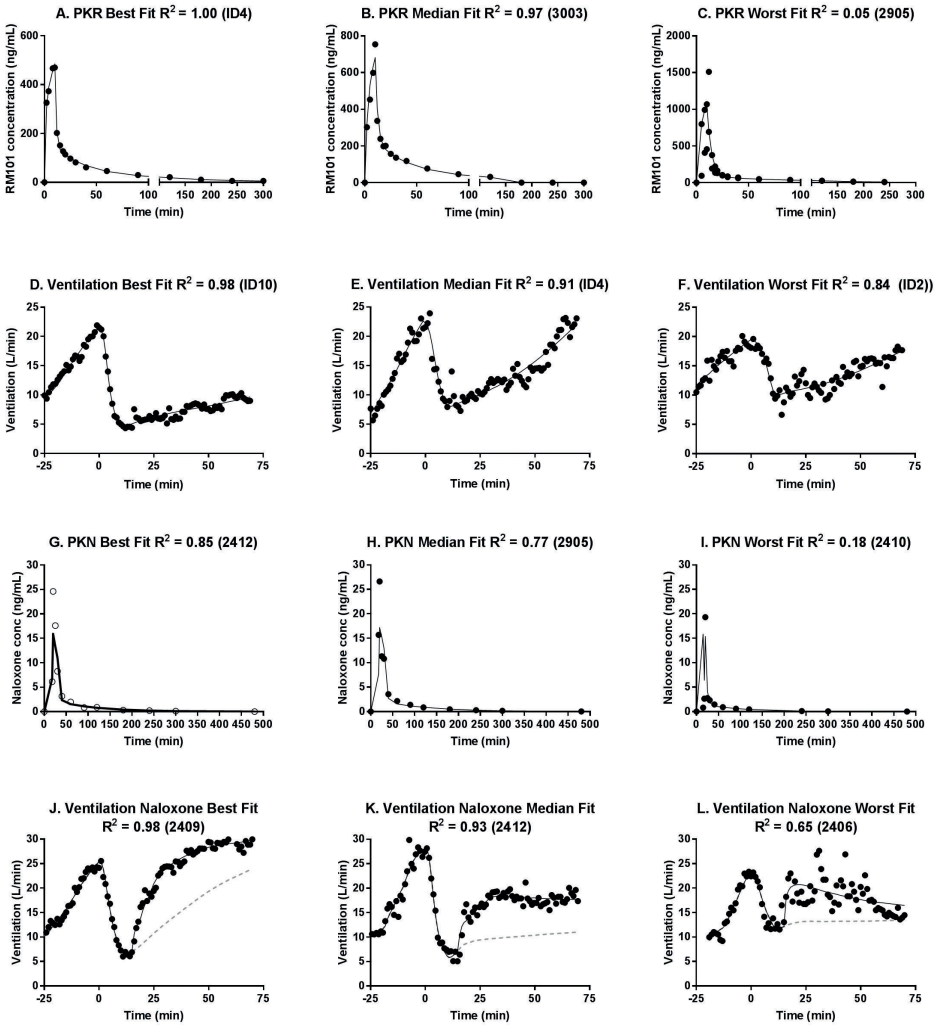

Figure 2. *RM101 pharmacokinetic (PK) data fits. Best* ***(A)****, median* ***(B)*** *and worst* ***(C)*** *pharmacokinetic data fits.* ***(D)****,* ***(E)*** *and* ***(F)*** *are the best, median and worst ventilation data fits.* ***(G)****,* ***(H)*** *and* ***(I)*** *are the best, median and worst naloxone PK data fits.* ***(J)****,* ***(K)*** *and* ***(L)*** *are the best, median and worst ventlation with naloxone data fits. Goodness-of-fit was based on the coefficient of determination.*

with 0.25 µg/kg, 8 subjects with 0.2 µg/kg) were included in the analysis. The average age of the subjects was 23.8 ± 0.4 years (mean ± SEM) and weight 78.5 ± 1.8 kg. In the reversal study, the average age was 23.5 ± 0.9 years and weight 81.3 ± 2.3 kg.

PHARMACOKINETIC ANALYSIS

In Fig. 1 the mean RM101 and naloxone plasma concentrations are shown. Peak arterial plasma RM101 concentration was reached after 10 min: 550 ± 52 ng/mL, 704 ± 16 ng/mL and 729 ± 46 ng/mL (mean ± SEM) for respectively 0.2 µg/kg, 0.25 µg/kg and 0.3 µg/kg RM101. The

	Estimate ± SEE	ω² ± SEE	Estimate ± SEE	ω² ± SEE
	RM101 PK parameter estimates		Naloxone PK parameter estimates	
V_1 (L)	5.59 ± 0.54	0.06 ± 0.04	25.3 ± 3.92	*
V_2 (L)	25.2 ± 2.95	0.05 ± 0.02	160 ± 11.1	*
V_3 (L)	56.1 ± 6.97	0.08 ± 0.05		
CL_1 (L/min)	1.12 ± 0.05	0.03 ± 0.02	3.66 ± 0.15	0.01 ± 0.01
CL_2 (L/min)	2.33 ± 0.20	0.09 ± 0.03	3.86 ± 0.73	0.13 ± 0.06
CL_3 (L/min)	0.50 ± 0.07	0.16 ± 0.08		
σ_1	0.14 ± 0.02		0.35 ± 0.02	
σ_2			8.2 ± 3.24	
	PKPD analysis: RM101 → Ventilation		PKPD analysis: RM101 + naloxone → Ventilation	
VN (L)	8.52 ± 0.43	0.04 ± 0.01	9.04 ± 0.43	0.05 ± 0.02
G (kPa)	5.12 ± 0.08	*	5.29 ± 0.11	0.01 ± 0.003
PB (kPa)	11.6 ± 1.47	0.12 ± 0.05	13.3 ± 1.08	0.11 ± 0.04
$t_{1/2}k_{eOVN}$ (min)	4.48 ± 1.11	0.40 ± 0.30	1.97 ± 0.22	*
$t_{1/2}k_{eOG}$ (min)	31.3 ± 3.95	0.17 ± 0.07	33.6 ± 12.2	1.52 ± 0.06
C_{50VN} (ng/mL)	400 ± 74.1	0.19 ± 0.07	353 ± 105	*
C_{50G} (ng/mL)	51.5 ± 6.51	*	33.3 ± 12.0	1.26 ± 0.48
γ_{VN}	0.82 ± 0.19	0.71 ± 0.23	1.11 ± 0.16	*
γ_G	2.46 ± 0.22	0.11 ± 0.04	1.73 ± 0.20	0.08 ± 0.03
$t_{1/2}k_{eONLX}$ (min)			22.8 ± 4.89	0.56 ± 0.40
$C_{100NLXVN}$ (ng/mL)			0.12 ± 0.04	*
$C_{100NLXG}$ (ng/mL)			1.24 ± 0.33	*
$t_{1/2}k_{eOP}$ (min)	10.4 ± 2.73	0.37 ± 0.13	7.28 ± 1.57	0.33 ± 0.13
σ	1.42 ± 0.08		1.69 ± 0.01	

Table 1. *Pharmacokinetic and pharmacodynamic parameter estimates of RM101 and naloxone. SE; standard error of the estimate, ω^2; variance of the model parameter across the population, V_1, V_2, V_3; volume of compartments 1, 2, and 3, CL; clearance of compartment V_1, CL_2 and CL_3; inter-compartmental clearances between compartments V_2 - V_1 and V_3 - V_1, σ_1; standard deviation of absolute residual variability, σ_2; standard deviation of relative variability, VN; normocapnic baseline ventilation, G; CO_2 sensitivity, PB; baseline end-tidal CO_2, $t_{1/2}k_{eOVN}$; the blood-effect-site equilibration half-life for normocapnic ventilation, $t_{1/2}k_{eOVG}$; the blood-effect-site equilibration half-life for CO_2 sensitivity, C_{50VN}; RM101 effect-site concentration causing 50% reduction of ventilation, C_{50G}; effect-site concentration causing 50% reduction in CO_2 sensitivity, Y_{VN} ; a shape parameter for baseline ventilation, Y_G; a shape parameter for CO_2 sensitivity, $t_{1/2}k_{eOVNLX}$; the blood-effect-site equilibration half –life for naloxone, C_{100N}; the naloxone concentration in which the C_{50} of RM101 doubles, $t_{1/2}k_{eOP}$; the blood-effect-site equilibration half-life for normocapnic ventilation, σ; standard deviation of the absolute residual.*

** Not estimable.*

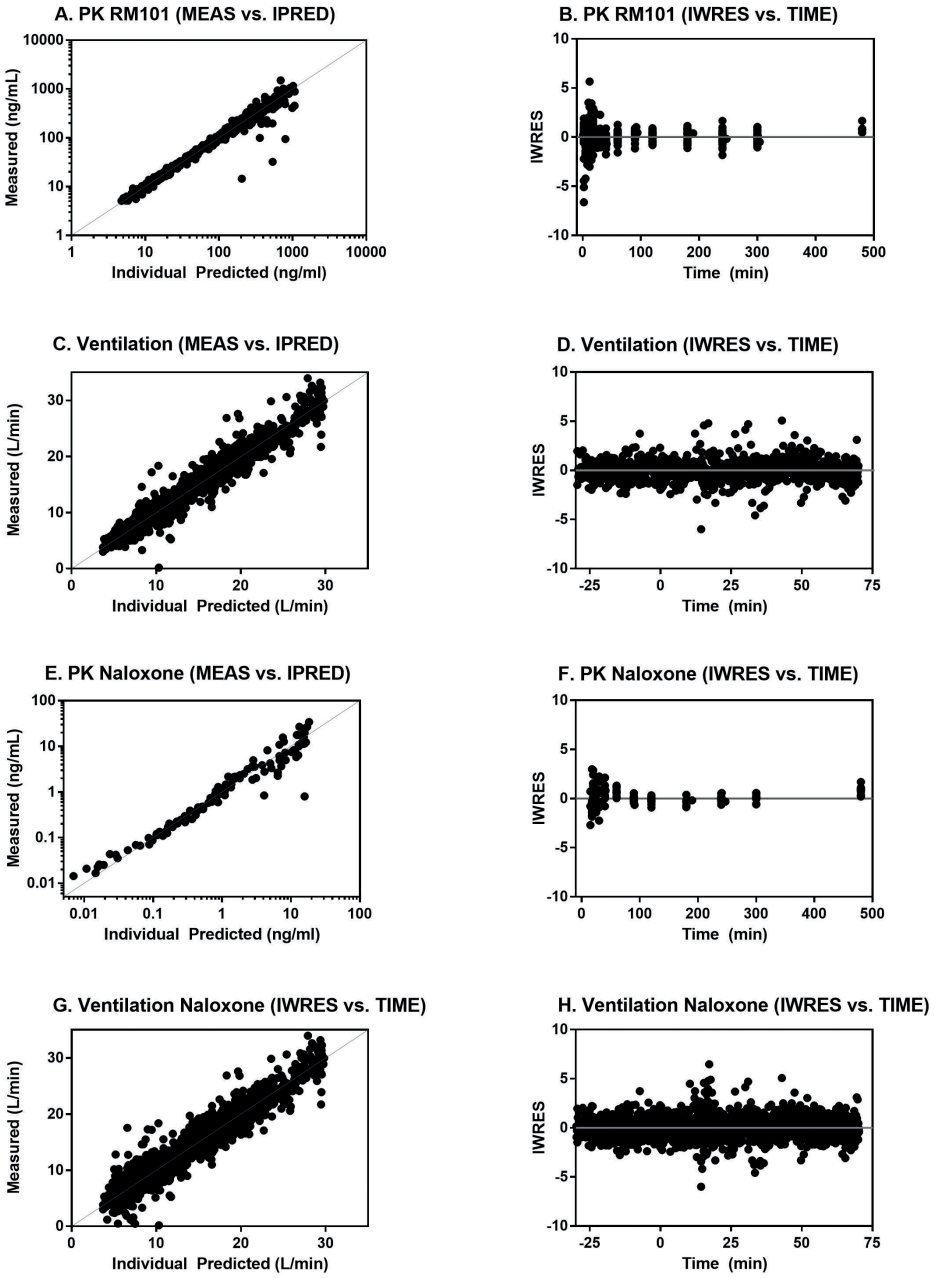

Figure 3. *Goodness of fit plots of the pharmacokinetic and pharmacodynamic analysis.* ***A.*** *RM101 concentration: measured versus individual predicted values.* ***B.*** *RM101 concentration: individual weighted residuals (IWRES) versus time.* ***C.*** *Ventilation: measured versus individual predicted values.* ***D.*** *Ventilation: individual weighted residuals (IWRES) versus time.* ***E.*** *Naloxone concentration: measured versus individual predicted values.* ***F.*** *Naloxone concentration: individual weighted residuals (IWRES) versus time.* ***G.*** *Ventilation with naloxone: measured versus individual predicted values.* ***H.*** *Ventilation with naloxone: individual weighted residuals (IWRES) versus time.*

pharmacokinetic model of RM101 consisted of a three-compartment model with one central (V_1) and two peripheral compartments (V_2 / V_3). The pharmacokinetic model for naloxone consisted of a two-compartmental model with one central (V_1) and one peripheral compartment (V_2). Covariate weight or dose did not improve the fits. The PK parameter estimates are shown in Table 1. Best, median and worst fits (based on the coefficient of determination) are shown in Fig. 2 (RM101 Figs. 2A-C, naloxone Figs. 2G-I). Goodness of fit plots are shown in Fig. 3 (RM101 Figs. 3A-B, naloxone Figs. 3E-F). Inspection of the individual data and the goodness of fit plots indicate that the PK data are adequately described by the pharmacokinetic models of RM101 and naloxone.

PHARMACODYNAMIC ANALYSIS

RM101 induced a rapid respiratory depressant effect within 15 min, which ranged from 54% (0.2 µg/kg) to 68% (0.3 µg/kg). Eight subjects demonstrated effective reversal of the opioid-induced respiratory depression (naloxone dose 0.4-1.6 mg); in 3 subjects naloxone was unable to reverse respiratory depression.

Inspection of the individual fits and the goodness of fit plots indicate that the model adequately described the data (Figs. 2 and 3). In Figure 2 best, median and worst fits are shown (data of both studies are included). RM101 had an effect on VN and G (CO_2 sensitivity). The effect on ventilation occurred rapidly with a $t_{1/2}k_{eOVN}$ of 4.5 ± 1.1 min and a C_{50VN} of 400 ± 74 ng/ml. The effect on CO_2 sensitivity was slower ($t_{1/2}k_{eOG}$ 31.3 ± 4 min), but took place at substantially lower plasma concentrations than the effect on ventilation (C_{50G} 51.5 ± 6.5 ng/ml).

At a steady-state arterial naloxone plasma concentration of 0.12 ± 0.04 ng/mL ($C_{100NLXVN}$), the arterial plasma concentration of RM101 causing a 50% depression of ventilation (C_{50VN}) doubled from 353 to 707 ng/mL. At an arterial plasma naloxone concentration of 1.24 ± 0.33 ng/ml ($C_{100NLXG}$), the arterial RM101 plasma concentration that induces a 50% decrease in CO_2 sensitivity (C_{50G}) doubled from 33 to 66 ng/mL. The equilibration half-life of naloxone's reversal on ventilation and CO_2 was 22.8 ± 4.9 min.

DISCUSSION

In this placebo-controlled, dose-escalating, double blind and randomized study, the effect of the novel MOR, KOR and DOR opioid agonist RM101 on ventilation was studied. The PK-PD analysis demonstrated that RM101 had two distinct actions: at low plasma concentrations a slow effect on CO_2 sensitivity, at higher plasma concentrations a more rapid effect on ventilation. We further observed that naloxone antagonized RM101-induced respiratory depression by doubling plasma RM101 concentrations needed to decrease ventilation with 50%.

Developments in the prevention of opioid-induced respiratory depression (OIRD) focus either on the reversal or prevention of OIRD through the co-administration of respiratory stimulants or on the development of an opioid without/with reduced effect on ventilation. Recently tested non-opioid respiratory stimulants are for example ampakines, 5HT receptor agonists, phosphodiesterase inhibitors and potassium channel blockers.[5,9-11] Opioids with reduced respiratory effects often act at multiple opioid or receptor systems or at opioid and non-opioid receptor systems.[5] Examples of the former are cebranopadol, a MOR and NOP receptor agonist that displays ceiling in respiratory depression due to a respiratory stimulatory effect due to NOP activation.[8] Another example of the latter is tapentadol, a MOR agonist that also inhibits cellular noradrenaline reuptake. Noradrenaline acts at alpha$_2$-adrenergic receptors within the spinal

dorsal horn. The activation of MOR and adrenergic pathways produces synergistic analgesic effects with a reduced side effect profile.[12] RM101 produced respiratory depression. Although Fig. 1D suggests ceiling in respiratory effect, our PK-PD analysis could not confirm this. Hence our results show that RM101 behaves differently from either buprenorphine or cebranopadol, although the respiratory effects are best viewed in light of RM101's analgesic effects. To that end analgesic studies (preferably performed in the same subject population but certainly in the same laboratory) need to be added allowing the construction of so-called safety or utility functions that estimate the probability of analgesia minus the probability of respiratory depression and consequently allow comparison among opioids.[13-15]

The study naloxone reversal of OIRD is highly relevant. Some opioids with a high affinity for the MOR are resistant to naloxone reversal (such as buprenorphine).[16,17] Moreover, since naloxone has a short half-life (15-20 min), renarcotization is possible following naloxone administration, especially when dealing with opioids with a slow onset and offset (such as morphine and buprenorphine).[5] Naloxone is a non-selective competitive antagonist at the μ-opioid receptor (MOR) with fast receptor association and dissociation kinetics.[18] In this study, naloxone was administered in four doses of 0.4 mg, with 4 minutes between each administration. The naloxone dose to reverse RM101-induced respiratory depression ranged from 0.4 to 1.6 mg. Importantly, in two subjects the respiratory depression was not reversed by naloxone. This could be a dosing effect with higher naloxone doses (> 1.6 mg) needed to cause reversal in these two subjects. This then suggests a relative high affinity of RM101 for the MOR (at least in these two receptors), similar to our earlier observation with buprenorphine, but more research is needed to fully understand this issue.

We estimated a value for $t_{1/2}k_{eOP}$ (time constant of the dynamic ventilatory response to CO_2) of 10.4 ± 2.7 min. This is relatively long compared to previous observations of a half-life of about 2-3 min. We have no explanation for this discrepancy. The ventilatory CO_2 half-life is determined by (1) cardiac output, (2) brain blood flow and wash-in of CO_2 into the brain compartment and (3) neuronal dynamics. Any of these factors may have been affected in our studies, possibly related to an interaction with the tested opioid.
We performed the study at a clamped end-tidal pCO_2 but due to the sometimes brisk respiratory effects, end-tidal control was suboptimal and end-tidal pCO_2 occasionally inclined above the clamped target value. The fluctuations may have underestimated the effect of RM101 but since they were rather small we argue that the effects were minimal.

In conclusion, the novel opioid analgesic RM101 produced respiratory depressant effects due to a rapid effect on ventilation and a slower effect on CO_2 sensitivity, without ceiling. Naloxone reversal was observed in most but not all subjects, which is best explained by a dosing effect and possibly a high affinity of the drug for the MOR. More research is needed to determine the utility or safety function of RM101 and to determine the naloxone dose that reverses RM101's respiratory effect in all subjects.

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Chapter 7

General Discussion, Summary and Conclusions

Chronic pain is quite prevalent in the Western world with an incidence of about 18% in the Netherlands, similar to estimates from other European countries and the United States of America (USA).[1] This indicates that on a population of 17,1 million, there are about 3 million pain patients in the Netherlands. Treatment of chronic pain generally consists of non-pharmacological interventions, for example physical or behavioural therapy, often combined with medication such as non-steroidal anti-inflammatory drugs or opioids. The intention of pain treatment in patients with moderate to severe acute and chronic pain is to reduce their symptoms and improve their quality of life. Opioids alleviate pain, however they can cause side effects which may be life threatening. Both in the US and the Netherlands there is a sharp rise in the prescription of opioids.[2-5] In the Netherlands, from 2004 to 2013, the number of opioid users doubled to 1 million individuals.[2] The majority of patients (80%) received an opioid for treatment of non-cancer pain, predominantly musculoskeletal pain such as lower back pain. Recent data show a further increase in opioid consumption in the Netherlands to 1,2 million users or 7% of the population in 2016. In the US the epidemic has led to a rise in drug deaths with 16,000 to 20,000 prescription opioid deaths per year.[3,5] Recent preliminary data suggest that in 2016 59,000-65,000 people died from drug overdoses in the US, of which more than 20,000 people died from fentanyl overdose.[6] Numbers of drug deaths in the Netherlands due to opioids are not known.

Deaths due to opioids are related to diversion, abuse, addiction and combining opioid use with other centrally acting drugs such as alcohol and benzodiazepines. The cause of death is almost always cardiorespiratory collapse secondary to opioid-induced respiratory depression and cardiac arrest. Due to the rise in opioid-related mortality, the development of a treatment for opioid-induced respiratory depression (OIRD) is necessary. Nearly half of all opioid overdose deaths involve a prescription opioid, all other overdose deaths occur due to the illicit use of illegal opioids and heroin.[7] Currently, in the United States (US) a campaign is launched (Turn The Tide Rx), which includes a pledge to educate clinicians to treat pain safely and effectively, screening patients for opioid use disorder and provide them with evidence-based treatment.[8] Although prevention is the first step to reduce mortality caused by OIRD, the only available treatment of OIRD is administration of naloxone. Naloxone is a non-selective opioid receptor antagonist which inhibits all pharmacological effects of opioids, benefit (analgesia) and harm (side effects such as OIRD). The extent and the duration of naloxone reversal of OIRD is highly variable and related to many factors as the specific opioid used, dose administered, administration mode, concurrent medication, underlying disease and the exogenous stimulatory factors.[9] With all opioids that have a longer plasma half-life than naloxone, there is a chance of recurrence of the OIRD, *i.e.* renarcotization. However this can be prevented by continuous administration of naloxone.[10] In the US firefighters, paramedics, police officers and relatives of opioid addicts carry naloxone in their cars in a FDA-approved nasal spray. The FDA approved naloxone nasal spray in 2015. Intranasal administration of naloxone demonstrated to be as effective or exceed intramuscular administration of naloxone.[11]

Due to the disadvantage of complete reversal of opioids activity by naloxone (OIRD as well as analgesia), there is a need for treatment of OIRD by non-opioid drugs as potassium channel blockers, AMPAkines and 5-HT receptor agonists. These drugs may restore breathing to an acceptable level, or even prevent OIRD, without affecting analgesia. The goal of this thesis was to study the ability to reverse OIRD with respiratory stimulants and to study drugs that

might prevent OIRD. In this final chapter, I will give a summary of the observations made during my thesis project. Here after, I will discuss the benefit-harm composite of drugs, followed by a recapitulation of the possible treatments of OIRD. Finally, the conclusions of this thesis are presented.

SUMMARY

In **chapter 2** the effect of the TASK1/TASK3 channel blocker doxapram was investigated on ventilation, analgesia and cardiac output. Eight healthy volunteers were included and a stepped-infusion of doxapram or placebo infusion was combined with alfentanil infusion on two separate occasions in random order. During administration poikilocapnic (ambient air) ventilation, cardiac output and analgesia were measured. Ventilation was measured breath-to-breath, cardiac output with a FloTrac/Vigileo connected to an arterial line in the radial artery and analgesia with a transcutaneous electrical stimulation (TES) on the lower leg. Arterial plasma samples of alfentanil and doxapram were taken. A PK-PD model was constructed in which the doxapram plasma concentrations (PK) were related to cardiac output (CO), whereafter the CO was related to alfentanil plasma concentrations (PK) and subsequently to pain responses (a PK-PD/PK-PD analysis). Compared to placebo treatment, alfentanil plasma concentration was reduced by 14% during low and 26% during high dose infusion of doxapram. This induced a reduction in antinociception of 25%. Our PK-PD model demonstrated that the reduction in alfentanil concentration is well explained by the doxapram-induced increase in CO due to increased intercompartmental and elimination clearances. Due to the reduction in alfentanil plasma concentration, there was a reduction in the level of antinociception.

In Chapter 3 and Chapter 4 the efficacy to reverse alfentanil-induced respiratory depression of novel agent GAL021 is determined. GAL021 inhibits calcium-activated potassium channels at the carotid bodies. In **Chapter 3** we describe the effect of GAL021 on ventilation, hemodynamic parameters and antinociception. Chapter 3 describes two studies: the first study determined the effect of GAL021 on alfentanil-induced respiratory depression under isohypercapnic conditions, in the second study the effect of GAL021 on poikilocapnic (ambient air) ventilation and other variables as hemodynamic effects, antinociception, sedation and adverse events were determined. In total, twelve healthy volunteers were included and received a stepped-drug infusion of GAL021/placebo and alfentanil. As in chapter 2, ventilation was measured on a breath-to-breath basis, analgesia with TES on the lower leg and cardiac output monitoring with a FloTrac/Vigileo on an arterial line in the radial artery. Sedation was measured with a NRS (numerous rating scale). In study 1, GAL021 showed to increase ventilation with 6.1 (3.6-8.6) L/min and 3.6 (1.5-5.7) L/min during high GAL021 infusion, combined with low alfentanil infusion, respectively high alfentanil infusion. This effect was due to an increase in respiratory rate and tidal volume. Poikilocapnic ventilation and arterial pCO_2 showed a significant difference with GAL021 *vs.* placebo treatment in the second study. No major side effects occurred during the study. No treatment differences were observed for plasma alfentanil concentrations, blood pressure, cardiac output, pain threshold and sedation. These data demonstrates stimulatory effect of GAL021 on ventilation and pCO_2 without serious adverse effects and might be used as a respiratory stimulant in the future.

To further understand the novel molecule GAL021, **chapter 4** describes a pharmacokinetic-

pharmacodynamic (PD-PD) analysis of the effect of GAL021 on alfentanil-induced respiratory depression in twelve healthy volunteers. It is a sub-analysis of the study described in Chapter 3. The data were analyzed with three different PK-PD models. Model 1 assuming a multiplicative interaction between alfentanil and GAL021 without ceiling in the effect of GAL021 on ventilation, Model 2 assuming a multiplicative interaction with ceiling in the effect of GAL021 on ventilation and Model 3 assuming an additive interaction between alfentanil and GAL021. Model selection was based on the minimum objective function (MOF) value, which favored the model in which GAL021 shows ceiling in its efficacy to reverse respiratory depression. This suggests that GAL021 efficacy is reduced at low ventilation levels. GAL021 onset/offset times are rapid with a blood-effect site equilibration half-life not different from zero.

In **chapter 5** a utility function of alfentanil is described. The utility function is developed to determine the opioid's toxicity (harm as respiratory depression, sedation, dizziness) in light of its benefit (analgesia) and to define an optimal dosing regimen. In this study the concept of utility function was further developed with the determination of the probabilities of four distinct conditions: probability of presence of benefit (analgesia) with or without severe toxicity (respiratory depression) and the probability of the inadequacy of benefit (inadequate analgesia) with or without severe toxicity, where the probability of analgesia was defined as $P(A > 0.25)$ and $P(A > 0.5)$ as a 25 and 50% increase in tolerated electrical current and $P(R > 0.5)$ as a 50% reduction in minute ventilation. In this analysis, the data of three previous PK-PD analysis were combined and forty-eight subjects were included in the analysis. The analysis demonstrated that the optimum analgesia probability without serious respiratory depression is reached at an effect-site concentration of 60 ng/mL. With increasing the effect-site concentration of alfentanil the probability of serious respiratory depression prevails.

Chapter 6 describes a population PK-PD modelling study in healthy volunteers to determine the effect of a novel opioid analgesic, RM101, on isohypercapnic ventilation. Furthermore, we analyzed the possibility to reverse the respiratory effect with naloxone. As previous mentioned, ventilation was measured on a breath-to-breath analysis in an isohypercapnic condition where after RM101 was administered in a dose-escalating manner. RM101 demonstrated to have a dual effect on ventilation, first a rapid effect on the minute volume, followed by a slower effect on CO_2 sensitivity, which did not to have a ceiling effect. Naloxone reversal was observed in most but not in all subjects, which is best explained by a dosing effect and possibly a high affinity of RM101 on MOR.

TREATMENT OF OIRD WITH NON-OPIOID DRUGS

Opioid-induced analgesia and respiratory depression arise from stimulation of μ-opioid receptors (MORs). Due to stimulation of MORs there is an increase in pCO_2 and a reduction in tidal and minute volume.[12] Partial pressure carbon dioxide (pCO_2) was the first respiratory stimulant discovered by Haldane. He concluded that the respiratory center adjusts to changes in arterial pCO_2, which was transferred to the respiratory center through arterial blood.[13] However, an increase in pCO_2 could cause depression of the central nervous system and administration of CO_2 for the treatment of respiratory depression did not become common practice. Since 1960s multiple drugs have been designed to alleviate OIRD with varying results, as caffeine, aminophylline, atropine, almitrine.[10,14,15] Most promising drugs are 5-hydroxytryptamine (5-HT,

serotonin) receptor agonists, ampkakines and potassiumchannel blockers as doxapram and GAL021. These respiratory stimulant drugs manipulate neuronal transmission to stimulate respiratory drive.

Serotonin receptor ligands are involved in multiple neuromodulatory processes in the nervous system. 5-HT is a respiratory stimulatory neurotransmitter. Different 5-HT receptor agonist demonstrated to alleviate OIRD in animals without an effect on antinociception.[16-19] Manzke et al. demonstrated that one half of all 5-HT4A receptors in the pre-Botzinger complex co-expressed MORs and stimulation of the 5-HT4A receptors alleviated fentanyl-induced respiratory depression in rats and maintained a stable respiratory rhythm without loss of fentanyl's analgesic effect.[16] Multiple 5-HT ligands have been tested, but the availability for human administration is limited. Two studies were performed, one with mosapride, a 5-HT4 receptor agonist and one with buspirone, a 5-HT1A receptor agonist. Mosapride failed to prevent morphine-induced respiratory depression in a double-blind cross over study in human volunteers, while combining morphine with naloxone demonstrated a reversibility in morphine-induced respiratory depression. The authors of the study explain the negative result by decreased plasma concentrations in the brain versus abdominal plasma concentrations after oral administration.[20] Buspirone (60 mg) co-administration with morphine did not prevent respiratory depression but led to a significant increase in intensity of nausea. Higher buspirone dosing was not possible due to the nausea. However, when comparing data to animal data (intravenous administration), a PKPD analysis demonstrated the negative result might be due to low effect site concentrations after oral ingestion.[21]

Ampakines are a group of compounds which modulate the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionis acid) receptor by increasing the duration of inward currents of glutamate.[22] It is hypothesized that ampakines modulate the central respiratory drive by glutamate-mediated transmission in the pre-Botzinger complex to generate respiratory rhythm through an increase in glutamate-mediated excitatory inspiratory drive.[23-25] AMPA receptor modulators do not interact with the receptor as an agonist, but binds to an allosteric site within the glutamate receptor complex. Different classes of AMPA receptor modulators have been described, but the most promising group are the benzamides (also known as ampakines; CX516, CX546, CX614, CX717).[10] CX717 is the most studied ampakine. It demonstrated to alleviate fentanyl-induced respiratory depression (60 µg/kg) without significantly altering analgesia in rats and when administered prior to fentanyl, it prevented the fentanyl-induced respiratory depression.[26] In vivo administration of CX717 with propofol provided protection against respiratory depression, but CX717 was not effective in rescuing propofol-induced respiratory effects.[27] In humans, CX717 was studied in a double-blind, placebo-controlled, crossover study to access the effect of pre-treatment with CX717 combined with alfentanil in a target concentration of 100 ng/mL. Oertel et al. demonstrated CX717 to prevent respiratory depression without effecting analgesia. Ventilatory response to hypercapnic challenge (VE55) was alleviated by CX717 (41% *vs* 54%) without effecting antinociception. However, CX717 led to a significantly more pronounced increase in sedation. Due to the oral formulation CX717 cannot be used in the peri-operative setting with a peak plasma concentration after 2 hours.[28] Potassium channel blocker doxapram is one of the oldest respiratory stimulant known. Doxapram inhibits background K^+ channels (TASK1, TASK 3, TASK 1/3) on type 1 carotid body cells. In response to hypoxia the carotid body glomus cells (type 1) are involved in a signaling

protein cascade with closure of the oxygen-sensitive K^+ channels, followed by depolarization of the membrane of the carotid body type 1 cells and Ca^{2+}-dependent neurotransmitter release.[29] Other channels possibly involved in the hypoxia-sensitive K^+ current in glomus cells are the Kv and BK channels, but the signaling pathways of the inhibition of hypoxia are not well defined (Kim, AMP on TASK 1-3 and Ca concentration ,2014). Antagonism of the hypoxia-sensitive K^+ current stimulates respiration. The effect of doxapram on ventilation is described in multiple species and it thought to increase ventilation predominantly due to an increase in tidal volume with little effect on respiratory rate.[14] After total intravenous anesthesia, doxapram showed to fasten arousal, return to spontaneous breathing, extubation with increased tidal volumes and respiratory rate in 90 patients for laparoscopic surgery.[30] In patients with obstructive sleep apnea doxapram 100 mg was administered intravenously before extubation after bariatric surgery with total intravenous anesthesia. Patients with doxapram had lower sedation scores, shorter mean recovery room stay and no patients developed post-operative respiratory complications (atelectasis, bronchospasm, hypopnea) *versus* 29% in the placebo group.[31] Doxapram was originally used as an analeptic with sympaticoexcitation, sweating, convulsions, anxiety, panic attacks, hypertension and increased cardiac output. In our study (Chapter 2), no respiratory effects of doxapram on alfentanil-induced respiratory depression were observed. Recently, two other TASK channel antagonist were discovered (PK-THPP and A1899). The magnitude of effect of PK-THPP and A1899 showed to exceed that of doxapram without any effect on mean arterial blood pressor.[32] These drugs have not yet been studied on human volunteers.

GAL021 is a novel potassium channel blocker. It blocks the BK_{Ca} channel. GAL021 stimulates ventilation and attenuates opioid-induced respiratory depression without effecting analgesia (Chapter 4 and [33,34]). GAL021 reverses drug-induced respiratory depression by isoflurane, propofol and midazolam. Ventilatory stimulation is accompanied by enhanced carotid sinus nerve activity, although carotid nerve transection does not completely abolish the respiratory effect of GAL021.[35] More studies need to be performed with GAL021 to determine the clinical applicability.

A novel development in the possible prevention of OIRD is the treatment of analgesia with biased ligands.[36] Biased ligands are ligands that selectively promotes pharmacological responses that differ from traditional agonist and circumvent the opioid respiratory pathway.[37] The biased ligands are envisioned to change the classic two-state model of receptor function (agonist, antagonist, later partial agonist and inverse agonist) to a more specific pharmacologic profile. For MOR a biased ligand is currently studied: TRV130. It is a biased ligand of the MOR that activates G protein signaling with little β-arrestin recruitment. In rodents TRV demonstrated potent analgesia with less gastro-intestinal dysfunction and respiratory depression in equianalgesic doses compared to morphine.[38] In healthy volunteers the effect of ligand-biased TRV130 has been analyzed for analgesic properties, respiratory depression has been analyzed with surrogated markers as oxygen saturation and capnometry. This study, not powered to determine the respiratory effects, demonstrated smaller reductions in hypercapnia-induced ventilatory thrive than morphine.[39] Yet the respiratory effects need to be studied in the future.

A BENEFIT-HARM COMPOSITE OF DRUG EFFECT

One way of understanding the complex behavior of drugs that produce wanted effects and

unwanted side effects is by integrating benefit and harm into a risk-benefit composite as a utility of safety function. Previously the drug toxicity was expressed as the therapeutic index, which equals the ratio of TD_{50} (dose causing toxicity in 50% of the population) and ED_{50} (dose causing benefit in 50% of the population). Sheiner and Melmon introduced a concept derived from economic decision theory that allows combining of different end-points into one number, the utility, which they defined as the benefit of a drug minus the harm it produces.[40] They applied their concept to describe the benefit and harm of antihypertensive therapy. The concept was later used by Cullberg to define the outcome of anti-thrombin therapy.[41] The utility function (UF) of opioids is defined as the probability of opioid analgesia minus the probability of OIRD, where the probability of analgesia (P(A)) is defined as an increase in pain tolerance of ≥ 50 % after electrical or thermal stimulus and the probability of OIRD (P(R)) as a reduction in minute ventilation of ≥ 50%.

$$UF = P(A \geq 50\%) - P(R \geq 50\%)$$

Thusfar some opioids have been described by a UF.[42-45] The UF is context-sensitive, meaning that the function will differ depending on the definition of analgesia and respiratory depression. Previous UFs have chosen to describe analgesia with a P(A>50%), which is an increase in pain tolerance of 50% of more. In chapter 5 we described a UF where we have described analgesia with P(A>50%) and P(A>25%). The UF might allow comparison among drugs to determine the best opioid prescription for each patient.

After a bolus of 0.13 mg/kg morphine, the UF comparing the probability of analgesia versus the probability of respiratory depression was positive, which indicates that the probability of developing analgesia exceeds the probability of developing respiratory depression.44 For fentanyl (i.v. bolus of 3.5 µg/kg) the UF was negative in the first 90 minutes after fentanyl administration (probability for respiratory depression was higher than for analgesia). After 90 minutes the probability of both end-points was in the same order of magnitude.43 Alfentanil had a biphasic UF after an intravenous bolus of 50 µg/kg. In the first 6 minutes after alfentanil administration the UF was negative compared to a positive UF at 22 minutes after the administration. When the UF was used to compare the probability of analgesia and respiratory depression between oxycodone immediate release 20 mg and tapentadol 100 mg it was clearly demonstrated that the UF of tapentadol was beneficial over the UF of oxycodone. The UF of oxycodone was negative in the first 3 hours after administration (higher probability of respiratory depression than of analgesia), after which the probability returned to zero (equal probability of respiratory depression and analgesia). The UF of Tapentadol was zero over the whole study period independent of time of administration.46 Besides comparing different opioids with the UF after a bolus administration, the UF can describe the probability as a function of plasma concentrations of the desired opioid. To determine this UF, pharmacokinetic parameters are necessary to define the effect-site concentration of the opioid. For example, we showed that when the plasma concentration of morphine is higher than 5 ng/mL the probability for analgesia exceeds that of respiratory depression, which continues to be positive up to a plasma concentration of 30 ng/mL. On the other hand, the UF of fentanyl is only positive up to a plasma concentration of 0.6 ng/mL. At higher plasma concentrations the UF becomes negative, which indicates that the probability for respiratory depression exceeds the probability for analgesia. Alfentanil had a positive UF over the effect-site concentration range of 0 – 300 ng/mL.

Although equianalgesic doses of oral opioids *versus* intravenous opioids is difficult to compare, comparison based on the utility function suggests that tapentadol and morphine have a more favorable respiratory profile compared to the other opioids. However the desired outcome of opioid treatment, i.e. analgesia, can be objectified with multiple assays as an electrical current, pressure or thermal measurements. At this moment, only the UF of fentanyl is analyzed with a thermal and an electrical assay, demonstrating more negative UF with thermal assay *versus* electrical assay. For comparison between different opioids, the analgesia effect of the opioid should be measured with a comparable nociceptive assay.
The disadvantage of the above mentioned formulation of the UF is that a positive UF does not mean that respiratory depression does not occur. Therefore, in chapter 5 we have remodeled the UF and added the probability of adequate analgesia without severe respiratory depression (U2, desirable condition) and the probability of inadequate analgesia with severe respiratory (U3, least desirable condition). This modification of the original UF has not been calculated for other opioids.
Thus far, the UF is based on data obtained in a healthy population (i.e. volunteers without pain) and are not calculated in patients with opioid treatment. Further studies should address the utility of different opioids in chronic pain patients or patients with postoperative pain to further objectify the UF and support the comparison of opioid treatment in patients.

CONCLUSIONS

From the data presented in this thesis, the following can be concluded:

1) Doxapram reduces alfentanil-induced antinociception, which was related to an increase in alfentanil distribution and elimination. These changes might be best explained by the doxapram-induced increase in cardiac output.

2) GAL021, a novel calcium-activated potassium channel blocker, produces an increase in tidal volume and respiratory rate during opioid-induced respiratory depression without affecting the obtained opioid analgesic effect

3) GAL021 efficacy on alfentanil-induced respiratory depression reduces at low ventilation levels and demonstrates ceiling in its efficacy to reverse opioid-induced respiratory depression

4) The utility function of alfentanil is further developed to assess specific conditions in terms of wanted and unwanted effects (analgesia with or without respiratory depression, no analgesia with or without respiratory depression). This refined utility function may be used to compare the respiratory effects of analgesics and might allow for comparison between different opioid treatments.

5) RM101 causes respiratory depression in all subjects with a rapid effect on ventilation and a slower effect on CO_2 sensitivity. Since complete reversal of the respiratory effect of RM101 is not observed in all subjects, RM101 might have a high affinity for the μ-opioid receptor.

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Chapter 8

Algemene Discussie, Samenvatting en Conclusies

Chronische pijn komt in de Westerse wereld frequent voor met een incidentie van 18% in Nederland, dit is gelijk aan schattingen in andere Europese landen en de Verenigde Staten (VS).[1] Op een bevolking van 17,1 miljoen mensen in Nederland zijn er 3 miljoen patiënten met pijn. De behandeling van chronische pijn is vaak door niet-farmacologische behandeling, zoals cognitieve of gedragstherapie, gecombineerd met medicatie zoals niet-steroide anti-inflammatoire geneesmiddelen of opioiden. In patienten met matige tot ernstige acute en chronische pijn is het doel van de behandeling om de symptomen te verlichten en de kwaliteit van leven te verbeteren. Opioïden zijn effectieve pijnstillers, maar gaan gepaard met bijwerkingen die levensbedreigend kunnen zijn. Zowel in de VS als in Nederland is er een forse toename in de hoeveelheid voorgeschreven opioiden.[2-5] In Nederland is het aantal gebruikers van opioïden tussen 2004 - 2013 verdubbeld tot 1 miljoen gebruikers.[2] De meeste patienten (80%) gebruiken opioïden voor pijn niet veroorzaakt door maligniteiten, veelal voor musculoskeletale pijn als lage rugpijn. Recente data toont verdere toename in het gebruik van opioiden in Nederland tot 1.2 miljoen gebruikers of 7% van de totale populatie in 2016. In de VS is er een toename in drugs/medicatie gerelateerde doden, in totaal zijn er 16.000 – 20.000 doden op basis van voorgeschreven opioïden per jaar geregistreerd.[3-5] Recente voorlopige resultaten suggereren dat in 2016 er 59.000 – 65.000 doden waren op basis van overdosis door drugs of medicatie, waarvan er meer dan 20.000 zijn overleden op basis van fentanyl overdosering.[6] Hoeveel personen in Nederland zijn overleden door opioïden is niet bekend.

Overlijden door opioïden is gerelateerd aan misbruik, verslaving en het gecombineerd gebruik van opioïden met andere centraal werkende medicatie zoals alcohol en benzodiazepines. De oorzaak van het overlijden is bijna altijd door cardiorespiratoire collaps op basis van opioïd-geïnduceerde ademdepressie (OIRD). Door de toename van opioïd gerelateerde mortaliteit is de ontwikkeling van een behandeling tegen OIRD nodig. Bijna de helft van alle overdoses op basis van opioïden zijn voorgeschreven door artsen, de overige komen door illegale opioïden en heroïne.[7] Momenteel is er in de VS een campagne (Turn the Tide Rx) om onderwijs te geven over de veilige en effectieve behandeling van pijn aan artsen, leren hoe opioïd verslaafden te herkennen zijn en hoe zij te behandelen zijn.[8] Hoewel preventie de eerste stap is in het verminderen van mortaliteit door OIRD, is momenteel de enige beschikbare behandeling met medicatie (naloxon). Naloxon is een niet-selectieve opioïd receptor antagonist wat alle farmacologische activiteiten van opioiden remt; de voordelen (analgesie) en de nadelen (bijwerkingen zoals OIRD). De mate en de duur van het effect van naloxon op ademhaling is erg variabel en gerelateerd aan vele factoren als het gebruikte opioïd, gebruikte dosis, manier van toediening, andere medicatie, onderliggende ziekte en exogene stimulantia.[9] Alle opioïden met een langere plasma halfwaardetijd dan naloxon, hebben een kans op het terugkeren van de OIRD, *i.e.* renarcotizeren. Dit kan voorkomen worden door een continue toediening van naloxon.[10] In de VS hebben brandweermannen, ambulance broeders, politieagenten, familie en vrienden van opioïd verslaafde naloxon in hun auto in de vorm van een FDA-goedgekeurde neusspray. De FDA heeft de naloxon neusspray goedgekeurd in 2015. Intranasale toediening van naloxone is even effectief of zelfs effectiever dan intramusculaire toediening van naloxon.[11]

Door het volledig antagoneren van het effect van opioïden door naloxon (zowel OIRD als analgesie) is er behoefte aan een behandeling van OIRD door niet-opioïde geneesmiddelen als kalium kanaal blokkers, AMPAkines en 5-HT receptor agonisten. Deze geneesmiddelen kunnen mogelijk de ademhaling herstellen tot een bepaald niveau of OIRD voorkomen zonder

effect te hebben op analgesia. Het doel van dit proefschrift was om de mogelijkheden te onderzoeken om OIRD te voorkomen met geneesmiddelen die ademhaling stimuleren en om geneesmiddelen te onderzoeken die mogelijk OIRD kunnen voorkomen.
In dit laatste hoofdstuk zal ik een samenvatting geven van de uitgevoerde studies tijdens mijn promotie onderzoek. Hierna zal ik analyse om de voor-/nadelen van verschillende geneesmiddelen bespreken en een samenvatting geven van de mogelijke (experimentele) behandelingen van OIRD. Uiteindelijk presenteer ik de conclusies van mijn proefschrift.

SAMENVATTING

In **hoofdstuk 2** is het effect van TASK1/TASK3-kanaal blokker doxapram beschreven op ventilatie, analgesie en hartminuutvolume. Acht gezonde vrijwilligers zijn geïncludeerd in de studie en hebben een gefaseerde infusie van doxapram of placebo ontvangen in combinatie met alfentanil op twee verschillende onderzoeksdagen. Tijdens het toedienen van de geneesmiddelen is de poikilocapnische (lucht zoals in dagelijks leven) ventilatie, hartminuutvolume en analgesia gemeten. De ventilatie is gemeten op breath-to-breath (*i.e.* iedere ademhalingsteug werd gemeten), hartminuutvolume door een FloTrac/Vigileo met een arterielijn in de arteria radialis en analgesia met een transcutane electrische stimulatie (TES) op het onderbeen. Arteriele plasma monsters van alfentanil en doxapram zijn afgenomen. In het PK-PD model is de plasma concentratie (PK) van doxapram van invloed op het hartminuutvolume (HMV), waarna het HMV van invloed is op de alfentanil plasma concentratie (PK) en dit uiteindelijk de analgesie beïnvloedt (een PK-PD/PK-PD model). Tijdens doxapram infusie in vergelijking met een placebo infusie was de alfentanil plasma concentratie verminderd met 14% tijdens lage dosering doxapram en 26% tijdens hoge dosering doxapram. Dit leidden tot een vermindering in analgesie van 25%. Het PK-PD model toont dat de vermindering in alfentanil concentratie verklaard kan worden door de doxapram-geïnduceerde toename in hartminuutvolume door een toename in intercompartmentale en eliminatie klaring. Door een vermindering in alfentanil plasma concentratie was er een vermindering in analgesie.

In hoofdstuk 3 en hoofdstuk 4 is de mogelijkheid onderzocht om de door alfentanil-geïnduceerde ademdepressie ongedaan te maken door toediening van GAL021. GAL021 remt de calcium-geactiveerde kalium kanalen in de receptoren in de carotiden. In **hoofdstuk 3** beschrijven we het effect van GAL021 op ventilatie, hemodynamische parameters en analgesie. Hoofdstuk 3 beschrijft twee studies: de eerste studie beschrijft het effect van GAL021 op alfentanil-geïnduceerde ademdepressie tijdens isohypercapnische (een op een vast niveau gefixeerde verhoogde pCO_2) condities en de tweede studie het effect van GAL021 op poikilocapnische ventilatie en andere variabele als hemodynamische parameters, analgesia, sedatie en bijwerkingen. In totaal zijn er twaalf proefpersonen geïncludeerd in de studie en hebben zij een stapsgewijze infusie gekregen van GAL021/placebo en alfentanil. Net als in hoofdstuk 2 is ventilatie gemeten op basis van iedere ademhaling, analgesie met TES en hartminuutvolume met een FloTrac/Vigileo monitor en een arterielijn in de arteria radialis. Sedatie is gemeten met een NRS (numerous rating scale, numerieke schaalverdeling). In studie 1 zorgde GAL021 voor een toename in ventilatie van 6.1 (3.6-8.6) L/min en 3.6 (1.5-5.7 L/min) tijdens hoge dosering GAL021 gecombineerd met lage alfentanil toediening danwel hoge alfentanil toediening. Dit effect werd veroorzaakt door een toename van ademhalingsfrequentie en teugvolumes. Poikilocapnische ventilatie en arteriele pCO_2 toonde een significant verschil tijdens GAL021 behandeling *vs.* placebo behandeling in de tweede studie. Er traden geen benoemenswaardige

bijwerkingen op tijdens de studie. Er werd geen verschil geobjectiveerd in alfentanil plasma concentraties, bloeddruk, hartminuutvolume, analgesie of sedatie. Deze data tonen een stimulerend effect van GAL021 op ventilatie en pCO_2 zonder serieuze bijwerkingen en GAL021 kan mogelijk in de toekomst worden gebruikt als een geneesmiddel om ademhaling te stimuleren.

Om meer over het experimentele medicijn GAL021 te weten te komen beschrijft **hoofdstuk 4** de farmacokinetische-farmacodynamische (PK-PD) analyse van het effect van GAL021 op alfentanil-geïnduceerde ademdepressie in twaalf gezonde vrijwilligers. Het is een subanalyse van de studie beschreven in hoofdstuk 3. De data zijn geanalyseerd door middel van drie verschillende PK-PD modellen. Model 1 gaat uit van een multiplicatieve interactie tussen alfentanil en GAL021 zonder 'ceiling' (plafond effect) in het effect van GAL021 op ventilatie, model 2 gaat uit van een multiplicatieve interactie met ceiling in het effect van GAL021 op ventilatie en model 3 gaat uit van een additief effect tussen alfentanil en GAL021. De selectie van het model (Model 2) is gebaseerd op de minimum objective function (MOF) waarde, waarbij GAL021 ceiling toont in het antagonere van de ademdepressie. Dit suggereert dat de effectiviteit van GAL021 is verminderd bij lage ademminnutvolumes. De effecten van GAL021 treden snel op, de bloed-effect equilibratie tijden zijn gelijk aan met nul.

Hoofdstuk 5 beschrijft een utility functie van alfentanil . De utility functie is bedacht om de toxiciteit van een opioïd (bijwerkingen als ademdepressie, sedatie, duizeligheid) *vs.* het voordeel (analgesie) te bepalen en een ideaal doseringsschema te definiëren. In deze studie hebben wij het concept utility functie verder ontwikkeld door de kans op vier verschillende mogelijkheden te formuleren: de kans op de aanwezigheid van het voordeel (analgesie) met of zonder ernstige toxiciteit (ademdepressie) en de kans op het niet aanwezig zijn van het voordeel (geen analgesie) met of zonder ernstige toxiciteit (ademdepressie), waarbij de kans op analgesie gedefiniëerd was als $P(A > 0.25)$ en $P(A > 0.5)$ zijnde 25 en 50% toename in toegediende elektrische pijnprikkel en $P(R > 0.5)$ zijnde 50% afname in ademminuutvolume. Voor deze analyse is de data van drie eerdere PK-PD analyses gecombineerd waarbij er achtenveertig proefpersonen zijn geïncludeerd in de analyse. De studie toonde dat de grootste kans op analgesie zonder ernstige ademdepressie bereikt wordt bij een effect-site concentratie van 60 ng/mL. Met een oplopende alfentanil effect-site concentratie neemt de kans op ernstige ademdepressie toe.

Hoofdstuk 6 beschrijft een PK-PD studie in gezonde vrijwilligers om het effect van een nieuw opioïd, RM101, op isohypercapnische ventilatie te bepalen. Verder analyseren we het effect om de ademdepressie veroorzaakt door RM101 te antagoneren met naloxon. Ventilatie werd gemeten op basis van iedere ademhaling in een isohypercapnische conditie waarna RM101 werd toegediend in een oplopende dosis. RM101 toonde op twee manieren effect te hebben op ventilatie; als eerste een snel effect op het ademminuutvolume, gevolgd door een langzaam effect op CO_2 gevoeligheid, wat geen ceiling effect toonde. Door naloxon werd de ademdepressie geantagoneerd in veel, maar niet in alle proefpersonen. Dit kan het beste worden uitgelegd door een dosis effect en mogelijk door een hogere affiniteit van RM101 op de MOR.

BEHANDELING VAN ADEMDEPRESSIE MET NIET-OPIOIDE GENEESMIDDELEN

Opioïd-geïnduceerde analgesie en ademdepressie komen door stimulatie van de μ-opioid receptor (MOR). Door stimulatie van de MOR is er een toename in pCO_2 en een vermindering in teug- en ademminuutvolume.[12] Het eerste geneesmiddel, uitgevonden door Haldane, wat ademhalings stimuleerde was pCO_2. Hij concludeerde dat de respiratoire centra in de hersenen reageerden op veranderingen in arteriele pCO_2.[13] Echter veroorzaakte een toename in pCO_2 depressie van het centrale zenuwstelsel en het gebruik van CO_2 voor de behandeling van ademdepressie werd niet meer toegepast. Sinds 1960 zijn er meerdere geneesmiddelen met wisselend succes ontworpen om opioid-geinduceerde ademdepressie te verminderen, zoals caffeïne, aminofylline, atropine en almitrine.[10,14,15] Meest belovende geneesmiddelen zijn de 5-hydroxytryptamine (5-HT, serotonine) receptor agonist, ampakines en kaliumkanaal blokkers als doxapram en GAL021. Deze respiratoire stimulerende geneesmiddelen manipuleren neuronale transmissie om de ademprikkel te stimuleren.

Serotonine receptor agonisten zijn betrokken bij meerdere neuromodulerende processen in het zenuwstelsel. 5-HT is een respiratoire stimulerende neurotransmitter. Verschillende 5-HT receptor agonisten hebben in dieren aangetoond om opioïd-geïnduceerde ademdepressie te verminderen zonder een effect te hebben op analgesie.[16-19] Manzke et al. toonde aan dat de helft van alle 5-HT4A receptoren in de pre-Botzinger complex ook MORs hebben en door stimulatie van de 5-HT4A receptoren fentanyl-geïnduceerde ademdepressie verminderd werd zonder effect te hebben op de analgetische effecten van fentanyl in ratten.[16] Meerdere 5-HT agonisten zijn getest, maar de beschikbaarheid voor menselijke toediening is beperkt. In totaal zijn er twee studies uitgevoerd, één met mosapride (5-HT4 receptor agonist) en één met buspirone (5-HT1A receptor agonist). Mosapride had geen effect op morfine-geïnduceerde ademdepressie in een dubbelblinde, cross-over studie in gezonde vrijwilligers, terwijl het combineren van morfine met naloxon de morfine-geïnduceerde ademdepressie antagoneerde. De auteurs van de studie verklaarde het negatieve resultaat door een verminderde plasma concentratie in de hersenen versus de plasma concentratie abdominaal na orale inname.[20] Buspirone (60 mg) toegediend in combinatie met morfine voorkwam geen ademdepressie, maar veroorzaakte wel een significante toename in misselijkheid. Hogere buspirone dosering was niet mogelijk in verband met de misselijkheid bij de proefpersonen. Bij het vergelijken van de data met proefdier data (intraveneuze toediening), toonde de PK-PD analyse dat het negatieve resultaat mogelijk veroorzaakt werd door een te lage effect-site concentratie na orale inname.[21]

Ampakines zijn een groep geneesmiddelen welke effect hebben op de AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionis acid) receptor door de duur van de inwaartse actiepotentiaal van glutamaat te verlengen.[22] De hypothese is dat ampakines de centrale ademhalingsprikkel moduleren door glutamaat-gemedieerde transmissie in het pre-Botzinger complex om een ademhalingsritme te creëren door een toename in glutamaat-gemedieerde excitatoire inspiratoire stimulans.[23-25] AMPA receptor modulatoren hebben geen interactie met de receptor als een agonist, maar hechten aan een allosterische binding in het glutamaat-receptor complex. Verschillende klasse van AMPA receptor modulatoren zijn beschreven, de meest belovende groep zijn de benzamides (ook bekend als ampakines: CX516, CX546, CX614, CX717).[10] De meeste studies zijn verricht met CX717. CX717 toonde aan fentanyl-geïnduceerde ademdepressie (60 μg/kg) te verminderen zonder effect te hebben op analgesie in ratten. Wanneer CX717 gecombineerd werd met fentanyl, voorkwam CX717 fentanyl-geïnduceerde

ademdepressie.[26] In vivo beschermt CX717 tegen propofol-geïnduceerde ademdepressie, maar CX717 was niet effectief in het antagoneren van propofol-geïnduceerde ademdepressie.[27] In mensen is CX717 in een dubbelblinde, placebo-gecontroleerde, cross-over studie onderzocht om het effect van CX717 in combinatie met toediening van alfentanil in een target concentratie van 100 ng/mL. Oertel et al. toonde aan dat CX717 ademdepressie voorkomt zonder effect te hebben op analgesia. De ventilatoire response op een hypercapnie (VE_{55}) was verminderd door CX717 (41% *vs* 54%) zonder effect te hebben op analgesie. Echter leidde CX717 tot een significante toename in sedatie. De orale toedieningsvorm van CX717 kan niet gebruikt worden peri-operatief in verband met een plasma concentratie piek na 2 uur.[28]
Kalium-kanaal blokker doxapram is één van de oudste ademhalingsstimulantia. Doxapram remt achtergrond K^+ kanalen (TASK1, TASK3, TASK 1/3) op type 1 cellichamen in de carotiden. Als reactie op hypoxie stimuleren de type 1 cellichamen in de carotiden een eiwit cascade door het sluiten van de zuurstof-gevoelige K^+ kanalen, waarna er een depolarisatie is van het membraam van de type 1 cellichamen en afgifte van Ca^{2+}-afhankelijke neurotransmitters.[29] Andere mogelijke kanalen die mogelijk betrokken zijn bij de zuurstof afhankelijke K^+ kanalen in de type 1 cellichamen zijn de Kv en BK kanalen, echter zijn de remmende signalen door hypoxie niet geheel duidelijk.[30] Antagoneren van de hypoxie gevoelige K^+ kanalen stimuleert ademhaling. Het effect van doxapram op ventilatie is beschreven in verschillende diersoorten. Het effect van doxapram op ademhaling is voornamelijk door een toename in teugvolume met minimaal effect op ademhalingsfrequentie.[15] Na intraveneuze anesthesie geeft doxapram eerder herstel van bewustzijn, sneller herstel van spontane ademhaling, grotere teugvolumes en ademhalingsfrequentie bij extubatie in 90 patiënten na laparoscopische chirurgie.[30] In patiënten met slaap-apneu syndroom is 100 mg doxapram intraveneus toegediend aan patiënten na bariatrische chirurgie voor extubatie. Deze patiënten hadden lagere sedatie scores, kortere verblijfsduur op de uitslaapkamer en geen enkele patiënt ontwikkelde postoperatieve respiratoire problemen (atelectase, bronchospasme, verminderde ademhaling) *versus* 29% van de patiënten in de placebo groep.[31] Doxapram was oorspronkelijk gebruik als analepticum met sympathico-excitatie, zweten, convulsies, angst/paniek aanvallen, hypertensie en een toegenomen hartminuutvolume. In onze studie (hoofdstuk 2) werden er geen respiratoire effecten van doxapram op alfentanil-geïnduceerde ademdepressie waargenomen. Onlangs zijn er twee andere TASK-kanaal antagonisten ontdekt (PK-THPP en A1899). Het effect van deze antagonisten overtreffen het effect van doxapram zonder de bloeddruk te beinvloeden.[32] Echter zijn deze geneesmiddelen nog niet getest op vrijwilligers.
GAL021 is een recent ontdekte K^+ kanaal blokker. Het blokkeert het BK_{Ca} kanaal. GAL021 stimuleert ventilatie en vermindert de OIRD zonder effect te hebben op analgesie (hoofdstuk 4 en [33,34]). GAL021 vermindert ademdepressie geïnduceerd door isoflurane, propofol en midazolam. Ademhalingsstimulatie vindt plaats door een toegenomen zenuwactiviteit in de carotiden, echter houdt GAL021 respiratoire effecten na het verwijderen van de carotiden en is het effect dus niet enkel op de carotiden.[35] Meer studies met GAL021 moeten verricht worden voor het gebruikt kan worden in de kliniek.

Een nieuwe ontwikkeling in het mogelijk voorkomen van OIRD is, zijn 'biased ligands'.[36] Biased ligands zijn moleculen die selectief het farmacologisch gewenste effect stimuleren en het ademhalingsdepressieve effect omzeilen.[37] De biased ligands beïnvloeden het klassieke model betreft receptor functie (agonist *vs* antagonist, later toegevoegd partiele agonist en tegenovergestelde agonist) naar een meer specifiek farmacologisch profiel. Voor MOR wordt

er momenteel een biased ligand onderzocht: TRV130. TRV130 activeert het G-eiwit met minimale β-arrestin betrokkenheid. TRV130 toonde in muizen dat het voldoende analgesie geeft met minder bijwerkingen (gastro-intestinaal en ademdepressie) dan in een equivalente dosering morfine.[38] In gezonde vrijwilligers is het analgetische effect van TRV130 bepaald, echter is het effect op ademhaling geanalyseerd met afgeleide meetwaardes als zuurstof saturatie en capnometrie. De studie toonde minder effect op hypercapnische geïnduceerde ademhalingsprikkel dan morfine.[39] De respiratoire effecten van TRV130 moeten in de toekomst beter onderzocht worden.

EEN BEREKENING VAN GENEESMIDDEL EFFECT: VOORDEEL *VS.* NADEEL

Een manier om inzicht te krijgen in het gedrag van geneesmiddelen met het oog op gewenste effecten en ongewenste effecten is het integreren van deze effecten in een berekening, als een utility functie. Eerder werd de toxiciteit van een geneesmiddel uitgedrukt met de therapeutische index, de ratio tussen de TD_{50} (dosis die in 50% van de populatie toxisch is) en de ED_{50} (dosis die in 50% van de populatie effectief is). Sheiner en Melmom hebben een concept, gebaseerd op een economische principe, geïntroduceerd om verschillend eindpunten te combineren in één berekening, waarbij het voordeel van het geneesmiddel minus de toxiciteit van het geneesmiddel gecombineerd werd. De eerste utility functions die zijn beschreven, hebben betrekking op antihypertensieve medicatie en anticoagulantia.[40,41] De utility functie van opioïden is gedefinieerd als de kans op analgesia minus de kans op OIRD, waarbij de kans op analgesia (P(A)) gedefinieerd is als een toename in pijn tolerantie van ≥ 50% na een elektrische of warmte stimulus en de kans op OIRD (P(R)) als een afname in ademhalingsvolume van ≥ 50%.

$$UF = P(A \geq 50\%) - P(R \geq 50\%)$$

Momenteel zijn er enkele opioïden beschreven door middel van een utility functie (fentanyl, morfine, oxycodon, cebranopadol en alfentanil).[42-45] De UF is context gevoelig, wat betekent dat de functie afhankelijk is van de definitie van analgesie en ademdepressie. Eerdere UFs hebben analgesia beschreven met een P (A > 50%), wat een toename in pijn tolerantie van 50% of meer is. In hoofdstuk 5 beschrijven wij een UF waarin analgesia is geanalyseerd met een P (A > 50%) en een P (A > 25%). De UF geeft wellicht de mogelijkheid om geneesmiddelen te vergelijken en zo de beste therapie voor elke patient te bepalen.
Na een bolus van 0.13 mg/kg morfine is de UF van morfine positief (kans op analgesie is groter dan de kans op ademdepressie).[43] Na een bolus van 3.5 µg/kg fentanyl intraveneus is de UF negatief gedurende de eerste 90 minuten waarna de kans op analgesie en ademdepressie even groot is.[42] Alfentanil heeft een bifasische UF na een bolus van 50 µg/kg. De eerste 6 minuten zijn negatief, waarna de UF significant toeneemt na 22 minuten (hoofstuk 5). Een vergelijking tussen oxycodon met directe afgifte en tapentadol 100 mg toonde een gunstigere UF voor tapentadol dan voor oxycodon. Oxycodon had een negatieve UF in de eerste 3 uur, waarna de UF herstelt naar nul. Tapentadol had continue een UF rond de nul, onafhankelijk van de tijd na inname.[45] Naast het vergelijken van verschillende UF na een bolus toediening, kan de UF ook de kans berekenen op basis van de plasma concentratie van het gewenste opioïd. Om deze UF te berekenen zijn farmacokinetische parameters noodzakelijk om de effect-site concentratie van het opioïd te bepalen. Een plasma concentratie van > 5 ng/mL morfine geeft meer kans op analgesie dan op ademdepressie. Fentanyl toont een minimaal positieve UF tot een plasma concentratie van 0.6 ng/mL, bij hogere plasma concentratie is de UF negatief en de kans op

ademhalingsdepressie groter dan de kans op analgesie. Alfentanil toont een positieve UF over de effect-site concentraties van 0 – 300 ng/mL.
Hoewel equianalgetische doseringen van orale opioïden *versus* intraveneuze opioïden lastig is om te vergelijken, toont een vergelijking op basis van UF dat tapentadol en morfine een gunstiger profiel heeft met betrekking tot ademdepressie dan andere opioïden. Echter kan het gewenste effect van opioïden, *i.e.* analgesie, geobjectiveerd worden met verschillende testen zoals elektrische, druk of warmte stimuli. Op dit moment is de UF van fentanyl geanalyseerd met warmte en elektrische stimuli met een meer negatieve UF met warmte stimuli *vs.* elektrische stimuli. Om verschillende opioïden te vergelijken moet het analgetische effect met dezelfde nociceptieve stimulus onderzocht worden. Het nadeel van de UF is dat een positieve UF niet betekent dat er geen ademdepressie optreedt. In hoofdstuk 5 hebben wij daarom aan de UF twee andere berekeningen toegevoegd: de kans op adequate analgesie zonder ernstige ademdepressie (U_2, gewenste uitkomst) en de kans op inadequate analgesie met ernstige ademdepressie (U_3, minst gewenste uitkomst). Deze toegevoegde analyse aan de UF is niet eerder voor andere opioïden berekend.
Tot op heden is de UF gebaseerd op data verkregen in een gezonde populatie (*i.e.* gezonde vrijwilligers zonder pijn) en niet gebaseerd op patiënten die behandeld worden met opioïden. Toekomstige studies moeten het gebruik van de UF met verschillende opioïden in chronische pijn patiënten of postoperatieve patiënten analyseren en de mogelijkheid geven tot het vergelijken van opioïd behandeling tussen patiënten.

CONCLUSIES

Uit de data gepresenteerd in dit proefschrift kunnen de volgende conclusies worden getrokken:

1) Doxapram vermindert alfentanil-geïnduceerde antinociceptie door een toegenomen alfentanil distributie en eliminatie. Dit kan het beste verklaard worden door de toename in hartminuutvolume na doxapram administratie.

2) GAL021, een nieuw calcium-geactiveerde kalium kanaal blokker, geeft een toename in teugvolume en ademhalingsfrequentie bij opioid-geïnduceerde ademhalingsdepressie zonder het pijnstillende effect van het opioid te beïnvloeden.

3) GAL021's effectiviteit op alfentanil-geïnduceerde ademhalingsdepressie vermindert bij lage ademminuutvolumes en toont een plafond aan in zijn effectiviteit om opioid-geïnduceerde ademhalingsdepressie te verminderen.

4) De utility functie van alfentanil is verder ontwikkeld om specifieke effecten naar gewenste en ongewenste effecten (analgesie met of zonder ademhalingsdepressie, geen analgesie met of zonder ademhalingsdepressie) te onderzoeken. Het kan mogelijk gebruikt worden om de effecten op ademhaling van opioïden en tussen opioïden te vergelijken.

5) RM101veroorzaakt ademhalingsdepressie in alle proefpersonen met een snel effect op ventilatie en een langzamer effect op CO_2 gevoeligheid. Gezien het effect op ventilatie met naloxon niet volledig geantagoneerd kon worden in alle proefpersonen, heeft RM101 mogelijk een hoge affiniteit voor de μ-opioid receptor.

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Curriculum Vitae
List of Publications
Symbols and Abbreviations

CURRICULUM VITAE

Maria Hermine Johanna (Margot) Roozekrans was born on June 1st, 1987 in Rotterdam, the Netherlands. She attended the Erasmiaans Gymnasium in Rotterdam and obtained her VWO degree in June 2005, whereafter she started her medical studies at the Utrecht University. In 2007 she started her clinical rotations. In her fourth year she obtained interest in research and did an internship in the Netherlands Institute of Neuroscience on the effect of stem cells on post-mortem brain tissue of Alzheimer patients. In her sixth year, she joined the research of the ALS center in the University Medical Center Utrecht where she studied the relationship between drug use and ALS (amyotropic lateral sclerosis). After her graduation in 2012, she was accepted as PhD student at the Anesthesiology department at the Leiden University Medical Center under supervision of prof. dr. A. Dahan. She did a two year fulltime PhD project, where after she combined her PhD project with her residency at the Anesthesiology department under the supervision of prof. dr. L.P.H.J. Aarts (started in July 2014).

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SYMBOLS AND ABBREVIATIONS

5-HT	5-hydroxytryptamine
AMPA	α-amino- 3- hydroxyl-5-methyl-4-isoxazolepropionate
BoF	Best of fit
C_{50}	Effect-site concentration causing 50% of effect
CB	Carotid bodies
C_E	Effect-site concentration
CI	Clearance
CO	Cardiac output
CO_2	Carbon dioxide
Cp	Plasma concentration
DOR	δ-opioid receptor
ED_{50}	Dose causing 50% of effect
EF_A	Predicted ventilation with alfentanil/placebo
EF_G	Predicted ventilation with alfentanil/GAL021
FDA	US Food and Drug Administration
GoF	Goodness of fit
IPRED	Individual predicted values
IWRES	Individual weighted residuals
KOR	κ-opioid receptor
mA	Milli Ampere
MEAS	Measured data
MOR	μ-opioid receptor
MOF	Minimum Objective Function
OIRD	Opioid Induced Respiratory Depression
P	Probability
pCO_2	Carbon dioxide partial pressure
PD	Pharmacodynamic
$PETCO_2$	End-tidal partial pressure of carbon dioxide
PK	Pharmacokinetic
pO_2	Oxygen partial pressure
Qn	Clearance of compartment n
R^2	Coefficient of determination
SEE	Standard error of estimate
SVPC	Standardized visual predictive checks
σ	Standard deviation of the error
$t_{1/2}k_{eO}$	Blood effect-site equilibration half-life
TES	Transcutaneous electrical stimulation
TASK	TWIK-related acid-sensitive potassium channels

THR	Pain threshold
TOL	Pain tolerance
UF	Utility function
V	Ventilation
V_n	Volume of compartment n
ω^2	Inter-subject variability
Y	Shape parameter