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Title :

Pharmacokinetics and Pharmacodynamics of Alfentanil in P-glycoprotein-Competent and P-glycoprotein-Deficient Mice: P-glycoprotein Efflux Alters Alfentanil Brain Disposition and Antinociception

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Abbreviations used: P-gp, P-glycoprotein; MPR, maximum possible response; HPLC, high-performance liquid chromatography; MS, mass spectrometry; CNS, central nervous system; PK-PD, pharmacokinetic-pharmacodynamic

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Abstract

Previous studies have indicated that P-glycoprotein (P-gp) attenuates the CNS penetration and central activity of some opioids. The impact of P-gp-mediated efflux on the disposition and efficacy of the synthetic opioid alfentanil currently is unknown. In this study, P-gp-competent [*mdr1a*(+/+)] and P-gp-deficient [*mdr1a*(-/-)] mice were used to investigate the impact of P-gp-mediated efflux on the systemic pharmacokinetics, brain disposition, and central activity of alfentanil. Equipotent doses of alfentanil were administered to *mdr1a*(+/+) and *mdr1a*(-/-) mice (0.2 and 0.067 mg/kg, respectively), and the time course of brain and serum concentrations, as well as antinociception, were determined. A pharmacokinetic-pharmacodynamic (PK-PD) model was fit to the data and used to assess the impact of P-gp on parameters associated with alfentanil disposition and action. The *mdr1a*(+/+) mice were less sensitive to alfentanil than *mdr1a*(-/-) mice, requiring a 3-fold higher dose to produce similar antinociception. PK-PD modeling revealed no differences in alfentanil systemic pharmacokinetics between P-gp expressers and nonexpressers. However, the steady-state brain-to-serum concentration ratio ($K_{p,brain,ss}$) was ~3-fold lower in *mdr1a*(+/+) mice compared to *mdr1a*(-/-) mice (0.19±0.01 versus 0.54±0.04, respectively). Consistent with the ~3-fold lower $K_{p,brain,ss}$, the antinociception versus serum concentration relationship in *mdr1a*(+/+) mice was shifted ~3-fold rightward compared to *mdr1a*(-/-) mice. However, there was no difference in the antinociception versus brain concentration relationship, or in the brain tissue EC_{50} (11±1.8 versus 9.2±1.7 ng/g), between *mdr1a*(+/+) and *mdr1a*(-/-) mice. These results indicate that alfentanil is an *in vivo* P-gp substrate, and are consistent with the hypothesis that P-gp-mediated efflux attenuates antinociception by reducing alfentanil $K_{p,brain,ss}$.

Introduction

P-glycoprotein (P-gp) is the 170-kD protein product of the multi-drug resistance gene (*mdr1*) first identified for its ability to confer multi-drug resistance in tumor cells (Juliano, 1976; Gros et al., 1986). P-gp mediates excretory and barrier functions in several tissue (e.g., proximal tubular cells of the kidneys, the canalicular membrane of hepatocytes in the liver, the apical membrane of intestinal enterocytes, and the luminal membrane of brain capillary endothelial cells) (Thiebaut et al., 1987; Cordon-Cardo et al., 1989; Cordon-Cardo et al., 1990). P-gp appears to play a protective role in intact mammals by attenuating absorption, facilitating excretion, and restricting distribution to several tissue sites, including the central nervous system (CNS), of many structurally diverse xenobiotics, including calcium channel blockers, HIV protease inhibitors, immunosuppressants, and opioids (Matheny et al., 2001).

Concomitant administration of P-gp inhibitors with P-gp substrates may lead to clinically significant drug interactions (Ho and Kim, 2005). For example, although the anti-diarrheal agent loperamide is a potent opioid agonist, it is not centrally active due, in part, to P-gp-mediated efflux at the blood-brain barrier (BBB) (Schinkel et al., 1996). However, when loperamide and the P-gp inhibitor quinidine were co-administered to subjects, respiratory depression was observed, which was attributed to an increase in loperamide brain concentration caused by P-gp inhibition (Sadeque et al., 2000). While the precise mechanism of this interaction has not been verified, the potential for enhanced central effects of P-gp substrates due to P-gp inhibition is nonetheless clear.

Studies have demonstrated that P-gp attenuates the brain distribution and central activity of several opioids. For example, (Thompson et al., 2000) showed that fentanyl, morphine, and methadone resulted in increased and prolonged antinociception in *mdr1a*(-/-) mice compared to

mdr1a(+/+) mice. Similarly, the cyclic peptide opioid DPDPE produced increased antinociception in *mdr1a*(-/-) mice as opposed to their P-gp-expressing counterparts (Chen and Pollack, 1998). The P-gp inhibitor GF120918 was able to restore DPDPE-mediated antinociception in *mdr1a*(+/+) mice to levels observed in the *mdr1a*(-/-) mice (Chen and Pollack, 1999). The P-gp inhibitor verapamil also was capable of increasing morphine brain concentrations and morphine-associated antinociception in *mdr1a*(+/+) mice (Zong and Pollack, 2000).

Alfentanil is a synthetic opioid used for the induction of surgical anaesthesia and the management of post-surgical pain. The alfentanil dose needs to be individualized based on numerous factors, including pathological condition, use of other medicines, and the type and duration of the surgical procedure (Scholz et al., 1996). Because alfentanil is a CYP3A4 substrate, CYP3A4 activity is another important determinant of the required alfentanil dose (Kharasch and Thummel, 1993). Many compounds are substrates of both CYP3A4 and P-gp. If alfentanil is a P-gp substrate, P-gp may be a determinant of the required alfentanil dose, a possible a source of inter-patient variability, and a potential locus of drug-drug interactions.

The impact of P-gp-mediated efflux on the pharmacokinetics and central pharmacodynamics of the alfentanil is unknown. Initial pilot experiments in this laboratory indicated P-gp-mediated efflux reduces alfentanil-associated antinociception. To investigate these observations further, and to evaluate whether P-gp efflux activity may contribute to inter-patient variability in alfentanil response or serve as a locus of drug-drug interactions, the present study was undertaken to determine the impact of P-gp-mediated efflux on the systemic pharmacokinetics, brain disposition, and central activity of alfentanil. A PK-PD modeling approach was employed

to assess the mechanism(s) by which P-gp-mediated efflux influences alfentanil-associated antinociception.

Material and Methods

Materials. Alfentanil was obtained from Taylor Pharmaceuticals (Decatur, IL) and loperamide was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were obtained from common sources and were of reagent grade or better.

Animals. Male CF-1 *mdr1a*(+/+) and *mdr1a*(-/-) mice (30-40 g; Charles River Laboratories, Inc. Wilmington, MA) were maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room with access to water and food ad libitum. All procedures involving mice were approved by The Institutional Animal Care and Use Committee of the University of North Carolina and were conducted in accordance with "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised in 1985).

PK/PD Study. Based on the results of pilot studies, 36 *mdr1a*(-/-) and 36 *mdr1a*(+/+) mice received equipotent subcutaneous doses of alfentanil in physiological saline (0.067 and 0.2 mg/kg, respectively). At 0.5, 1, 2, 4, 8, 15, 30, 45 and 60 min post-administration, antinociception was assessed, and 4 *mdr1a*(-/-) and 4 *mdr1a*(+/+) mice were sacrificed by decapitation for collection of brain tissue and trunk blood. Trunk blood was collected in 1.5-ml microcentrifuge tubes and was allowed to clot for $\geq$ 30 min at room temperature. Serum was harvested following centrifugation. Brain and serum samples were stored at -20°C until analysis by HPLC-MS/MS.

Assessment of Antinociception. Antinociception was assessed with the hot plate latency test as described elsewhere (Chen and Pollack, 1997). Prior to administration of alfentanil, baseline hotplate latency was determined for each animal in triplicate. Hotplate latency was defined as the time interval between placement on the hot plate (55°C; Columbus Instruments, Columbus, OH) and first observation of a jump or lick of the hind limb(s). Animals with an average baseline

latency < 25 sec were used in the study. A cut-off latency of 60 sec was used to avoid tissue damage. The degree of antinociception was calculated as:

$$\%\text{MPR} = \frac{\text{test latency} - \text{control latency}}{60 - \text{control latency}} \times 100\% \qquad (1)$$

Quantitation of Alfentanil in Serum and Brain. Brain samples were homogenized in water (1:2 v/v) with a sonic probe. A 25-µl aliquot of homogenate or serum was transferred to a HPLC vial, and protein was precipitated with 100 µl methanol containing internal standard (loperamide, 5 ng/ml). The sample was vortex-mixed, centrifuged, and the supernatant was analyzed by HPLC-MS/MS. Samples were injected (3 µl; CTC Analytics autosampler, Zwingen, Switzerland) onto a Phenomenex 2.0 30 mm, 5 µm Gemini 110A column (Phenomenex, Torrance, CA) maintained at room temperature. The total run time was 3 min. Analytes were eluted with a linear gradient consisting of ammonium acetate (pH 6.8; 10 mM) ["A"] and methanol ["B"] produced by two Shimadzu LC-10ADVP binary pumps. An initial condition of 5% "B" was ramped to 95% "B" over 2 min, held for 0.5 min, and then returned initial condition of 5% "B" in a single step to re-equilibrate the column. During the run, the flow rate was increased from 750 to 1500 µl/min over the first 2 min, held at 1500 µl/min for 1 min, and then returned the initial flow rate of 750 µl/min in a single step. The entire column effluent was diverted from the source of the PE-Sciex API-4000 "quadrupole mass spectrometer (Turbo V Ionspray source, 700˚C, PerkinElmerSciex Instruments, Boston, MA) for the first 1 min and last 0.5 min of the run. Alfentanil and loperamide were measured in positive ionization mode using multiple reaction monitoring (417.3→268.3 and 477.4→266.0, respectively). Standards were prepared in brain homogenate and serum and fitted with a quadratic equation with 1/y weighting (0.1-500 ng/ml). Accuracy of standards was within ± 15%.

Pharmacokinetic-Pharmacodynamic Analysis. A compartmental modeling approach with distribution between serum and brain tissue was used to describe alfentanil pharmacokinetics. The pharmacokinetic model shown schematically in Figure 1 was fit simultaneously to the serum and brain concentration data from both *mdr1a*(-/-) and *mdr1a*(+/+) mice using nonlinear least-squares regression (WinNonlin 4.1; Pharsight Corporation, Mountain View, CA). The absorption rate constant (k_a), central volume (V_c), and systemic clearance (Cl) did not differ between *mdr1a*(-/-) and *mdr1a* +/+ mice, and therefore were assumed to be identical when fitting the model to the data from both mouse strains simultaneously; the brain uptake (Cl_{up}) and brain efflux (Cl_{efflux}) clearances were allowed to vary between *mdr1a*(-/-) and *mdr1a*(+/+) mice**.** The brain volume (V_b) was determined experimentally as 13.4 ml·kg^{-1}, assuming a specific gravity of 1.0 ml. The pharmacodynamic parameters, EC_{50} and γ, were determined directly from fitting a sigmoidal E_{max} model to the antinociception versus brain concentration (C) data:

$$\%\mathrm{MPR} = \frac{E_{max} \cdot C^{\gamma}}{EC_{50}{}^{\gamma} + C^{\gamma}} \qquad (2)$$

E_{max} was defined as 100%, and γ was constrained to the same value for *mdr1a*(-/-) and *mdr1a*(+/+) mice. The time course of the brain-to-serum concentration ratio ($K_{p,brain}$) was used to estimate the brain equilibration rate constant (k_{eq}) and steady-state brain-to-serum ratio ($K_{p,brain,ss}$) according to:

$$K_{p,brain} = K_{p,brain,ss}\left(1 - e^{-k_{eq} \cdot t}\right) \qquad (3)$$

The brain equilibration half-life ($t_{1/2eq,brain}$) was obtained from k_{eq}:

$$t_{1/2eq,brain} = \frac{\ln(2)}{K_{eq}} \qquad (4)$$

Results

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Alfentanil Pharmacokinetics. Alfentanil was absorbed rapidly following subcutaneous administration, with peak serum and brain concentrations achieved in less than 10 minutes (Figure 2). Alfentanil clearance was high (~equivalent to hepatic blood flow), assuming complete absorption from the subcutaneous site, and half-life was short ($t_{1/2}$ < 15 min). Alfentanil serum concentrations were 3-fold lower in the *mdr1a*(-/-) mice, consistent with those animals receiving a 3-fold lower dose than their transporter-competent counterparts. However, the time course of brain concentrations in the *mdr1a*(-/-) and *mdr1a*(+/+) mice were nearly superimposable (Figure 2). Both the systemic and brain tissue pharmacokinetics were capable of being described by the pharmacokinetic model (Figure 2 and Table 1). Pharmacokinetic-parameter estimates obtained from the pharmacokinetic model are reported in Table 1.

Alfentanil Pharmacodynamics. Pilot experiments indicated that, at equivalent doses, antinociceptive activity was lower in *mdr1a*(+/+) mice than in *mdr1a*(-/-) mice (data not shown). However, at a 3-fold higher dose (0.20 mg/kg vs 0.067 mg/kg), the magnitude and duration of antinociception in *mdr1a*(+/+) were identical to those in *mdr1a*(-/-) mice (Figure 3). In both *mdr1a*(+/+) and *mdr1a*(-/-) mice, alfentanil had a rapid onset of antinociception, a peak effect of ~85% MPR, and a rapid offset of action with nociceptive response returning to baseline within 60 min of administration. Consistent with a lower alfentanil potency in *mdr1a*(+/+) mice (due to P-gp-mediated efflux from the brain) , there was a 3-fold rightward shift in the serum concentration-effect relationship in transporter-competent versus transporter-deficient mice (Figure 4). There was no difference in the brain concentration effect relationship, nor brain EC_{50}s, between the *mdr1a*(+/+) and *mdr1a*(-/-) mice (Figure 5; Table 1). The PK-PD model adequately described the time course of antinociception, the serum concentration-effect relationships, and the brain concentration-effect relationships in both mouse strains (Figures 3, 4,

and 5, respectively). The PK-PD model indicated the presence of a slight counterclockwise hysteresis in the antinociceptive effect versus serum concentration relationship (Figure 4). However, there was no hysteresis in the antinociceptive effect versus brain concentration relationship (Figure 5). Pharmacodynamic parameter estimates obtained from the PK-PD model are reported in Table 1.

Alfentanil Brain Disposition. Equilibration of alfentanil between brain and serum occurred rapidly, with state-steady $K_{p,brain}$ reached within approximately 4 min. The time course of alfentanil $K_{p,brain}$ is shown in Figure 6. The $K_{p,brain,ss}$ was less than unity for both *mdr1a*(+/+) and *mdr1a*(-/-) mice, and the $K_{p,brain,ss}$ in *mdr1a*(-/-) mice was 3-fold higher than in *mdr1a*(+/+) mice (Table 1; Figure 6). Estimates of Cl_{up} and Cl_{efflux} differed between *mdr1a*(+/+) and *mdr1a*(-/-) mice, with Cl_{up} increased (1.6-fold) and Cl_{efflux} decreased (1.6-fold) in *mdr1a*(-/-) mice. The $t_{1/2eq,brain}$ was short ($\leq$ 1.5 min) in both *mdr1a*(+/+) and *mdr1a*(-/-) mice. However, $t_{1/2eq,brain}$ was ~1.5-fold longer in *mdr1a*(-/-) mice (Table 1). Consistent with implicit assumptions of the PK model, the ratios of Cl_{up}/Cl_{efflux} (0.18 and 0.47 in *mdr1a*(+/+) and *mdr1a*(-/-) mice, respectively) were comparable to the respective $K_{p,brain,ss}$ values (Table 1).

Discussion

The ATP-dependent efflux transporter P-gp is the protein product of the *mdr1* gene, and is expressed in variety of tissues including the luminal membrane of the BBB (Cordon-Cardo et al., 1989; Cordon-Cardo et al., 1990). Several studies have indicated that some opioids have reduced brain penetration and attenuated central activity due to P-gp-mediated efflux (Chen and Pollack, 1998; Thompson et al., 2000; Zong and Pollack, 2000; Dagenais et al., 2004). The influence of P-gp on the pharmacokinetics and central pharmacodynamics of the synthetic opioid alfentanil had not been explored previously. Pilot experiments in this laboratory indicated that alfentanil produced less antinociception in *mdr1a*(+/+) mice than in *mdr1a*(-/-) mice, consistent with P-gp-mediated efflux at the BBB. In this study, the time course of antinociception, as well as serum and brain concentrations of alfentanil, were evaluated in *mdr1a*(+/+) and *mdr1a*(-/-) mice in order to investigate the impact of P-gp-mediated efflux on the systemic pharmacokinetics, brain disposition, and central activity of alfentanil.

In order to achieve a similar degree of antinociception in both *mdr1a*(+/+) and *mdr1a*(-/-) mice, the dose administered to *mdr1a*(+/+) mice was three-fold higher than that in transporter-deficient animals. Even though the doses were different, pharmacokinetic modeling indicated no difference in systemic pharmacokinetics between *mdr1a*(+/+) and *mdr1a*(-/-) mice (Table 1). This result was not unexpected since P-gp often has minimal impact on systemic pharmacokinetics following subcutaneous or intravenous administration (Chen et al., 2003).

In contrast to the serum pharmacokinetics, P-gp had a pronounced effect on alfentanil brain pharmacokinetics. The $K_{p,brain,ss}$ of *mdr1a*(+/+) mice was ~3-fold lower compared to *mdr1a*(-/-) mice (0.19 vs. 0.54). The decrease in $K_{p,brain,ss}$ was accompanied by a ~1.6-fold decrease in Cl_{up} and ~1.6-fold increase in Cl_{efflux}. These observations are consistent with the hypothesis that P-gp

decreases $K_{p,brain,ss}$ by both attenuating brain uptake and enhancing brain efflux. Similar observations have been reported for other P-gp substrates (Kusuhara et al., 1997). The brain and serum concentrations of alfentanil equilibrated rapidly, with a $t_{1/2,eq,,brain} \leq 1.5$ min. This value is similar to previously reported estimates from humans (Lotsch, 2005). Unexpectedly, the $t_{1/2,eq,,brain}$ was shorter in the *mdr1a*(+/+) mice (Table 1). This observation may be explained by the fact that $t_{1/2,eq,,brain}$ is inversely proportional to Cl_{efflux}, and that P-gp increased Cl_{efflux} (~1.6-fold) thereby causing a proportional decrease in the $t_{1/2,eq,,brain}$ in *mdr1a*(+/+) mice (~1.4-fold). Interestingly, this result implies that P-gp-mediated efflux may reduce equilibration time between brain and systemic concentrations. Previously, the short $t_{1/2,eq,,brain}$ of alfentanil had been attributed in part to a small $K_{p,brain,ss}$ (Upton et al., 1997). In this study, the $K_{p,brain,ss}$ of alfentanil was less than unity for both *mdr1a*(+/+) and *mdr1a*(-/-) mice, indicating two important points: first, that a small $K_{p,brain,ss}$ may indeed facilitate rapid equilibrium between systemic and brain concentration, and second, that a $K_{p,brain,ss}$ greater than unity may not be needed, or even desirable, for CNS drug with rapid onset and offset of action.

PK-PD modeling indicated a ~3-fold rightward shift in the antinociception versus serum concentration relationship for *mdr1a*(+/+) mice compared to *mdr1a*(-/-) mice. PK-PD modeling also revealed a slight counterclockwise hysteresis in the antinociception versus serum concentration relationship for both *mdr1a*(+/+) and *mdr1a*(-/-) mice. However, there was no hysteresis in the antinociception versus brain concentration relationship, and the brain tissue EC_{50}s between *mdr1a*(+/+) and *mdr1a*(-/-) mice were not different. These observations are consistent with brain concentrations driving antinociception, and provide compelling evidence that P-gp efflux attenuates alfentanil antinociception by reducing $K_{p,brain,ss}$.

This study is the first to show that alfentanil is a P-gp substrate. In contrast, an earlier study that examined the transcellular flux of alfentanil across L-MDR1 (expressing P-gp) and LLC-PK1 cell monolayers concluded alfentanil was not a P-gp substrate and had low affinity towards P-gp (IC_{50} > 50 μM) (Wandel et al., 2002). There are at least two possible explanations for the difference in results between these two studies. First, even though murine-human differences in P-gp substrate recognition and transport appear modest for most substrates (Yamazaki et al., 2001; Hochman et al., 2002; Takeuchi et al., 2006), there might be species differences in the P-gp-mediated transport of alfentanil,. This study evaluated the *in vivo* effects of murine P-gp (*mdr1a*), whereas the previous work studied the human form of P-gp (*MDR1*) *in vitro.* Secondly, *in vitro* systems often are less sensitive than intact animal models for identifying weak P-gp substrates (Polli et al., 2001; Raub et al., 2006). In the Wandel et al. study, the basal activity of endogenous efflux transporter(s) in the LLC-PK1 and L-MDR1 cell lines may have masked P-gp-mediated transport of alfentanil, for flux was higher in the basolateral-to-apical direction in both the P-gp-expressing L-MDR1 and control LLC-PK1 cell monolayers.

Alfentanil is a CYP3A4 substrate in humans, and has been used as a noninvasive clinical probe to evaluate CYP3A4 activity (Kharasch et al., 2005). The degree of miosis produced by alfentanil has been shown to correlate well with alfentanil plasma concentrations, and as such alfentanil-pupilometry studies have been used to evaluate CYP3A4 activity and to conduct drug-drug interaction studies. An assumption of such studies is that any increase or decrease in alfentanil-induced miosis is due primarily to changes in CYP3A4 activity (inhibition or induction). The present results showing that alfentanil is a P-gp substrate indicate that alfentanil-pupilometry studies may have the potential to detect alterations in P-gp activity. Previous pupilometry studies conducted with the P-gp substrates morphine, fentanyl, methadone, and

loperamide have shown that inhibition of P-gp at the BBB by the P-gp inhibitor quinidine is modest (Kharasch et al., 2003; Skarke et al., 2003; Kharasch et al., 2004a; Kharasch et al., 2004b). Since quinidine is one of the most potent compounds capable of inhibiting P-gp that is in clinical use, the likelihood of significant inhibition of P-gp at the BBB appears remote. However, future drug-drug interaction studies conducted with alfentanil should be assessed carefully to ensure that any observed drug-drug interaction is not caused by P-gp inhibition. The clinical significance of alfentanil being a P-gp substrate is not known, but may be modest considering that only a 3-fold P-gp effect was observed in mice, that all human *MDR1a* ploymorisms identified to date retain most functional activity, and that clinically significant inhibition of P-gp at the BBB has not been well documented (Ho and Kim, 2005; Kerb, 2006).

In summary, the present study indicated that alfentanil is a P-gp substrate, and that P-gp-mediated efflux attenuates alfentanil antinociception by reducing $K_{p,brain,ss}$. These observations may have important implications regarding inter-individual differences in alfentanil pharmacodynamics and for the risk of drug-drug interactions. Additional studies may be warranted to assess the clinical relevance of P-gp efflux as a determinant of alfentanil pharmacotherapy.

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Footnotes

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Legends to Figures

Figure 1. Pharmacokinetic-pharmacodynamic model for alfentanil disposition and antinociception in mice. Pharmacokinetic parameters were obtained by fitting the above model to the time course of serum and brain concentrations of *mdr1a*(-/-) and *mdr1a*(+/+) mice following subcutaneous administration of alfentanil. The absorption rate constant (K_a), central volume (V_c), and systemic clearance (Cl) were held constant between *mdr1a*(-/-) and *mdr1a*(+/+) mice; whereas, the brain uptake (Cl_{up}) and brain efflux clearances (Cl_{efflux}) were allowed to vary between *mdr1a*(-/-) and *mdr1a*(+/+) mice**.** The brain volume (V_b) was fixed. The effect parameters, EC_{50} and γ, were obtained by fitting a sigmoidal E_{max} model to the brain concentration versus antinociception data. E_{max} was defined as 100%, and γ was constrained to the same value for *mdr1a*(-/-) and *mdr1a*(+/+) mice.

Figure 2. Time course of serum (●) and brain (▲) concentrations following a 0.067- or 0.2-mg/kg s.c. dose of alfentanil in *mdr1a*(-/-) (open symbols) or *mdr1a*(+/+) (solid symbols) mice, respectively. Data are presented as mean ± S.E. ($n \geq 3$). Dashed and solid lines represent the fit of the PK model to the concentration data for *mdr1a*(-/-) and *mdr1a*(+/+) mice, respectively.

Figure 3. Time course of antinociception following a 0.067- or 0.2-mg/kg s.c. dose of alfentanil in *mdr1a*(-/-) (open symbols) or *mdr1a*(+/+) (solid symbols) mice, respectively. Data are presented as mean ± S.E. ($n \geq 3$). Dashed and solid lines represent the fit of the PK-PD model to the antinociception data for *mdr1a*(-/-) and *mdr1a*(+/+) mice, respectively.

Figure 4. Relationship between antinociception and serum concentration of alfentanil following a 0.067- [*mdr1a*(-/-); ○] or 0.2- [*mdr1a*(+/+); ●] mg/kg s.c. dose**.** Data are presented as mean ±

S.E. [concentration data (n ≥ 3); antinociception (n = 4 to 33)]. Dashed and solid lines represent the fit of the PK-PD model to the antinociception and serum concentration data for *mdr1a*(-/-) and *mdr1a*(+/+) mice, respectively. A slight counterclockwise hysteresis is present for both *mdr1a*(-/-) and *mdr1a*(+/+) mouse strains.

Figure 5. Relationship between antinociception and brain concentration of alfentanil following a 0.067- [*mdr1a*(-/-); ○] or 0.2- [*mdr1a*(+/+); ●] mg/kg s.c. dose**.** Data are presented as mean ± S.E. [concentration data (n ≥ 3); antinociception (n = 4 to 33)]. Dashed and solid lines represent the fit of a sigmoidal E_{max} model to the effect data obtained from *mdr1a*(-/-) and *mdr1a*(+/+) mice, respectively. Gamma was constrained to the same value for both mouse strains.

Figure 6. Time course of alfentanil $K_{p,brain}$ in mice following a 0.067- [*mdr1a*(-/-); ○] or 0.2- [*mdr1a*(+/+); ●] mg/kg s.c. dose. Data are presented as mean ± S.E. (n ≥ 3). Dashed and solid lines represent the fit of a kinetic model to the *mdr1a*(-/-) and *mdr1a*(+/+) data, respectively.

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Table 1. PK-PD parameters for alfentanil in *mdr1a*(-/-) and *mdr1a*(+/+) mice

Parameter	weighting	*mdr1a*(-/-)	*mdr1a*(+/+)
K_a (min^{-1})		0.35 ± 0.03	
Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)	1/y	82 ± 3	
V_c ($ml \cdot kg^{-1}$)	1/y	1000 ± 60	
$t_{1/2}$ (min)	-	12 ± 0.6 [a]	
Cl_{up} ($ml \cdot min^{-1} \cdot kg^{-1}$)	1/y	7 ± 5	4 ± 3
Cl_{efflux} ($ml \cdot min^{-1} \cdot kg^{-1}$)	1/y	13 ± 12	22 ± 17
V_b ($ml \cdot kg^{-1}$)	-	13.4 (fixed)	
EC_{50} (ng/g)	uniformed	9.2 ± 1.7	11 ± 1.8
γ	uniformed	1.8 ± 0.4	
$K_{p,brain}$	1/y	0.54 ± 0.04	0.195 ± 0.008
K_{eq} (min^{-1})	1/y	0.46 ± 0.10	0.64 ± 0.09
$t_{1/2eq,brain}$ (min)	-	1.5 ± 0.3	1.08 ± 0.16

[1] Parameter estimate ± S.E. from nonlinear least-squares regression analysis of pooled *mdr1a*(-/-) or *mdr1a*(+/+) mouse data.

[2] Ka, Cl, Vc, Vb, and γ were constrained to the same value for *mdr1a*(-/-) or *mdr1a*(+/+) mice.

[3] $t_{1/2}$ and $t_{1/2eq,brain}$ were calculated from $0.693/(V_c/Cl)$ and $0.693/K_{eq,brain}$, respectively.

[a] Propagation of error was used to calculated SE.

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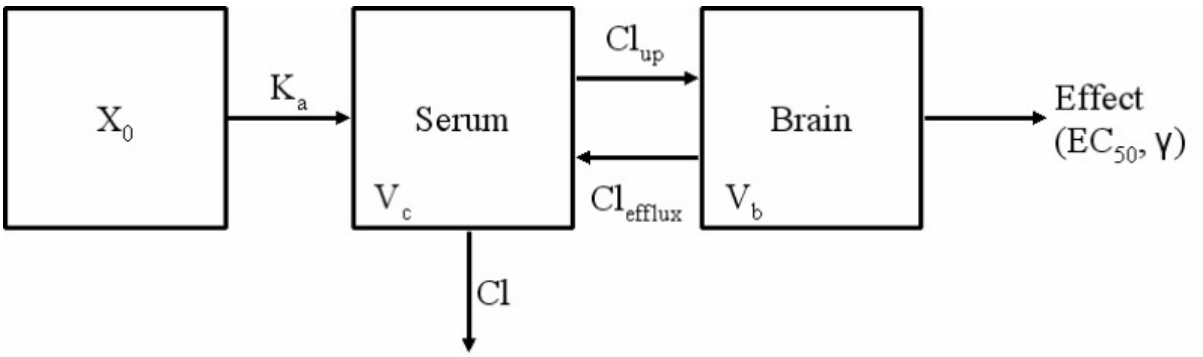

Figure 1

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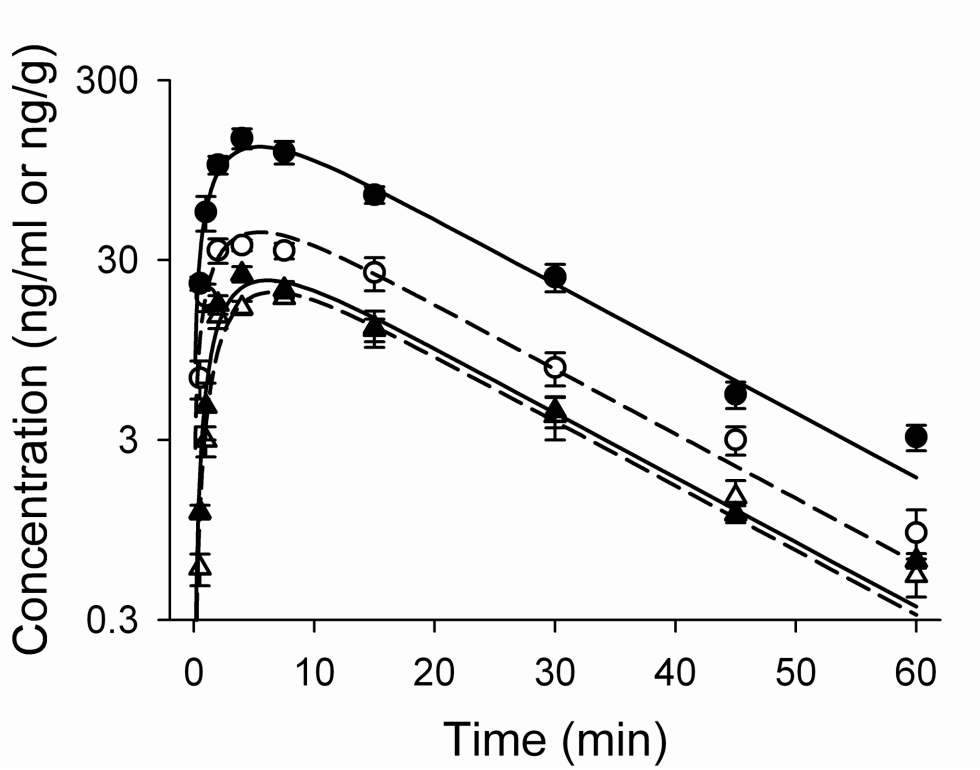

Figure 2

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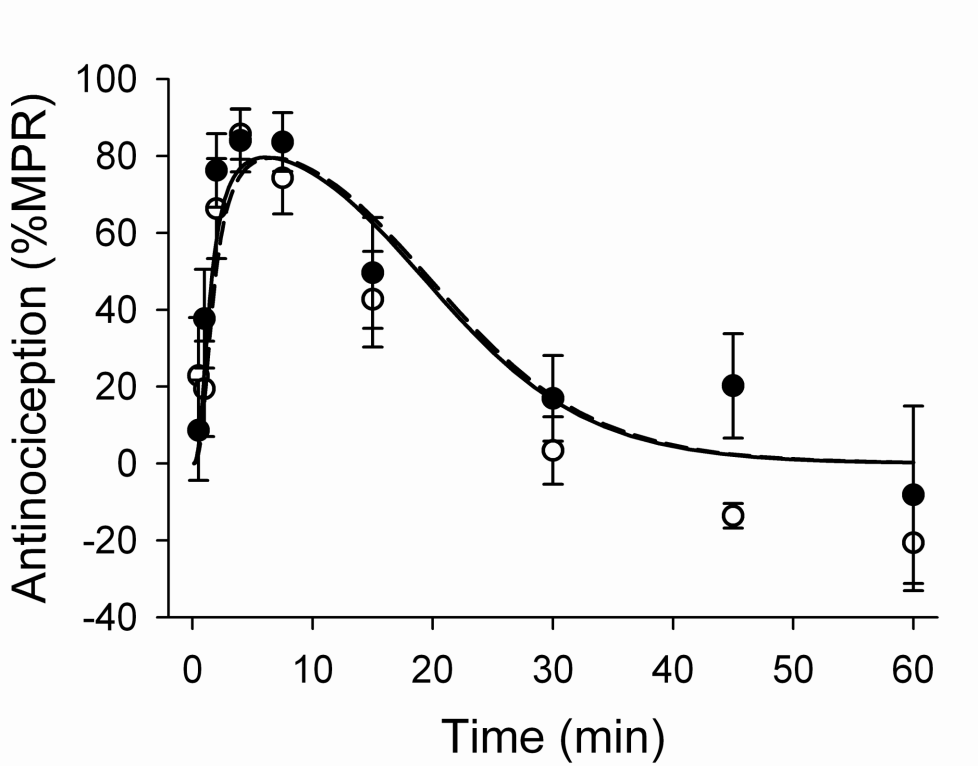

Figure 3

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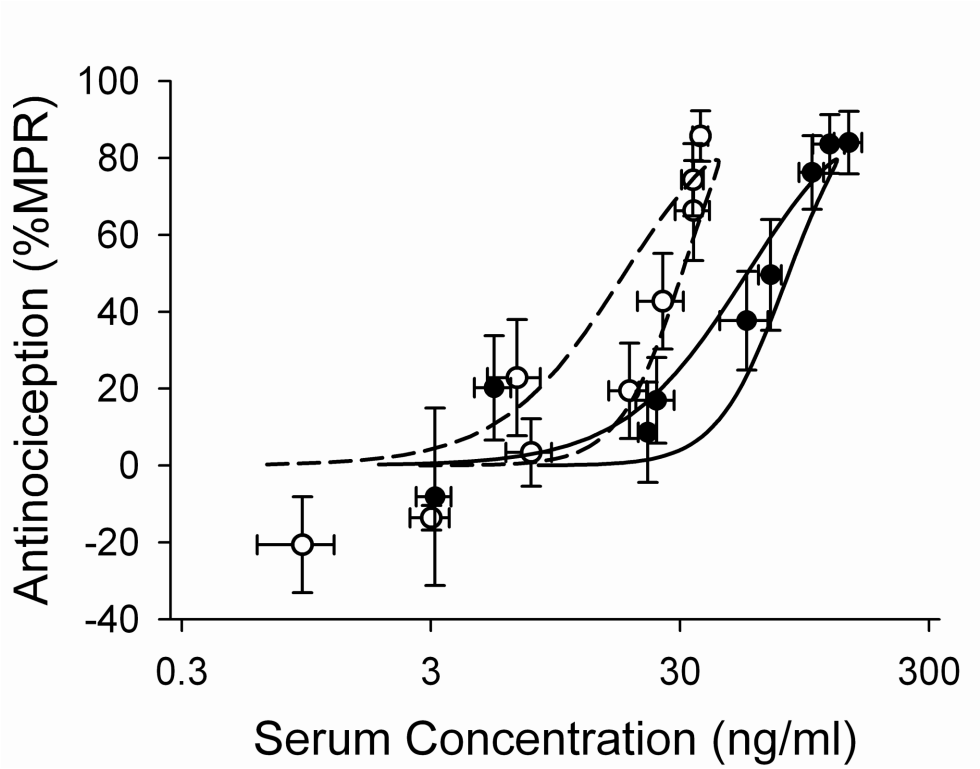

Figure 4

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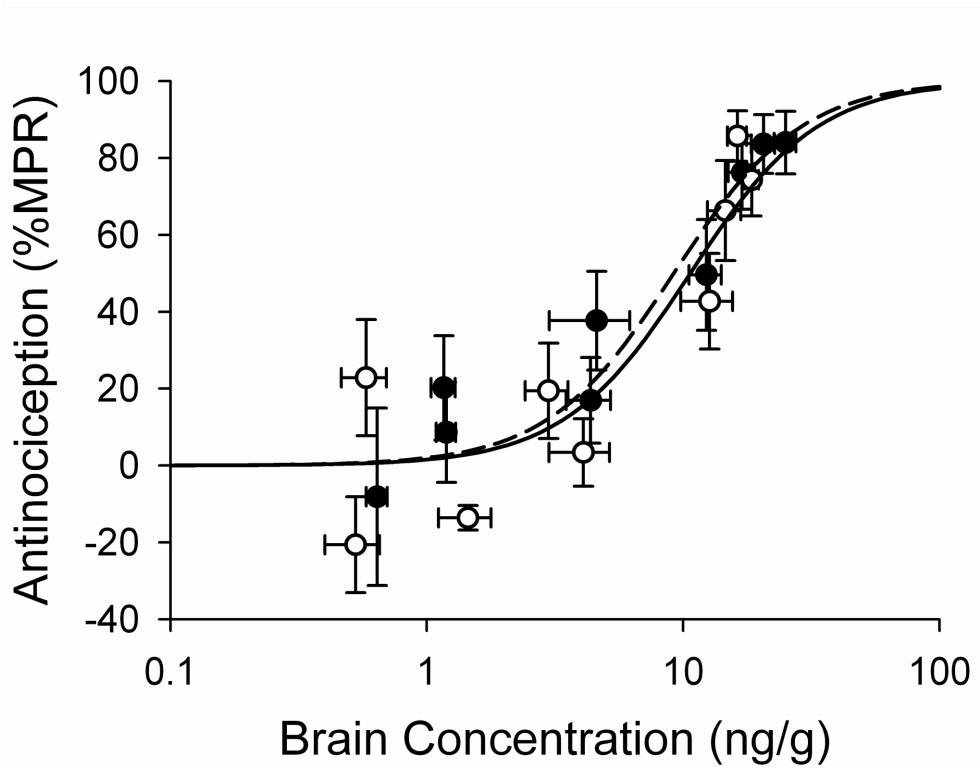

Figure 5

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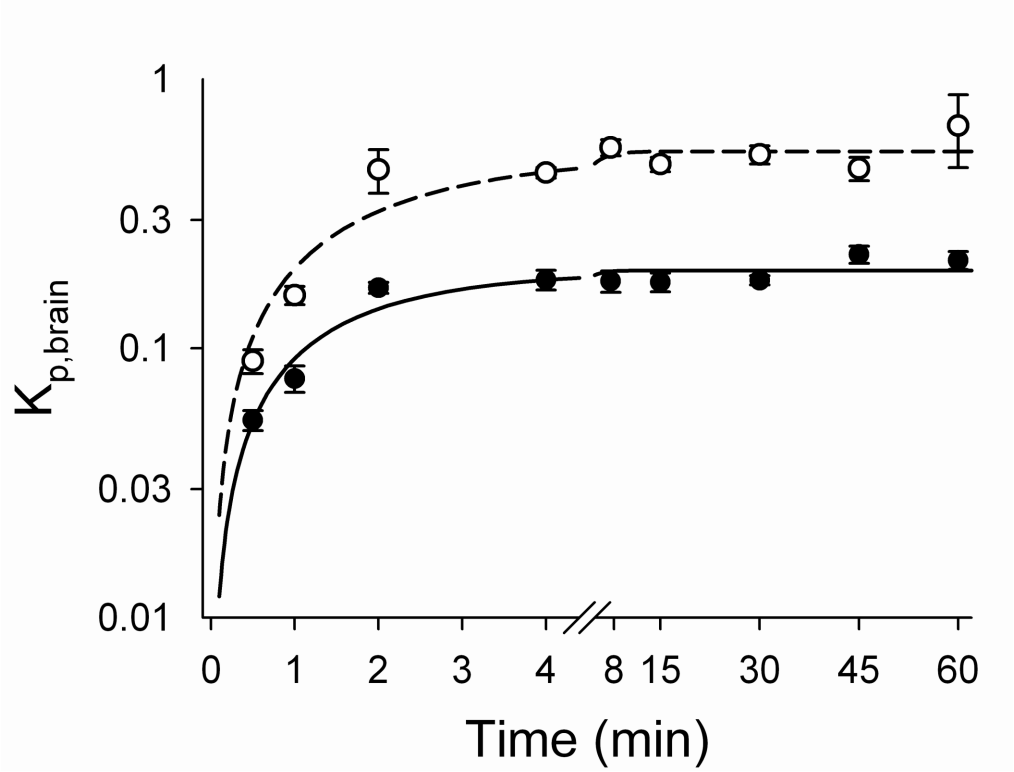

Figure 6