Opioid-induced respiratory depression: implications & prevention

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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]

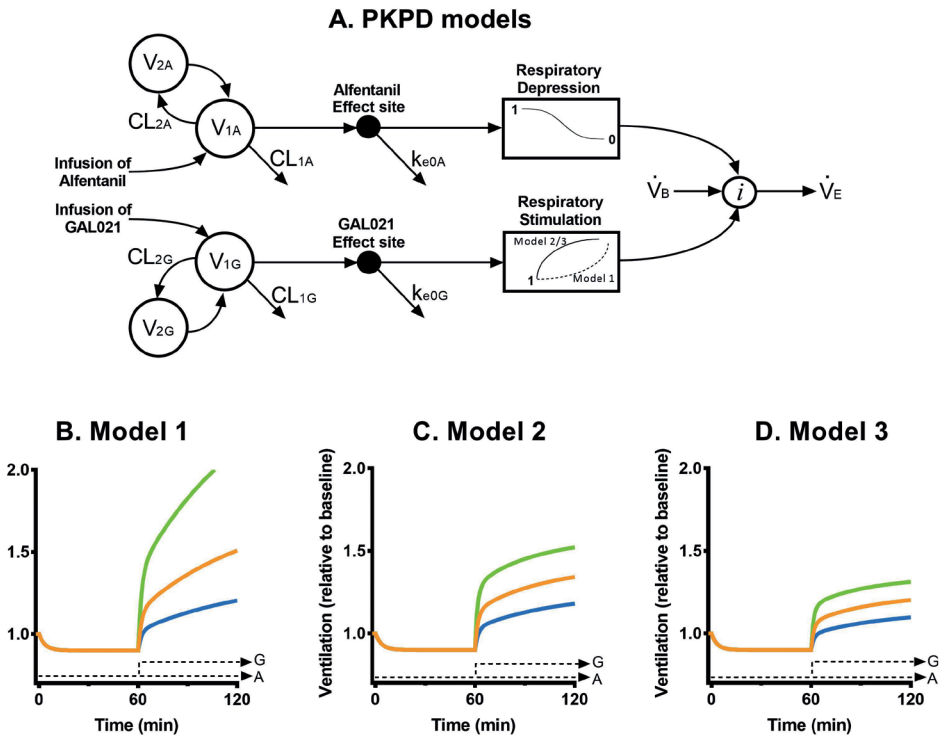

Figure 1. A. *Schematic representation of the pharmacokinetic (PK) nad pharmacodynamic (PD) models used to analyse the effects of alfentanil (A) and GAL021 (G) on ventilation. V_x is the volume of PK compartment x, CL_x clearance from PK compartment X, k_{eOA} the rate constant of equilibration of the 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.*

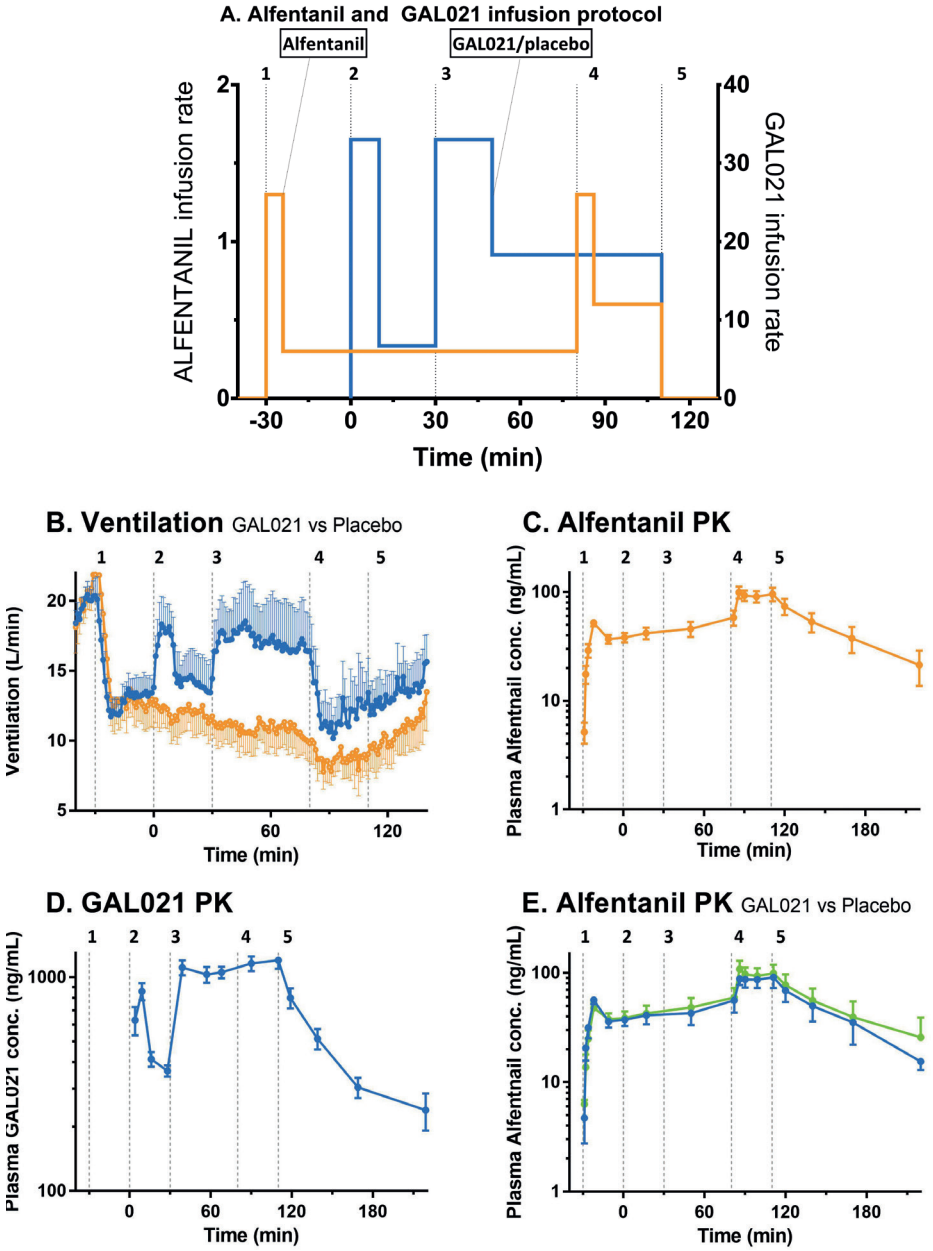

Figure 2. A. *Protocol of alfentanil and GAL021 infusions.* ***B.*** *Measured ventilation during infusion of alfentranil and GAL021 (blue) or placebo (orange).* ***C.*** *Mean plasma alfentanil concentrations (data of both GAL021 and placebo arems are included).* ***D.*** *Plasma GAL021 concentrations.* ***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

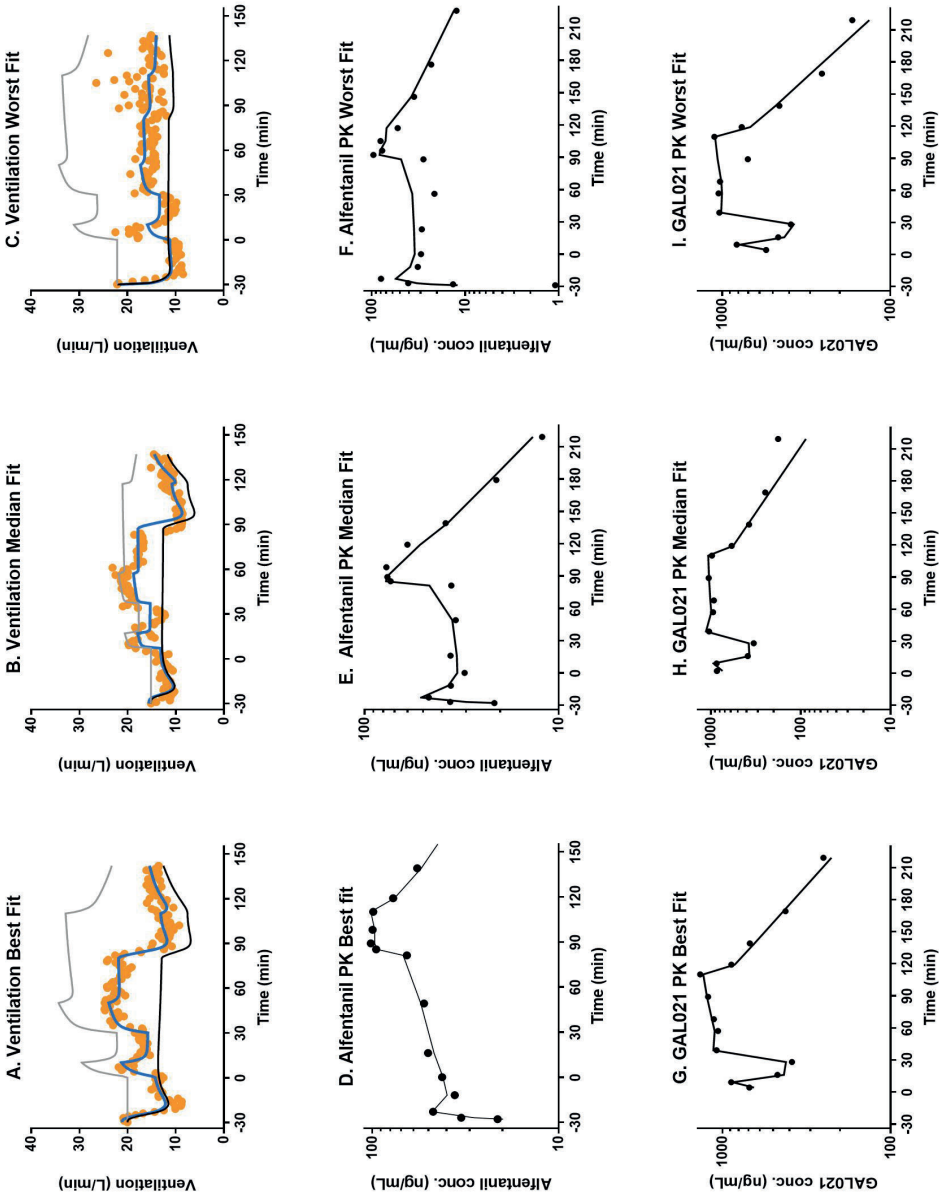

Figure 3. *Effect of GAL021 on ventilation. Best* ***(A)****, median* ***(B)*** *and worst* ***(C)*** *data fits. The orange dots are measured ventilation data points, the blue lines are individual predicted ventilation, the black thin lines are the alfentanil contribution to the predicted ventilation (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

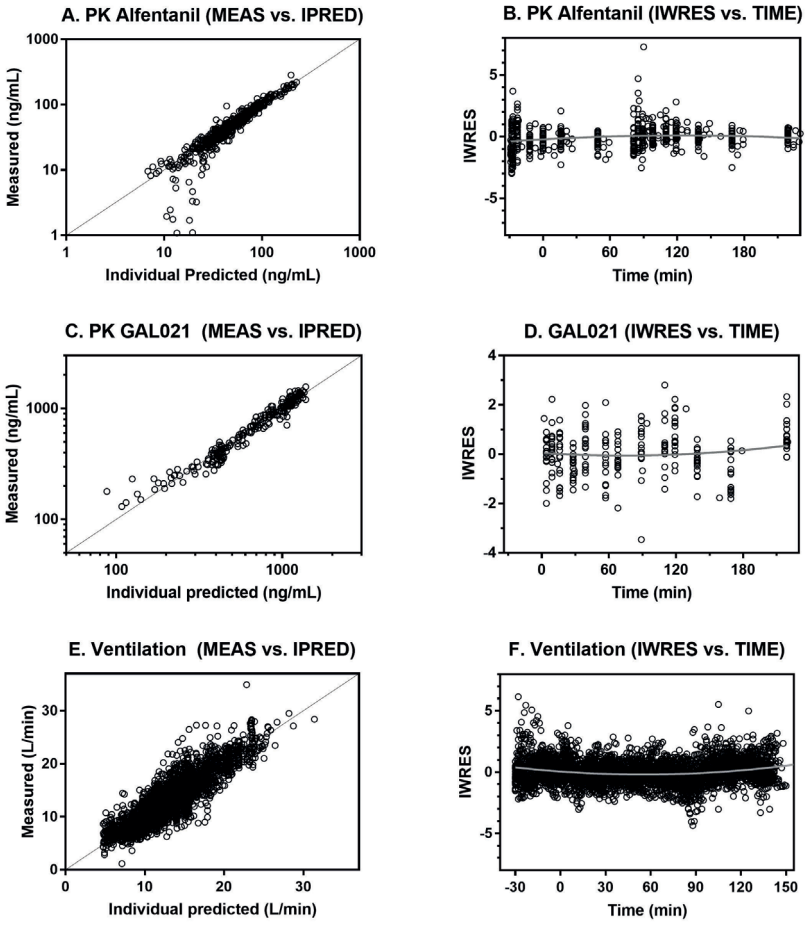

Figure 4. *Goodness-of-fits plots.* ***A*** *and* ***B*** *alfentanil pharmacokinetic (PK) analysis;* ***C*** *and* ***D*** *GAL021 PK analysis;* ***E*** *and* ***F*** *ventilation. MEAS is measured data, IPRED is the individual predicted values and IWRES is the individual weighted residuals. Through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

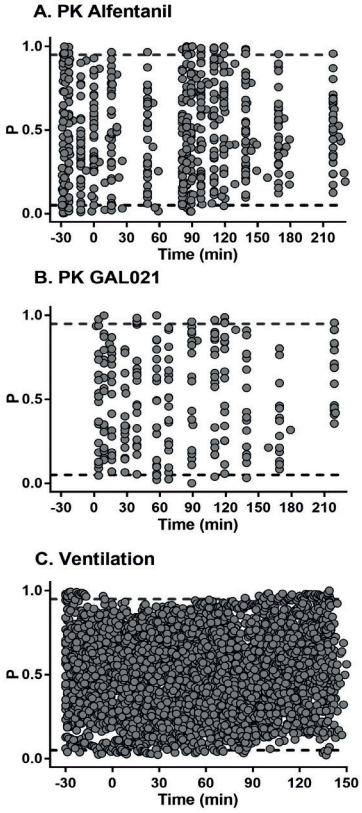

Figure 5. *Standardized visual predictive checks for alfentanil pharmacokinetic (PK) data* ***(A)****, the GAL021 PK data* ***(B)*** *and ventilation* ***(C)****. The broken lines represent the 2.5 and the 97.5% confidence limits. For all three measures, the 95% of the data lies within the 95% confidence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

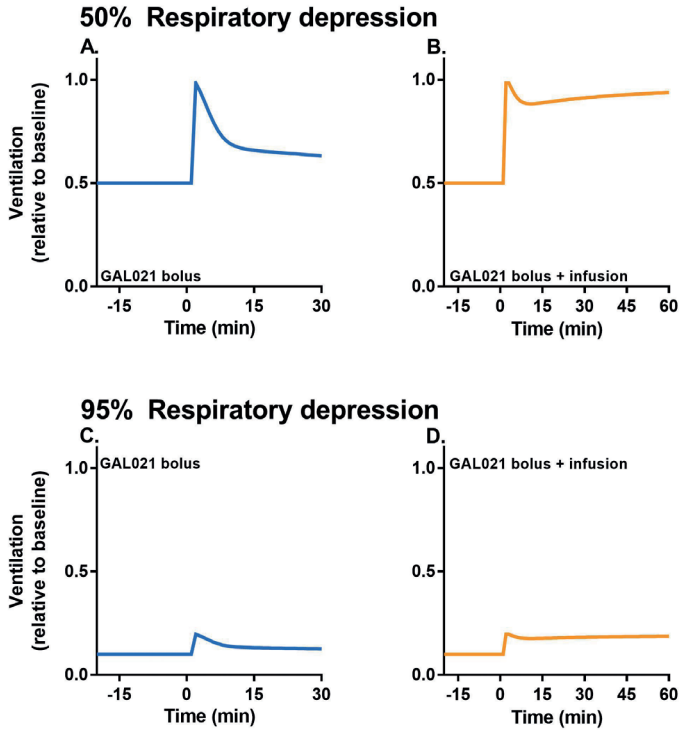

Figure 6. *Simulation of the effect of bolus and combined bolus and continuous GAL021 infusions on alfentanil-induced repsiratory depression. In these simulations, the effects of GAL021 on moderate (50%, **A** and **B**) and severe (90%, **C** and **D**) respiratory depression are simulated. At t = 0, 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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