

Pharmacodynamic Effect of Morphine-6-glucuronide versus Morphine on Hypoxic and Hypercapnic Breathing in Healthy Volunteers

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Background: Morphine-6-glucuronide (M6G) is an active metabolite of morphine that is generally associated with less respiratory depression than morphine. Because M6G will be on the market in the near future, the authors assessed the time profile and relative potency of M6G's effect *versus* morphine's effect on carbon dioxide-driven and hypoxic breathing.

Methods: In nine healthy female volunteers, the effects of 0.2 mg/kg intravenous M6G, 0.13 mg/kg intravenous morphine, and intravenous placebo were tested on ventilation at a fixed end-tidal pressure of carbon dioxide (P_{ETCO_2}) of 45 mmHg (V_{I45}) and on the acute hypoxic ventilatory response (AHR). All subjects participated in all three arms of the study. Respiratory studies were performed at 1-h intervals for 7 h after drug infusion. The data were analyzed using a population dose-driven approach, which uses a dose rate in function of time as input function driving the pharmacodynamics, and a population pharmacokinetic-pharmacodynamic (PK/PD) approach in which fixed pharmacokinetic parameter values from the literature were used as input function to the respiratory model. From the latter analysis, the authors obtained the blood effect-site equilibration half-life ($t_{1/2}k_{e0}$) and the effect-site concentration producing 25% depression of V_{I45} and AHR (C_{25}). Values reported are mean \pm SE.

Results: Placebo had no effect on V_{I45} or AHR over time. Both analysis approaches yielded good descriptions of the data with comparable model parameters. M6G PK/PD model parameters for V_{I45} were $t_{1/2}k_{e0}$ 2.1 ± 0.2 h and C_{25} 528 ± 88 nM and for AHR were $t_{1/2}k_{e0}$ 1.0 ± 0.1 h and C_{25} 873 ± 81 nM. Morphine PK/PD model parameters for V_{I45} were $t_{1/2}k_{e0}$ 3.8 ± 0.9 h and C_{25} 28 ± 6 nM and for AHR were $t_{1/2}k_{e0}$ 4.3 ± 0.6 h and C_{25} 16 ± 2 nM.

Conclusions: Morphine is more potent in affecting hypoxic ventilatory control than M6G, with a potency ratio ranging from 1:19 for V_{I45} to 1:50 for AHR. At drug concentrations causing 25% depression of V_{I45} , M6G caused only 15% depression of AHR, whereas morphine caused greater than 50% depression of AHR. Furthermore, the speed of onset/offset of M6G is faster than morphine by a factor of approximately 2. The

authors discuss some of the possible mechanisms for the observed differences in opioid behavior.

MORPHINE-6-GLUCURONIDE (M6G) is an important active metabolite of morphine in humans. Approximately 6-10% of morphine is glucuronidated in the liver to M6G, and M6G is, like morphine, a μ -opioid receptor agonist.¹ Data from clinical studies suggest that M6G contributes significantly to the analgesic effect observed after long-term morphine administration.²⁻⁴ Animal studies show profound and long-term antinociception and respiratory depression after M6G injections (especially when given centrally) through its action at the μ -opioid receptor.⁵⁻⁹ Initial clinical studies indicated excellent pain relief by intravenous M6G with reduced respiratory depression as compared to morphine,^{10,11} although recent studies that focused on single bolus or short-term infusions are equivocal with respect to M6G's benefit for acute pain relief.¹²⁻¹⁵ Part of the lack of analgesic effect in these studies may be related to the relatively low M6G doses tested (maximum bolus dose 0.1 mg/kg).

There are few human studies on the influence of M6G *versus* morphine on the control of breathing.^{11,15,16} The results of these studies indicate that M6G produced less respiratory depression than morphine. In one study, M6G caused a slight stimulatory effect on breathing.¹¹ In common with the analgesic studies, the M6G doses tested in these studies were relatively low (maximum bolus dose 0.07 mg/kg). The results of the respiratory studies are difficult to interpret for the following reasons: (1) morphine and M6G doses tested were not equipotent with respect to analgesic responses; (2) the respiratory effects were not related to the plasma or effect-site M6G or morphine concentrations; (3) respiratory testing was based on the inhalation of no or just one concentration of carbon dioxide, and none of these studies report the actual arterial or end-tidal carbon dioxide concentrations during carbon dioxide inhalation. The last item may have led to comparisons among agents and times at different end-tidal and arterial carbon dioxide concentrations. These "closed-loop" conditions may have significantly influenced the exposure of respiratory depression caused by the opioids tested.

Morphine-6-glucuronide is currently undergoing phase III clinical trials and will be marketed as an analgesic for postoperative and chronic (non)malignant pain. Therefore, knowledge on its respiratory effects is of importance. Because the respiratory effect of morphine is well

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documented,¹⁷⁻¹⁹ we compared the effect of M6G against morphine on hypercapnic and hypoxic breathing in a group of healthy female volunteers. To perform experiments without the confounding influence of changes in end-tidal carbon dioxide concentrations, we performed respiratory studies at constant end-tidal pressure of carbon dioxide (P_{ETCO_2} ; over time and among agents) by using the computer-driven dynamic end-tidal forcing technique.²⁰ We chose a morphine dose of 0.13 mg/kg because it causes potent and long-lasting respiratory depression and analgesia in female volunteers.^{17,21} As assessed by a pilot study, the M6G dose (0.2 mg/kg) was such that it caused depression of the ventilatory response to carbon dioxide similar to 0.13 mg/kg morphine in magnitude and duration. The data were analyzed using a pharmacokinetic-pharmacodynamic (PK/PD) approach in which the pharmacokinetic data were estimated from data obtained earlier in our laboratory. This allows us the comparison of potencies (in terms of effect-site concentrations causing 25 or 50% depression of breathing) and speed of onset/offset (*i.e.*, the blood effect-site equilibration half-life) of both opioids. We further analyzed the data using a dose-driven pharmacodynamic analysis, which uses a dose rate as a function of time rather than the pharmacokinetic profile as input driving the pharmacodynamics.^{22,23} This allows the comparison of drug effect in terms of the infusion rate that yields 25% or 50% depression of breathing at steady state. The design of the study was double blind, randomized, and placebo controlled.

Methods

Subjects and Apparatus

Nine healthy female volunteers were recruited after approval of the protocol by the local Human Ethics Committee (Leiden, The Netherlands). Oral and written consent was received from all volunteers. All subjects were healthy and did not have a history of tobacco or illicit drug use, and all took oral contraceptives. The subjects were asked to have a normal night of sleep and not to eat or drink for at least 6 h before the study. During the studies, the subjects were allowed to drink water but not to eat. Subjects were comfortably seated in a hospital bed. All experiments started at 8:30 AM.

The subjects were comfortably seated in a hospital bed and breathed through a facemask (Vital Signs, Totowa, NJ). The gas flows were measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump (stroke volume 1 l, at a frequency of 20/min). Corrections were made for the changes in gas viscosity because of changes in oxygen concentration of the inhaled gas mixtures. The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture

with a flow of 50 l/min from a gas mixing system, consisting of three mass flow controllers (Bronkhorst High Tech BV-F202, Veenendaal, The Netherlands) with which the flow of oxygen, carbon dioxide, and nitrogen could be set individually at a desired level. A personal computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations (P_{ETO_2} and P_{ETCO_2}) to follow a specified pattern in time, independent of the ventilatory response. The inspired and expired oxygen and carbon dioxide concentrations and the arterial hemoglobin-oxygen saturation (SpO_2) were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). P_{ETCO_2} , P_{ETO_2} , inspired minute ventilation (V_i), and SpO_2 were collected and stored on disc for further analysis.

Pilot Study Design

We decided to assess the effect of morphine and M6G on the ventilatory response to hypoxia at doses of each drug that would cause similar depression of the ventilatory response to carbon dioxide. Therefore, we performed an initial study in seven of our nine subjects testing the effect of 0.13 mg/kg intravenous morphine *versus* 0.2 mg/kg intravenous M6G. In a previous study, we observed that 0.13 mg/kg morphine causes 30–40% depression of the carbon dioxide sensitivity as well as potent analgesia for more than 6 h in a similar population of healthy female volunteers.^{17,21} The dose of 0.2 mg/kg M6G was based on a preliminary analgesia study performed in our laboratory in 32 healthy females. In that study, 0.2 mg/kg M6G caused potent and long-lasting analgesia (> 6 h; R. Romberg, M.D., and A. Dahan, M.D., Ph.D., unpublished observation, July 2001).

The subjects were tested twice with at least 2 weeks between studies. The ventilatory response to carbon dioxide was assessed at times $t = -30$ min (the control response), $t = 1$ h, and $t = 4$ h) after the drug infusion. The order of opioid testing was random, but the study was open label to the researchers. Three to four increases in P_{ETCO_2} were applied to obtain data points for the steady state ventilatory response. All data points were the mean value of 10 breaths taken after 7–8 min of inhaling the hypercapnic gas mixture. The increases varied from 3 to 19 mmHg and were performed with a background of normoxia ($P_{ETO_2} = 110$ mmHg). This procedure yields three to four steady state data points. We expressed V_i as a linear function of P_{ETCO_2} ,²⁰ $V_i = S [P_{ETCO_2} - B]$, where S is the slope of the hypercapnic ventilatory response and B is the apneic threshold or the intersection with the x-axis (at zero V_i).

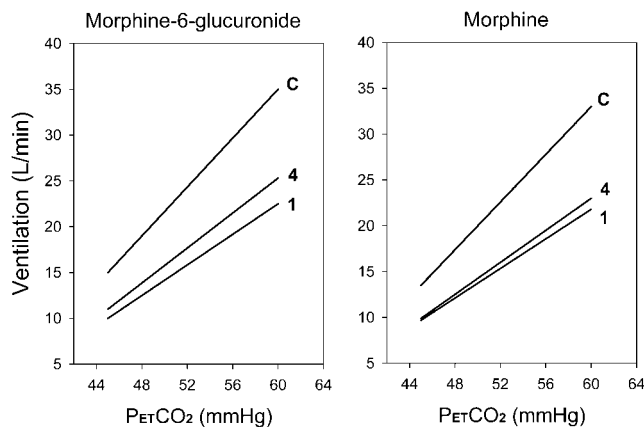


Fig. 1. Influence of 0.2 mg/kg intravenous morphine-6-glucuronide (left) and 0.13 mg/kg intravenous morphine (right) on the ventilatory response to carbon dioxide. *C* is the population response obtained before any drug given, and *1* and *4* are the population responses 1 and 4 h after the bolus drug infusions, respectively. The effect of morphine and morphine-6-glucuronide on the V_i carbon dioxide responses were similar over the 4-h time span. $PETCO_2$ = end-tidal pressure of carbon dioxide.

The results of the pilot study were as follows (see also fig. 1): M6G and morphine *S* control values were 1.31 ± 0.32 and $1.27 \pm 0.27 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ (mean \pm SD; morphine *vs.* M6G: not significant, paired *t* test). The values at 1 h after the 90-s drug infusions were M6G 0.83 ± 0.24 and morphine $0.80 \pm 0.28 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ (not significant). Finally, the values at 4 h after the drug infusions were M6G 0.90 ± 0.21 and morphine $0.92 \pm 0.27 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ (not significant). Neither M6G nor morphine had an effect on parameter *B* (data not given). Because these results indicated a similar effect of 0.13 mg/kg M6G and 0.2 mg/kg morphine on *S* at 1 and 4 h after drug infusion, these doses were chosen in the respiratory studies.

Study Design

Drugs. The study had three arms: (1) placebo (5 ml normal saline), (2) 0.2 mg/kg M6G (a solution of 5 ml), and (3) 0.13 mg/kg morphine (5 ml). Each subject participated in all three arms with at least 3 weeks between studies. The local pharmacy performed randomization and prepared the syringes on the day before the experiment. Morphine was locally produced by the hospital pharmacy, and M6G was obtained from CeNeS Ltd. (Cambridge, United Kingdom). Both opioids were dissolved in normal saline. The M6G solution contained no morphine or morphine-3-glucuronide as tested by the local toxicology laboratory. Intravenous drug infusions were made over 90 s. The respective molecular weights of morphine-6-glucuronide and morphine are 461 and 285 g/mol.

Induction of Hypoxia. Hypoxia was induced with the dynamic end-tidal forcing technique.²⁰ Steps from normoxia ($PETO_2$ 110 mmHg for 8 min) into hypoxia ($PETO_2$ 45 mmHg—values reached within four to six

breaths) were applied. Because peak hypoxic responses occur within 3 min,²⁴ hypoxia was maintained for 3 min, after which hyperoxia was introduced for 5 min (inspired oxygen fraction > 0.5). $PETCO_2$ was maintained at 45 mmHg (~ 5 mmHg above individual resting values) to offset any depressant effect of the opioids on $PETCO_2$.

Study Sequence. The study had a randomized double-blind design. Before drug infusion, control or baseline hypoxic responses were obtained. Next, the drugs were infused. Subsequently, ventilatory responses were obtained at 1-h intervals for 7 h. Total duration of the ventilatory testing was 16 min, and testing started 8 min before the hour and lasted until 8 min after the hour. Between ventilatory studies, the subjects were able to read or walk around.

Data Analysis

Ventilatory Response to Hypoxia. The breath-to-breath data of the last 10 breaths of normoxia (because $PETCO_2$ was fixed at 45 mmHg, we use the abbreviation $V_i(45)$) and the last 10 breaths of hypoxia, $V_i(\text{hypoxia})$, were averaged. Because the relation between ventilation and arterial oxygen saturation is linear,²⁵ we calculated the difference between the hypoxic and normoxic V_i and the SpO_2 data points and expressed the acute hypoxic ventilatory response (AHR) or sensitivity as follows²⁵:

$$AHR = \frac{V_i(\text{hypoxia}) - V_i(45)}{SpO_2(\text{normoxia}) - SpO_2(\text{hypoxia})} \quad (1)$$

(units: l/min per percent desaturation).

Ensemble Average. Initially, we performed a simple descriptive analysis. To assess the effect of single treatments relative to predrug baseline levels on $V_i(45)$ and AHR, we calculated the respective population value \pm 95% confidence intervals for each data point in time. When the value of the baseline data point was within the 95% confidence interval of any of the subsequent data points, we considered that specific data point not different from baseline.²⁴

Pharmacometric Analysis. A pharmacodynamic analysis was performed on the M6G and morphine respiratory data. Two respiratory parameters were tested: normoxic ventilation at an increased fixed $PETCO_2$ level of 45 mmHg ($V_i(45)$) and AHR.

Because morphine and M6G concentrations were not measured, the pharmacokinetics of these drugs were estimated using a dose-driven pharmacodynamic approach^{22,23} and by using fixed pharmacokinetic parameter values from the literature (references 21 and 26, see also table 1; PK/PD analysis). The dose-driven approach uses a dose rate as a function of time rather than the pharmacokinetic profile as input driving the pharmacodynamics.^{22,23} In this approach, compartments (with free parameters) are added until a parsimonious model is

Table 1. Pharmacokinetic Parameters from the Literature Used in the Pharmacokinetic-Pharmacodynamic Analysis

	Morphine	M6G
V_1 , l/kg	0.077	0.052
V_2 , l/kg	0.159	0.068
V_3 , l/kg	1.640	0.067
Cl_1 , l·min ⁻¹ ·kg ⁻¹	0.026	0.0019
Cl_2 , l·min ⁻¹ ·kg ⁻¹	0.014	0.0088
Cl_3 , l·min ⁻¹ ·kg ⁻¹	0.022	0.0010

Data are from references 21 (morphine) and 26 (morphine-6-glucuronide [M6G]).

obtained, *i.e.*, adding more components does not improve the goodness of fit. Details are given in the Appendix.

To eliminate the hysteresis between opioid plasma concentration and respiratory effect, an effect compartment was postulated. This effect compartment equilibrates with the plasma compartment with a time constant $t_{1/2k_{e0}}$ (blood effect-site equilibration half-life).

The relation between effect-site opioid concentration and respiratory effect was modeled by using the following pharmacodynamic model²⁷:

$$f(x) = \alpha \cdot (1 - x^\gamma) \quad (2)$$

By substituting E_0 , which is predrug V_i45 or AHR, for α , and substituting $U^\gamma \cdot 0.25$ for x^γ and $U = C/C_{25}$, where C_{25} is the effect-site concentration causing 25% decrease of E_0 , we obtain

$$E(t) = E_0 \cdot \left[1 - \left(\frac{C_E(t)}{C_{25}} \right)^\gamma \cdot 0.25 \right] \quad (3)$$

where $E(t)$ is effect at time t , $C_E(t)$ is effect-site concentration at time t , and γ is a dimensionless-shape parameter that determines the steepness of the dose-response curve. Finally, for variable V_i45 but not AHR, $E(t)$ is not allowed to become negative and fixed to 0 when $E(t)$ is less than 0.

The model was fitted to the data with NONMEM version V, level 1.1 (a data analysis program for nonlinear mixed effects modeling; UCSF, San Francisco, CA), using a population approach.²⁸ Because central volume and C_{25} are not both identifiable in the dose-driven analysis approach, an alternative measure of potency was estimated, the dose driving rate (DODR) that was defined as the infusion rate that yields 25% effect in steady state.^{22,23} To make a sensible comparison possible between the potency parameters obtained from both approaches, we also calculated DODR as the product of elimination clearance (Cl_1) and C_{25} from the PK/PD analysis.

Likelihood ratio tests were performed to determine whether γ equaled 1. Except for the pharmacokinetic part of the PK/PD model, the presence of first-level random effects (η) was tested on each of the model parameters giving %CV (percent coefficient of variation,

a measure of between subject variability). P values less than 0.01 were considered significant. Values reported are population value \pm SE.

Results

All subjects completed the protocol without major side effects. Nausea occurred in five subjects after morphine, one subject after M6G, and in none after placebo. The symptoms were mild and did not necessitate treatment. The mean age of the subjects was 24 ± 2 yr, mean weight was 74 ± 8 kg, and mean height was 174 ± 8 cm. All of the subjects completed the three arms of the study.

Ensemble Average

Predrug V_i45 and AHR did not differ among the three treatment levels (analysis of variance; fig. 2). During the hypoxic studies, SpO_2 values were $80 \pm 2\%$ and $PETCO_2$ values were 45.4 ± 0.2 mmHg among subjects. Over time, hypoxic $PETCO_2$ levels did not deviate from predrug values. As determined from the ensemble averaging procedure, placebo had no effect on V_i45 or AHR (95% confidence intervals included baseline value at all measured times; fig. 2). Both morphine and M6G caused significant depression of V_i45 with nearly identical time courses (maximum depression relative to $t = 0$ h was 30% at $t = 2$ h; duration of effect was 5–6 h; fig. 2). With respect to AHR, while the absolute magnitude of effect did not differ between M6G and morphine (M6G 40% depression at $t = 1$ h relative to $t = 0$ h; morphine 50% depression at $t = 4$ h relative to $t = 0$ h). M6G showed a more rapid return to baseline AHR values (M6G data points not different from control from $t = 3$ h on; morphine data points not significant from control from $t = 7$ h on; fig. 2).

Pharmacometric Analysis

Both approaches yielded adequate descriptions of the data. In the dose-driven pharmacodynamic analysis, a one-compartment model with effect compartment was identified. Addition of peripheral compartments did not significantly lower the objective function. In both approaches, the value of γ was not different from 1 (both for V_i45 and AHR). This indicates a linear relation among M6G, morphine, and effect (at least over the dose range studied). Examples of (best, median, and worst) data fits for V_i45 and AHR are given in figure 3 for both analysis approaches. The population estimates of the model parameters are given in table 2. Potency parameters from both approaches did not differ significantly (recall, $DODR = Cl_1 \cdot C_{25}$). Approximately twice the infusion rate of M6G was necessary to obtain a 25% depression of AHR compared to hypercapnic breathing, while similar infusion rates were needed for morphine to depress AHR and hypercapnic breathing by 25%. In terms of effect-site

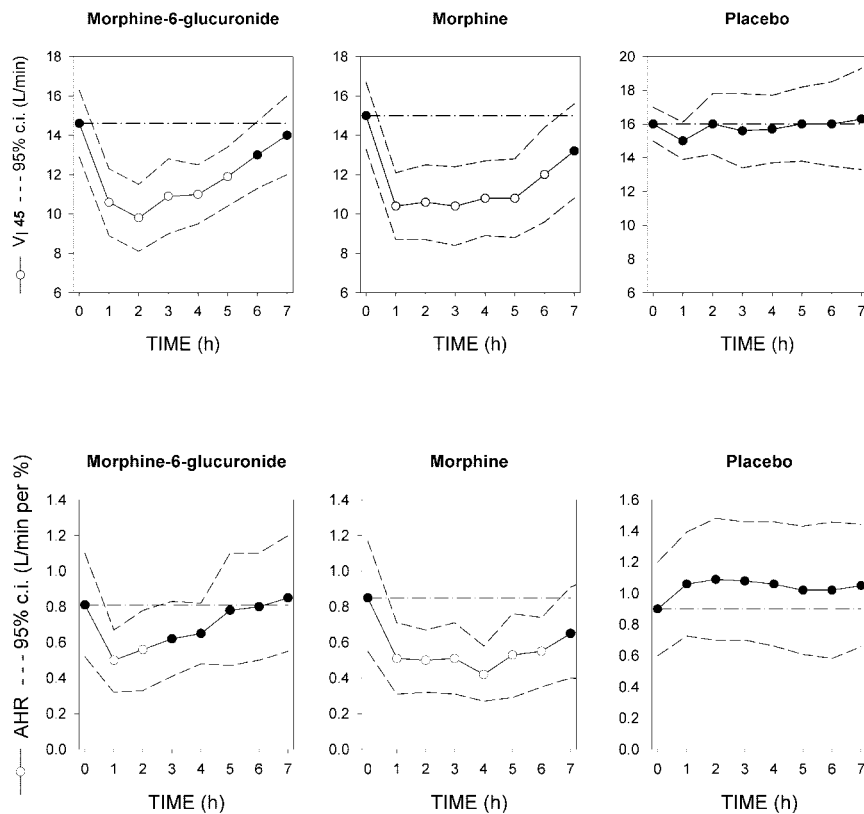


Fig. 2. Ensemble averages \pm 95% confidence intervals (c.i.) of normoxic ventilation at a fixed end-tidal pressure of carbon dioxide (P_{CO_2}) of 45 mmHg (\dot{V}_{i45} ; top) and the acute hypoxic response (AHR; bottom). Morphine-6-glucuronide (left), morphine (middle), and placebo (right) were infused at time $t = 0$. The data points at $t = 0$ are the control data points obtained just before drug infusion. The dash-dot bar is the extension of the control data point. Open symbols = data points significantly different from control.

concentration, we observed that compared to M6G, morphine is approximately 19 times more potent in depressing \dot{V}_{i45} and approximately 50 times more potent in depressing the ventilatory response to acute hypoxia ($P < 0.01$ vs. \dot{V}_{i45}). For M6G, greater effect-site concentrations were needed to depress AHR by 25% relative to \dot{V}_{i45} ; the reverse was true for morphine (fig. 4).

Although time constants ($t_{1/2k_{e0}}$) were generally smaller when obtained from the PK/PD analysis, they were not significantly different from the values obtained from the dose-driven analysis. The larger standard errors of $t_{1/2k_{e0}}$ derived from the dose-driven analysis (table 2) indicate a larger uncertainty in the estimation of this parameter when using the dose-driven pharmacodynamic analysis compared to the PK/PD analysis. As determined from PK/PD analysis, M6G had a faster speed of onset/offset relative to morphine; this was true for \dot{V}_{i45} and AHR, both by a factor of approximately 2.

Discussion

Despite their many side effects, opioids remain the only agents for alleviation of severe pain. Because they are associated with sometimes life-threatening respiratory depression,^{29,30} the development and use of opioid analgesics that produce less respiratory depression than commonly used opioids, such as morphine, is of importance. It has been claimed that M6G given intravenously causes less respiratory depression compared to mor-

phine.^{11,15,16,31} It is argued that this is related to the lower M6G affinity for the μ_2 -opioid receptor, relative to morphine.³¹ The μ_1 -opioid receptor is held responsible for the analgesic effect of opioids; the μ_2 receptor is held responsible for their respiratory effects.³² Indeed, from the receptor affinity profile, it is expected that M6G ($\mu_1 > \mu_2$) causes analgesia with less respiratory effect than morphine.³¹ Note, however, that most of these data are obtained from animals.

Experimental Considerations and Limitations

M6G Dose. The M6G dose of 0.2 mg/kg was chosen because it caused comparable depression of the \dot{V}_i carbon dioxide response over a 4-h time period (fig. 1). More recent studies from our laboratory show that 0.2 mg/kg M6G causes rapid-onset analgesia significantly greater than placebo in a group of 10 male and 10 female volunteers (R. Romberg, M.D., and A. Dahan, M.D., Ph.D., unpublished observation, November 2002). Whether the M6G dose tested produces equianalgesia relative to 0.13 mg/kg morphine remains unknown.

Sex Differences. In our study, we tested the effect of morphine and M6G in women exclusively rather than studying a group of mixed sexes. We did so for the reason that we previously observed important sex differences in the effect of morphine on both analgesic and respiratory effect.^{17,18,21} We observed greater depression of the AHR and slope of the response to inspired carbon dioxide in women compared to men and greater

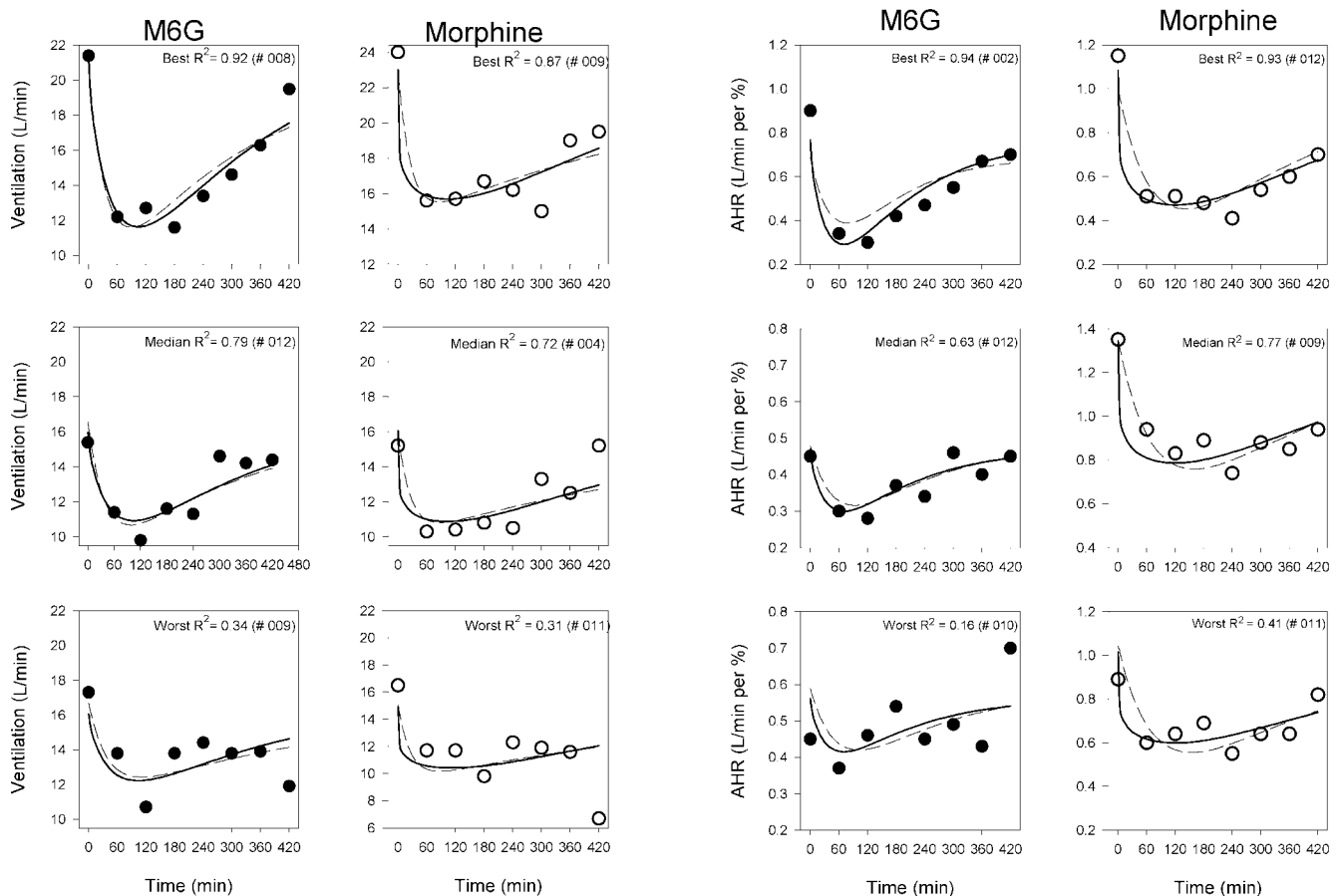


Fig. 3. The influence of intravenous morphine-6-glucuronide (M6G) and morphine on normoxic ventilation at a fixed end-tidal pressure of carbon dioxide (P_{ETCO_2}) of 45 mmHg ($V_{\text{E}45}$; left) and the acute hypoxic response (AHR; right). Measured (circles) and predicted parameters derived from the pharmacokinetic-pharmacodynamic (continuous lines) and the dose-driven pharmacodynamic approaches (broken lines). Best, median, and worst fits obtained from the pharmacokinetic-pharmacodynamic analysis. Goodness of fit was assessed by the coefficient of determination (R^2).

morphine analgesic potency in women.^{17,18,21} Our current observation on morphine is in agreement with our previous findings in women. Whether the current results also apply to men is unknown and deserves further study.

PK/PD versus Dose-driven Pharmacodynamic Analysis. We did not measure plasma morphine and M6G concentrations. Instead, we used two separate approaches to overcome this potential shortcoming. We applied a dose-driven pharmacodynamic analysis that enables the dynamic description of data when pharmacokinetic data are missing. However, this approach is restricted. The potency of the drug cannot be determined apart from an indirect measure, the infusion rate that would yield a certain effect at steady state (DODR). The dynamics of the virtual pharmacokinetic and effect compartments are confounded so that estimates of drug elimination (clearance) and drug plasma effect-site concentration equilibration ($t_{1/2k_{e0}}$) may be biased and dependent on the dosage form. In our PK/PD analysis, the pharmacokinetic parameters used to describe morphine's and M6G's pharmacokinetics were fixed to pub-

lished data obtained from our own laboratory in a comparable group of subjects and after infusion of similar doses of morphine and M6G.^{21,26} In fact, most of the subjects from the current study participated in the previous study on M6G pharmacokinetics.²⁶ Hence, although the pharmacodynamic parameters from the PK/PD analysis may also be biased and variances of their interindividual variabilities may be overestimated, we expect the bias to be small (at least smaller than the bias obtained using the dose-driven pharmacodynamic approach). Babenco *et al.*³³ used a PK/PD approach similar to ours (with fixed pharmacokinetic data from the literature) to reliably estimate the onset/offset time constant and C_{50} of remifentanyl's effect on ventilatory control.

To test the capability of our two models to predict respiratory effect after a dosing paradigm different from that used in the current study (a true test of the models), we performed six additional experiments. Six female subjects, aged 18–24 yr, received either morphine or M6G, and carbon dioxide-driven ventilation was measured for 7 h at 1-h intervals. Three subjects received a dose of M6G of 0.1 mg/kg at time $t = 0$ and a second

Table 2. Population Model Parameter Estimates for Normoxia Ventilation at a Fixed PETCO₂ of 45 mmHg and the Ventilatory Response to Acute Hypoxia

	Morphine-6-glucuronide			Morphine		
	Estimate	SE	%CV	Estimate	SE	%CV
Ventilation at a fixed PETCO ₂ of 45 mmHg						
PK/PD analysis						
Baseline, l/min	14.1	1.1	20	13.5	1.2	17
t _{1/2} k _{e0} , h	2.1	0.2	*	3.8	0.9	10
C ₂₅ , nM	528	88	37	28.0	5.9	*
C ₅₀ , nM†	1,056			56.0		
Dose-driven analysis						
Baseline, l/min	14.5	1.1	20	15.6	1.2	17
t _{1/2} k _{e0} , h	4.9	1.4	37	7.9	4.2	*
k _{el} , min ⁻¹	0.026	0.009	*	0.040	0.017	*
DODR, μg · kg ⁻¹ · min ⁻¹	21.4	3.2	*	9.0	1.6	*
Ventilatory response to hypoxia						
PK/PD analysis						
Baseline, l/min per %	0.80	0.10	38	0.90	0.11	25
t _{1/2} k _{e0} , h	1.0	0.1	*	4.3	0.6	*
C ₂₅ , nM	873	81	66	16.5	2.1	17
C ₅₀ , nM†	1,746			33.0		
Dose-driven analysis						
Baseline, l/min per %	0.80	0.10	38	0.92	0.12	29
t _{1/2} k _{e0} , h	2.6	1.4	29	3.8	2.2	18
k _{el} , min ⁻¹	0.017	0.009	*	0.013	0.006	*
DODR, μg · kg ⁻¹ · min ⁻¹	37.0	5.0	*	8.7	2.7	*

* Parameter not included in the statistical model. † C₅₀ is an extrapolated value.

C₂₅ and C₅₀ = concentrations causing 25% and 50% reduction of responses; %CV = percent coefficient of variation; DODR = dose driving rate; k_{el} = drug elimination rate; PETCO₂ = end-tidal pressure of carbon dioxide; PK/PD analysis = pharmacokinetic-pharmacodynamic analysis using pharmacokinetic data from the literature (see table 1); t_{1/2}k_{e0} = blood effect-site equilibration half-life.

dose of 0.1 mg/kg at t = 4 h. The three other subjects received 0.07 mg/kg morphine at times 0 and 4 h. The results are given in figure 5, showing that the predictions of respiratory depression after repeated dosing derived from both analysis approaches were good. The median prediction errors (bias) and median absolute prediction errors (inaccuracy) were 3.4 and 8.6% (PK/PD approach) and 4.7 and 9.8% (dose-driven approach) for the M6G repeated dose protocol. The equivalent values for morphine were 0 and 6.4% (PK/PD approach) and 0.6 and 6.4% (dose-driven approach). Taking into account these observations as well as the large estimation errors of t_{1/2}k_{e0} derived from the dose-driven analysis (SE and

possible bias, see previous paragraph), we will focus the remainder of the discussion on the results obtained from the PK/PD approach.

Pharmacodynamic Model. The power function we used to model respiratory effect (equations 2 and 3) has been applied previously to assess the steady state ventilatory effects of alfentanil and remifentanyl.^{27,34} The current study shows that our model may also be used to analyze non-steady state respiratory data. Although we and others have used a classic sigmoid Emax model to describe the relation between drug and respiratory effect,^{33,35} we believe that the model used in this study has various advantages over the sigmoid Emax model. In

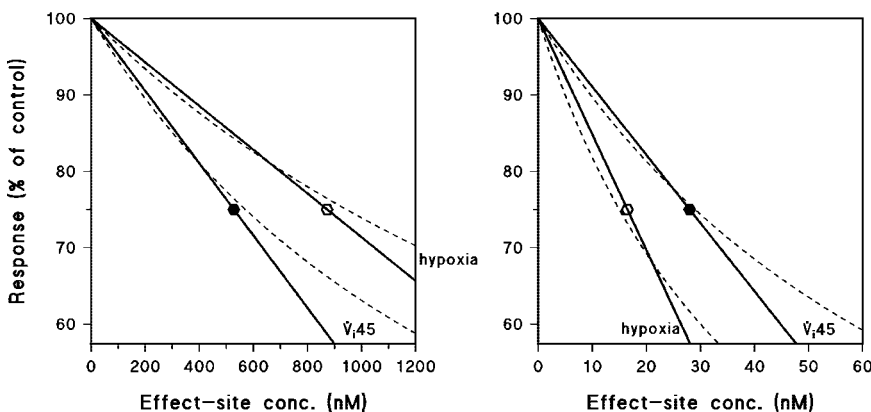
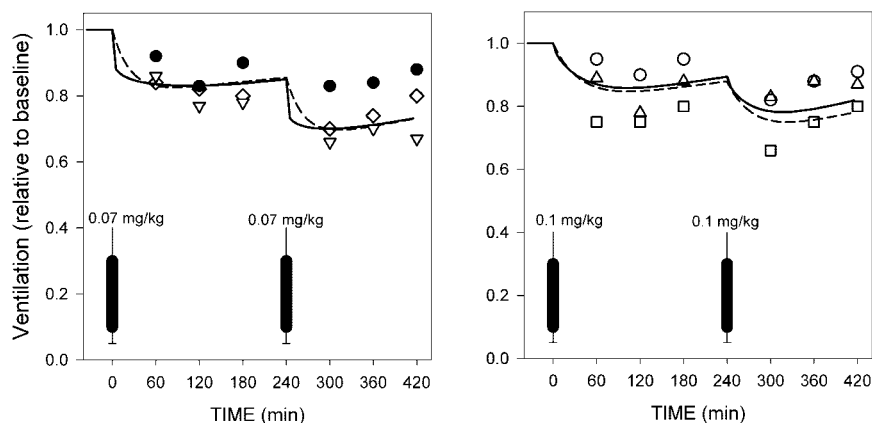


Fig. 4. Model prediction of the effect-site morphine-6-glucuronide (left) and morphine (right) concentrations versus hypercapnic ventilation (V_i45) and the ventilatory response to acute hypoxia. The analysis was performed using the pharmacodynamic model of equation 3 (power model; continuous line) with corresponding C₂₅ values (closed symbol for V_i45, open symbol for hypoxia), and by using a classic inhibitory sigmoid Emax model (broken lines). Note the good correspondence of the two models over the concentration ranges used in this study (NONMEM objective functions differed by < 2). At greater (extrapolated) concentration ranges, the sigmoid Emax model underestimates respiratory depression as

compared to the power model. Note further the higher morphine sensitivity for effect on the hypoxic response relative to hypercapnic ventilation. The reverse is true for morphine-6-glucuronide.

Fig. 5. The influence of 0.07 mg/kg intravenous morphine given at times $t = 0$ and $t = 4$ h in three female subjects (*left*) and the influence of 0.1 mg/kg intravenous morphine-6-glucuronide (M6G) given at times $t = 0$ and $t = 4$ h in three other female subjects (*right*) on ventilation at a fixed end-tidal pressure of carbon dioxide (P_{ETCO_2}) of 45 mmHg. Respiratory measurements were obtained at times $t = 1, 2, 3, 5, 6,$ and 7 h after the first infusion. Each symbol represents one subject. The *continuous line* is the model prediction of respiratory depression using the pharmacokinetic–pharmacodynamic model; the *broken line* is the prediction derived from the dose-driven model. Both models predicted the respiratory effects of a double infusion of morphine and M6G well. The median prediction errors and median absolute prediction errors were 3.4 and 8.6% (pharmacokinetic–pharmacodynamic model) and 4.7 and 9.8% (dose-driven model) for M6G, and 0 and 6.4% (pharmacokinetic–pharmacodynamic model) and 0.6 and 6.4% (dose-driven model) for morphine.



reference 27, these advantages are discussed to some extent. In brief, in contrast to the sigmoid Emax model, our model is able to predict negative effect as possibly may occur when an opioid causes a reduction rather than an increase in ventilation in response to hypoxia.³⁶ Furthermore, in contrast to the sigmoid Emax model, our model is able to predict apnea at finite drug concentrations. Especially at high opioid concentrations, periodic breathing and/or apnea are bound to occur. We reanalyzed our current data using an inhibitory sigmoid Emax model (*broken lines* in fig. 5). Over the concentration range applied in this study, the sigmoid Emax model performed equally well compared to the power model (differences in NONMEM's objective functions < 2). As expected, at relatively high opioid concentrations ($C_E > C_{25}$), the sigmoid Emax model underestimated respiratory depression compared to our model. Reanalysis of previous data sets from our laboratory using relatively high opioid doses (sevoflurane–alfentanil and propofol–remifentanil studies)^{27,34} revealed significantly better fits using our pharmacodynamic model rather than the sigmoid Emax model. Further studies applying greater M6G and morphine doses are needed to resolve which of the two models predicts the respiratory effect of these opioids best. However, for now, viewing the results of our analyses (power and sigmoid Emax model) and taking into account the reanalyses of the alfentanil and remifentanil data sets, our current approach seems best.

Potency

M6G versus Morphine. Morphine-6-glucuronide is less potent than morphine in causing respiratory depression. The potency ratios determined from C_{25} are for V_{i45} and AHR 1:19 and 1:50, respectively. Appreciable respiratory depression of V_{i45} was observed in all subjects. In this respect, our data are in disagreement with previous studies showing little to no respiratory effect of M6G in humans.^{11,15,16} These differences in study outcomes may be related to differences in experimental

paradigm (“open-loop” vs. “closed-loop” conditions) as well as to the relatively very low doses previously tested. Note that none of these studies examined the effect of M6G on hypoxic ventilatory responses.

Our observation that morphine is 19–50 times more potent than M6G in depressing respiratory parameters is in agreement with a recent finding showing that morphine is 11–48 times more potent than M6G judged by their effects on pupil size.³⁷ The M6G C_{50} value of that study was of the same order of magnitude as the extrapolated V_{i45} C_{50} values derived from our experiments (reference 37: 832 nM for M6G, *cf.* table 2).

Man versus Mice. Our findings are in sharp contrast to mice studies finding higher M6G than morphine potency for respiratory effect.^{7,8} For example, in mice, we recently observed a morphine to M6G potency ratio of 1:12 for depression of breathing frequency.⁷ Possible reasons to explain the low M6G potency in our current study—apart from the evident variation in the species—include the development of acute tolerance and/or activation of non- μ -opioid receptors associated with respiratory stimulation.^{8,37} We argue that this latter mechanism may be equivalent to opioid-induced hyperalgesia due to activation of *N*-methyl-D-aspartate (NMDA) pain facilitatory receptors.⁸ Because NMDA receptors play an important role in the control of breathing, especially in the development of AHR,³⁸ further studies are needed to examine the relation among M6G, NMDA receptors, and the modulation of the ventilatory response to hypoxia.

AHR versus V_{i45} . At the concentration morphine causing 25% depression of V_{i45} , the acute hypoxic response was affected by more than 50% (fig. 4). For M6G, depression of AHR was less than 25% at its C_{25} for V_{i45} . We consider the latter observation the most important result of our study because most if not all μ opioids affect AHR at lower concentrations than V_{i45} .^{27,34} These differences in opioid behavior (*i.e.*, M6G versus morphine and other μ -opioid receptor agonists) are difficult

to explain but may be related to the underestimation of the M6G effect on AHR (*i.e.*, a type II error) or to a true difference in opioid behavior, such as (1) differences in morphine and M6G distribution within the brain compartment,³⁹ (2) the activation of distinct effector pathways (involving different G-protein receptor complexes) by morphine and M6G,^{40,41} or (3) activation of stimulatory receptors by M6G but not by morphine in pathways involved in hypoxic control of breathing (see *Man versus Mice* section).

Contribution of M6G to Morphine-induced Respiratory Depression. Assuming that the fraction of morphine clearance which results in M6G formation is 6–10%,^{1,21} we calculated that the contribution of M6G to morphine respiratory depression (C_{25}) in humans with a normal renal function is less than 5% in the steady state. Evidently, because M6G is excreted *via* the kidneys only, renal failure increases the M6G contribution significantly because of M6G accumulation. However, because M6G respiratory potency is limited (table 2), relatively high steady state M6G plasma concentrations are needed to cause the abolishment of ventilatory responses (*e.g.*, steady state concentrations > 2,000 nM for V_{i45} and > 3,000 nM for AHR). These calculations are in agreement with a recent report showing that a patient with severe renal failure and M6G plasma concentrations of approximately 1,000 nM after repeated morphine administrations had a respiration frequency greater than 8 breaths/min and no signs of oxygen desaturation.⁴²

Speed of Effect Onset/Offset

Morphine-6-glucuronide has a faster speed of onset/offset than morphine. The $t_{1/2k_{e0}}$ values of morphine were greater than those of M6G by a factor of approximately 2 for both AHR and V_{i45} as derived from the PK/PD analysis (table 2). This was not expected, taking into account that it is traditionally suggested that M6G penetrates the blood–brain barrier much slower than morphine because of the hydrophilic nature of the molecule.^{43,44} To the best of our knowledge, there are only two PK/PD studies on M6G's effect that are available in the literature.^{37,43} In rats, Gårdmark *et al.*⁴³ observed that M6G had a considerably longer time delay than morphine with respect to *analgesic* effect (half-lives 1.4 h and 0.4 h for M6G and morphine, respectively). In humans, values of $t_{1/2k_{e0}}$ obtained from pupil size measurements were 6.4 and 2.8 h for M6G and morphine, respectively (values are medians).³⁷ As recently reviewed,³¹ there is growing evidence that despite its polarity, M6G brain uptake may be faster than previously assumed. This is related to the observation that under certain conditions (*e.g.*, in media of low polarity such as the blood–brain barrier) the M6G molecule is able to fold and mask its polar groups, increasing its lipophilicity.⁴⁵ These mechanisms may yield M6G molecules with similar lipophilicity as morphine molecules. Recent stud-

ies in rats further suggest that only part of the effect delay of morphine and M6G is caused by diffusion across the blood–brain barrier.^{46,47} For example, half of M6G's antinociceptive effect delay is the result of drug distribution within the brain tissue, rate-limiting mechanisms at the receptor level, and neuronal dynamics.⁴⁷ Taking into account all of the above, we believe that the observed differences in $t_{1/2k_{e0}}$ between morphine and M6G are related to a complex of factors occurring beyond the blood–brain barrier (*i.e.*, within the brain compartment) rather than factors related to their washin and washout into and out of the brain compartment.

In conclusion, this study is the first to compare the respiratory pharmacodynamics of morphine and its metabolite, morphine-6-glucuronide, in humans. We observed that morphine has a more potent influence on the control of breathing than M6G in a group of healthy female volunteers free of pain, stress, and inflammation. Most important, morphine and M6G have differential pharmacodynamic effects on the ventilatory response to hypoxia when compared to their effect on carbon dioxide-driven ventilation (V_{i45}). Higher effect-site concentrations of M6G are needed to depress the V_i response to acute hypoxia relative to concentrations needed to depress V_{i45} . Morphine, on the other hand, affects the ventilatory response to hypoxia at a lower effect-site concentration than V_{i45} . This is important because the chemoreflex response to hypoxia is a vital and sometimes life-saving reflex causing central nervous system arousal and an increase in oxygen uptake. The reasons for the observed differences between morphine and M6G remain unknown, but our data suggest that morphine and M6G act *via* distinct and complex pathways. Evidently, further studies are needed to examine whether there are clinically relevant differences in respiratory events in patients on morphine *versus* M6G for acute and chronic pain relief.

Appendix: Dose-driven Pharmacodynamic Analysis

Plasma concentration after bolus administration, modeled by n compartments, can be written as:²²

$$C_p(t) = \frac{\text{dose}}{V} \cdot \sum_{i=1}^n \alpha_i \cdot \exp(-\lambda_i \cdot t) \quad (4)$$

where dose is the bolus administered, V is the volume of the central compartment, and α_i are intercepts and λ_i are rate constants that can be calculated from k values or volumes and clearances using well-known formulas.⁴⁸ In NONMEM, these equations were solved by ADVAN7. When an effect compartment is postulated, it can be derived that the effect-site concentration $C_{ie}(t)$ equals:

$$C_{ie}(t) = \frac{\text{dose}}{V} \cdot \sum_{i=1}^n \frac{k_{e0}}{k_{e0} - \lambda_i} \cdot [\exp(-\lambda_i \cdot t) - \exp(-k_{e0} \cdot t)] \quad (5)$$

where k_{e0} is the central effect-site concentration equilibration rate.⁴⁹ When $n = 1$, this equation reduces to:

$$C_e(t) = \frac{\text{dose}}{V} \cdot \frac{k_{e0}}{k_{e0} - k_{e1}} \cdot [\exp(-k_{e1} \cdot t) - \exp(-k_{e0} \cdot t)] \quad (6)$$

where k_{e1} is the drug elimination rate. An inhibitory pharmacodynamic model relating concentration to effect reads as:

$$E(t) = E_0 \cdot \left[1 - \left(\frac{C_e(t)}{C_{25}} \right)^\gamma \cdot 0.25 \right] \quad (7)$$

where C_{25} is the effect-site concentration causing a 25% decrease in effect. When no concentration measurements are available, it is impossible to estimate both C_{25} and V , as becomes clear from equations 6 and 7. Following references 22 and 23, we introduce the parameter DODR, the dose driving rate that causes a 25% decrease in effect in steady state, which is given by:

$$\text{DODR} = k_{e1} \cdot V \cdot C_{25} \quad (8)$$

Substituting both DODR and $C_e(t)$ in equation 7 gives:

$$E(t) = E_0 \cdot \left[1 - \left(\frac{\text{dose} \cdot k_{e1}}{\text{DODR}} \cdot \frac{k_{e0}}{k_{e0} - k_{e1}} \cdot [\exp(-k_{e1} \cdot t) - \exp(-k_{e0} \cdot t)] \right)^\gamma \cdot 0.25 \right] \quad (9)$$

As noted by Fisher and Wright,⁵⁰ in the absence of concentration data, the parameters k_{e1} and k_{e0} do not have their usual interpretation. Here, k_{e1} relates dose to the concentration in a hypothetical driving compartment, and k_{e0} in turn relates concentration in that driving compartment to effect. These two relations may not be well described by single rate constants for complex time dependencies. We chose to test extensions of the relation between dose and concentration in the driving compartment, *i.e.*, by using equation 5 instead of equation 6 with increasing n until a parsimonious model was obtained. The other—less plausible—possibility, namely extending the relation between concentration and effect (*e.g.*, by adding rate constants), was not explored.

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