Coadministration of P-Glycoprotein Modulators on Loperamide Pharmacokinetics and Brain Distribution

Rita Nieto Montesinos, Brice Moulari, Jessica Gromand, Arnaud Beduneau, Alf Lamprecht, and Yann Pellequer

Laboratory of Pharmaceutical Engineering, University of Franche-Comté, Besançon, France

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ABSTRACT

The efflux transporter P-glycoprotein, expressed at high levels at the blood-brain barrier, exerts a profound effect on the disposition of various therapeutic compounds in the brain. A rapid and efficient modulation of this efflux transporter could enhance the distribution of its substrates and thereby improve central nervous system pharmacotherapies. This study explored the impact of the intravenous co-administration of two P-glycoprotein modulators, tariquidar and elacridar, on the pharmacokinetics and brain distribution of loperamide, a P-glycoprotein substrate probe, in rats. After 1 hour postdosing, tariquidar and elacridar, both at a dose of 1.0 mg/kg, increased loperamide levels in the brain by 2.3- and 3.5-fold, respectively. However, the concurrent administration of both P-glycoprotein modulators, each at a dose of 0.5 mg/kg, increased loperamide levels in the brain by 5.8-fold and resulted in the most pronounced opioid-induced clinical signs. This phenomenon may be the result of a combined noncompetitive modulation by tariquidar and elacridar. Besides, the simultaneous administration of elacridar and tariquidar did not significantly modify the pharmacokinetic parameters of loperamide. This observation potentially allows the concurrent use of low but therapeutic doses of P-gp modulators to achieve full inhibitory effects.

Introduction

Since its discovery in 1976 (Juliano and Ling, 1976), P-glycoprotein (P-gp) has been the most extensively studied ATP-binding cassette (ABC)-dependent efflux transporter. This protein is often regarded as a model to understand the biochemical mechanism of some ABC transport proteins. Two factors make P-glycoprotein (P-gp) the most critical efflux transporter: 1) its broad substrate specificity, which results in multidrug resistance (MDR) (Ambudkar et al., 1999), and 2) the prominent expression of P-gp in most excretory and barrier-function tissues (Loscher and Potschka, 2005). The relevant expression of P-gp at the blood-brain barrier (BBB) exerts a profound effect on the brain distribution of human immunodeficiency virus protease inhibitors, anticaner drugs, opioids, some psychotropics and other drugs, which leads to the failure of various clinical treatments for brain diseases (Loscher and Potschka, 2005; Linnet and Ejsing, 2008; Varatharajan and Thomas, 2009). The inhibition of P-gp-mediated efflux could enhance the distribution of these substrates into the brain and thereby improve central nervous system (CNS) pharmacotherapies.

The identification of some P-gp substrates that also had the ability to block the P-gp-mediated efflux led to the synthesis of their analogs to minimize effects not related to their inhibition of P-gp-mediated efflux. Unfortunately, these compounds, known as first- and second-generation P-gp modulators, caused undesirable pharmacokinetic profiles as a result of their nonspecificity toward the P-gp (Ecker and Chiba, 1995). With the purpose of avoiding these limitations, third-generation P-gp modulators have been developed. To be therapeutically effective, these compounds should be noncompetitive and sufficiently potent to achieve inhibitory effects at nontoxic plasma concentrations and sufficiently selective for P-gp to minimize effects on overall drug pharmacokinetics (Anderson et al., 2006). In vivo studies demonstrated that elacridar and tariquidar, third-generation P-gp modulators, significantly increased the brain distribution of several P-gp substrates without pharmacokinetic interactions (Choo et al., 2006; Hubensack et al., 2008). In contrast, recent studies promote the use of significantly high doses of these P-gp modulators to efficiently modulate the P-gp-mediated efflux at the BBB (Bauer et al., 2013). However, when coadministered with P-gp substrates, these doses may be associated with pharmacokinetic interactions and toxic profiles, thus limiting the use of these agents. This escalating doses approach could reflect the same drawbacks of the first- and second-generation P-gp modulators.

Unnecessary exposure to P-gp modulators could be minimized and potential drug-related side effects might be reduced if, instead of using one P-gp modulator at a high dose, a combination of P-gp modulators with different drug binding sites were used at lower and safe doses. Martin et al. (2000) described the presence of at least four distinct interaction sites on P-gp and the binding of tariquidar to site II (a transport and regulatory site) and elacridar to site IV (an exclusive
Materials and Methods

Loperamide hydrochloride and tetraglycol were obtained from Sigma Aldrich (St. Quentin Fallavier, France), elacridar was synthesized by the Laboratory of Pharmaceutical Chemistry at the University of Bonn in Germany, and tariquidar was purchased from API Services Inc. Polyethylene glycol 600 (PEG600) was obtained from Interchimie (Bobigny, France). Trisodium citrate solution was purchased from BD Vacutainer®80 (Franklin Lakes, NJ).

Ketoconazole (internal standard for loperamide) and chlorpromazine hydrochloride (internal standard for elacridar and tariquidar), tert-butyl methyl ether (t-BME), analytical-grade ammonium acetate and glacial acetic acid were obtained from Sigma-Aldrich (France). HPLC grade methanol and acetonitrile (ACN) were purchased from Carlo Erba Réactifs (Val-de-Reuil, France). Ultrapure water was freshly obtained before use from a Purelab Prima 7/15/20 Ultra Purelab Ultra Mk 2 from Elga LabWater (Wasquehal, France).

Ether (t-BME) was obtained from Interchimie (Bobigny, France). Trisodium citrate and tariquidar was purchased from API Services Inc. Polyethylene glycol 600 (PEG600) was obtained from Interchimie (Bobigny, France). Trisodium citrate solution was purchased from BD Vacutainer®80 (Franklin Lakes, NJ).

Animals. Behavioral observation, pharmacokinetic, and brain distribution studies were conducted in male Sprague-Dawley rats (Janvier, Le Genest Saint Isle, France). All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, Washington, DC). All the animals were allowed to acclimate for 1 week and were 7 weeks old (230–280 g) at the time of the experiment. The animals were maintained under a 12-hour light/dark cycle and a temperature-controlled environment. Food and water were provided ad libitum. The studies were approved by the Institutional Animal Care and Use Committee of the University of Franche-Comte.

Drug Solutions. The drug solutions were prepared on the day of the experiment. Elacridar was dissolved in tetraglycol at an initial concentration of 20 mg/ml. Loperamide and tariquidar were dissolved separately in a mixture of saline and PEG600 (3:1) at concentrations of 2 mg/ml. For each treatment, loperamide, elacridar, and tariquidar solutions were diluted with saline and PEG600 (3:1). All the solutions were completely transparent, indicating the full solubility of loperamide and both P-gp modulators in the vehicle.

Study Design. This study was carried out using a rat model, which is a promising model to predict P-gp activity by elacridar and/or tariquidar, loperamide, a μ-opioid agonist without central effects, can become a drug that produces substantial anti-nociception.
Statistical Analysis. The statistical analysis was carried out using SigmaStat 3.5 software. Analyses of statistical significance between two groups were examined by Student’s t test and between many groups by one-way analysis of variance (ANOVA) with the Holm-Sidak post-hoc test. P < 0.05 was considered significant. Moreover, the variance of the AUC_{inf} in each treatment group was estimated according to the Bailar method (Bailer, 1988; Yuan, 1993), which is based on the variability of the concentrations at each sampling time. A Z-test was used for pairwise comparison of AUCs. P < 0.05 was considered significant.

Results

Analysis of Loperamide, Elacridar, and Tariquidar by LC-MS. Based on structural similarities, solubility, recovery efficiency, and previous successful data (Kemper et al., 2001; Yu et al., 2004), ketoconazole was a satisfactory internal standard for loperamide, as chlorpromazine was for elacridar and tariquidar. The developed LC-MS method described in this article was linear over the concentration range 5.0–1000 ng/ml for all the three analytes: loperamide, elacridar, and tariquidar (r^2 ≥ 0.9990). Using 100 μl of rat plasma or tissue homogenate, the validated lower limit of quantification for each compound was the lowest concentration of standard on the calibration curves, 5.0 ng/ml. Intraday and interday accuracy and precision were within 15% for the three analytes. The specificity of the method was confirmed by the absence of interferences from endogenous compounds. In this study, the sample preparation procedure using ACN and t-BME (1:1) demonstrated absolute recovery values from rat plasma and brain samples higher than 90% for loperamide, ketoconazole, elacridar, tariquidar, and chlorpromazine. Furthermore, stability tests demonstrated that the analytes were stable under the storage conditions. The current validated method (data not shown) was then used for the simultaneous quantitation of loperamide, elacridar, and tariquidar in plasma and brain samples.

Influence of the Coadministration of Elacridar and Tariquidar on Their Plasma and Brain Levels. At a first stage, the groups that received elacridar at 1.0 mg/kg and/or tariquidar at 1.0 mg/kg (groups I, II, and III) were used to compare whether the concurrent administration of both P-gp modulators influenced their own plasma and brain distributions (Figs. 1 and 2).

No modification in the plasma AUC_{inf} of elacridar alone or co-administered with tariquidar (31.9 ± 2.7 versus 32.2 ± 3.4 nmol/h/ml) and in the plasma AUC_{inf} of tariquidar alone or coadministered with elacridar (37.8 ± 1.9 versus 37.0 ± 2.8 nmol h/ml) were observed (Table 1). These values indicate that the coadministration of these P-gp modulators at 1.0 mg/kg each had no observable effects on each other plasma concentrations. The elacridar AUC_{inf} for the brain remained unchanged after concurrent administration with tariquidar (3.1 ± 0.1 versus 3.6 ± 0.4 nmol/h/g). Vice versa, the tariquidar AUC_{inf} for the brain increased from 0.8 ± 0.1 to 1.6 ± 0.1 nmol/h/g (2.0-fold) in the presence of elacridar (Table 1). This increase was associated with a twofold higher K_{p}, for tariquidar. These findings suggest that when both P-gp modulators are coadministered, elacridar could interfere with the active transport of tariquidar at the BBB.

Influence of the Coadministration of Elacridar and Tariquidar on Loperamide Plasma Levels. To evaluate the effects of the co-administration of both P-gp modulators on loperamide pharmacokinetics, the groups that received a total dose of 1.0 mg/kg of P-gp modulators (groups I, II, and IV) were compared. Group V served as a control.

In rats receiving 0.5 mg/kg of loperamide alone, the concentration of loperamide in plasma (C_{max}) after 1 hour (T_{max}) was approximately 4.0% of the administered dose, which reflects a rapid metabolism of loperamide during this first hour (Fig. 3; Table 2). The mean elimination half-life of a single loperamide administration in this study was 3.6 ± 0.3 hours, and it was not significantly altered in presence of elacridar and/or tariquidar. Likewise, the AUC, MRT, CL, and V_{Dss} were not significantly different in any of the treatments using one or two P-gp modulators. These results confirmed that neither elacridar nor tariquidar altered the pharmacokinetic parameters of loperamide.

Influence of the Coadministration of Elacridar and Tariquidar on Loperamide Concentrations in the CNS. To evaluate the effects of the coadministration of both P-gp modulators on the brain distribution of loperamide, the groups that received a total dose of 1.0 mg/kg of P-gp modulators (groups I, II, and IV) were compared. Group V served as a control.

Previous studies (Elkiweri et al., 2009; Kawamura et al., 2011) showed that low doses of P-gp modulators and loperamide were taken up into the brain. In agreement with these results, in the present study,...
these doses demonstrated sufficient degree of P-gp inhibition at the BBB (Tables 3 and 4). Immediately after administration, a few animals from the loperamide-treated groups that received tariquidar at 1.0 mg/kg or elacridar at 1.0 mg/kg showed lethargy, piloerection, and shallow breathing. However, these animals were able to respond if handled, and by 15 minutes post treatment, they recovered normal activity. According to our clinical score, 1.0 mg/kg of tariquidar (total score = 13) and 1.0 mg/kg of elacridar (total score = 26) slightly promoted the central effects of the loperamide. More than 50% of the animals from the group that received loperamide coadministered with elacridar and tariquidar, each at 0.5 mg/kg, not only immediately exhibited the same clinical signs but also demonstrated whole-body tetany and eye protrusion. In addition, two of the 12 animals of this group showed the Straub reaction, which is characterized by the rigidity of the tail, held in an S-shaped curve across the back of the animal (Bilbey et al., 1960). These animals recovered normal activity approximately 30 minutes later. These clinical signs indicate that coadministration of the two P-gp modulators (total score = 102) at a total dose of 1.0 mg/kg significantly potentiated the opioid brain effects of loperamide (Table 3).

The administration of loperamide alone resulted in very low levels (10.53 ± 0.51 ng/g) in the brain after 1 hour (Fig. 3). However, the coadministration of the P-gp modulators significantly increased the concentration of loperamide in the brain at this time point. Tariquidar and elacridar, each at 1.0 mg/kg increased loperamide levels in the brain by 2.3- (22.48 ± 2.93 ng/g) and 3.5-fold (33.84 ± 3.95 ng/g), respectively. However, the concurrent administration of both P-gp modulators at half doses increased the concentration of the loperamide in the brain by 5.8-fold (47.26 ± 6.09 ng/g). After 6 hours, loperamide was undetectable in brains from animals that had not received either P-gp modulator and nearly 10.0 ng/g in the other three groups. After 12 and 24 hours, loperamide was not detectable in any group. The differences in the loperamide AUC_{inf} for the brains and the K_{p} values were even more marked than the effects at the individual times (Table 4). All these results suggest a greater inhibition of the P-gp-mediated efflux by elacridar than by tariquidar and a possible synergistic effect of both P-gp modulators when they are coadministered.

**Discussion**

Given that the use of relatively high doses of the third-generation P-gp modulators (Salama et al., 2005) may be limited by the same drawbacks of the first- and second-generation P-gp modulators, this study evaluated the potential of combining the administration of two P-gp modulators to determine the influence on the efflux activity of the P-gp at the BBB.

The coadministration of elacridar and tariquidar did not significantly increase the plasma concentrations of each agent relative to the values obtained for administration of the single agents. Their respective AUCs suggest that at the doses used in the current work, neither P-gp modulator interferes with the elimination pathway of the other. The K_{p} obtained for individual doses of elacridar and tariquidar are low as a result of the low concentrations of either P-gp modulator in the brain. These results contrast with prior studies that showed that the levels of tariquidar and elacridar were much higher in the brain than in the plasma (Bankstahl et al., 2008; Kuntner et al., 2010), but those experiments used between 3.0- and 15-fold higher doses of P-gp modulators compared with the current study. These differences were properly explained before (Kawamura et al., 2011; Sane et al., 2013), where murine models revealed that at higher doses of elacridar, a higher distribution of the P-gp modulator was attained in the brain. For instance, at 1 hour post injection, the brain-to-plasma concentration ratio of elacridar increased from ~0.4 at 0.5 mg/kg to ~5.2 at 2.5 mg/kg (Sane et al., 2013). This dose-dependent distribution relationship was also observed for tariquidar (Bankstahl et al., 2013). A supplementary explanation for the low brain distribution of elacridar and tariquidar in this study is based on the pharmacokinetic behavior of these compounds at low doses. At nanomolar doses, both P-gp modulators are actively transported not only by the P-gp but also by the breast cancer resistance protein at the BBB (Bankstahl et al., 2013). Thus, the amount transported by these two proteins would be higher than the amount that arrives to the brain by passive diffusion, resulting in increased plasma concentrations and decreased brain concentrations of these compounds.

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**TABLE 1**

Area under the concentration-time curves (AUC_{inf}) and brain-to-plasma partition coefficient (K_{p}) of elacridar and tariquidar

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Elacridar 1.0 mg/kg (1773.05 nmol/kg)</th>
<th>Elacridar 1.0 mg/kg (+ Tariquidar 1.0 mg/kg)</th>
<th>Tariquidar 1.0 mg/kg (1545.60 nmol/kg)</th>
<th>Tariquidar 1.0 mg/kg (+ Elacridar 1.0 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{inf}_plasma (nmol·h/ml)</td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>3.1</td>
<td>0.27</td>
<td>3.22</td>
<td>0.34</td>
<td>37.8</td>
</tr>
<tr>
<td>AUC_{inf}_brain (nmol·h/g)</td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>0.098</td>
<td>0.01</td>
<td>0.115</td>
<td>0.021</td>
<td>0.022</td>
</tr>
</tbody>
</table>
Nevertheless, when the two compounds are coadministered, elacridar may reduce or delay the active transport of tariquidar by both proteins (Kannan et al., 2011), thus significantly increasing the \( K_P \) of tariquidar. An important issue to consider when evaluating the distribution of low doses of P-gp modulators in the brain is the species differences in P-gp transport activity, which appear to be substrate-dependent (Lin and Yamazaki, 2003). Several chemical entities that were P-gp substrates in mice were also P-gp substrates in rats, but the brain distribution of these compounds is not always the same in both species. In one clear example, whereas the \( K_P \) of N-desmethyl-venlafaxine was the same in mice and rats, the \( K_P \) of risperidone was 2.36-fold higher in mice than in rats (Bundgaard et al., 2012). These data can also account for the higher brain distribution of relative low doses of P-gp modulators in mice compared with our rat model.

To evaluate the effects of the coadministration of both P-gp modulators at a total dose of 1.0 mg/kg on P-gp activity, loperamide was chosen as a P-gp substrate probe. The mean half-life of loperamide was 3.6 ± 0.3 hours, which is different from a previous study (Zamek-Gliszczynski et al., 2012), where less than 1.0%/ml of the intravenously-administered dose of loperamide was monitored at 5 minutes postdosing. This difference can be attributed to the low solubility of loperamide in the vehicle used in that study. However, our \( K_P \) values of loperamide are in agreement with another study, where the \( K_{P(0-1h)} \) of the loperamide was 0.006 (Elkiweri et al., 2009). In the current investigation, the half-life as well as the AUC, MRT, CL, and Vd\(_{a}\) were not significantly modified when loperamide was coadministered with elacridar or tariquidar or both P-gp modulators at a total dose of 1.0 mg/kg. The lack of alterations in the pharmacokinetic parameters of loperamide in the different groups confirms the minimal modulation on the P-gp transport activity, which appear to be substrate-dependent (Lin and Yamazaki, 2003). Several chemical entities that were P-gp substrates in mice are also P-gp substrates in rats, but the brain distribution of these compounds is not always the same in both species. In one clear example, whereas the \( K_P \) of N-desmethyl-venlafaxine was the same in mice and rats, the \( K_P \) of risperidone was 2.36-fold higher in mice than in rats (Bundgaard et al., 2012). These data can also account for the higher brain distribution of relative low doses of P-gp modulators in mice compared with our rat model.

### TABLE 2

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>No P-gp Modulator</th>
<th>Tariquidar 1.0 mg/kg</th>
<th>Elacridar 1.0 mg/kg</th>
<th>Elacridar 0.5 mg/kg+ Tariquidar 0.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>( T_{max} ) (h)</td>
<td>1.0</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>( C_{max} ) (ng/ml)</td>
<td>5052</td>
<td>800</td>
<td>5371</td>
<td>660</td>
</tr>
<tr>
<td>AUC(_{inf} ) (ng.h/ml)</td>
<td>28900</td>
<td>4554</td>
<td>34365</td>
<td>4800</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>3.6</td>
<td>0.3</td>
<td>3.9</td>
<td>0.5</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>5.0</td>
<td>0.4</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
<td>CL (ml/h/kg)</td>
<td>4.4</td>
<td>0.7</td>
<td>3.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Vd (ml/kg)</td>
<td>22.1</td>
<td>2.6</td>
<td>20.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*ANOVA: Significantly different compared with the group that received no P-gp modulator.

### TABLE 3

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>No P-gp Modulator</th>
<th>Tariquidar 1.0 mg/kg</th>
<th>Elacridar 1.0 mg/kg</th>
<th>Elacridar 0.5 mg/kg+ Tariquidar 0.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>AUC(_{inf} ) (ng.h/g)</td>
<td>10.5</td>
<td>0.5</td>
<td>124.5( ^a )</td>
<td>20.1</td>
</tr>
<tr>
<td>( K_P )</td>
<td>0.0004</td>
<td>0.0001</td>
<td>0.0037( ^c )</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

\(^{a}\)Bailer method: significantly different compared with the group that received no P-glycoprotein (P-gp) modulator.

\(^{b}\)Bailer method: significantly different compared with the group that received no P-gp modulator.

\(^{c}\)ANOVA: significantly different compared with the group that received elacridar or tariquidar at 1.0 mg/kg.

\(^{d}\)ANOVA: significantly different compared with the group that received elacridar or tariquidar at 1.0 mg/kg.
that of the P-gp substrate [3H]vinblastine (Martin et al., 1999). Thus, despite the active transport, it appears that both P-gp modulators were able to bind at their corresponding drug binding sites and the complex allosteric communication resulted in a possible synergistic interaction. Nevertheless, the dose-limiting opioid effects of loperamide preclude assessing this strategy with higher doses of loperamide coadministered with higher doses of the P-gp modulators. Taking into account that synergism can be different at different dose levels (Chou, 2006), these preliminary synergistic effects should be further confirmed using radiolabeled [1H or 14C]loperamide associated to higher doses of P-gp modulators. Moreover, many other P-gp substrates with different therapeutic effects and several doses of elacridar and tariquidar remain to be explored and extrapolated to different species to define the synergistic interaction between both P-gp modulators. The synergistic phenomenon observed herein resulted in strong pharmacodynamic effects by loperamide, a potent CNS agent. However, this approach could be restricted in infectious or cancer diseases, where high brain concentrations of the therapeutic agents are needed and a synergism at high effect levels is more relevant than at low effect levels (Chou, 2006). Other P-gp modulators can also be used for these studies, provided that the steric hindrance of one does not affect the binding of the other.

Since this preliminary study support the synergistic modulation of P-gp using low doses of elacridar and tariquidar, this approach may represent a potential step forward to avoid the use of high, nearly toxic doses of P-gp modulators without significant pharmacokinetics interactions. Furthermore, because the distribution and the permanence of these P-gp modulators in the brain are dose-dependent, the rapid decrease of the P-gp modulators in the brain as observed in this work represents an advantage. Our approach could avoid the entry of harmful compounds after a long-lasting P-gp inhibition at the BBB.

Acknowledgments

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Authorship Contributions

Participated in research design: Nieto Montesinos, Beduneau, Lamprecht, and Pellequer.

Conducted experiments: Nieto Montesinos, Gromand.

Contributed new reagents or analytic tools: Nieto Montesinos, Mouliar, Pellequer.

Performed data analysis: Nieto Montesinos, Lamprecht, Pellequer.

Wrote or contributed to the writing of the manuscript: Nieto Montesinos, Lamprecht, Pellequer.

Contributions

Tariquidar 1.0 mg/kg

Elacridar 0.5 mg/kg

Elacridar 1.0 mg/kg

Central nervous system

<table>
<thead>
<tr>
<th>Clinical Signs (1 h postdose)</th>
<th>No P-gp Modulator</th>
<th>Elacridar 1.0 mg/kg</th>
<th>Tariquidar 1.0 mg/kg</th>
<th>Elacridar 0.5 mg/kg+ Tariquidar 0.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethargy</td>
<td>0/12 0</td>
<td>6/12 2</td>
<td>4/12 2</td>
<td>9/12 3</td>
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<tr>
<td>Whole-body tetry</td>
<td>0/12 0</td>
<td>0/12 0</td>
<td>0/12 0</td>
<td>9/12 2</td>
</tr>
<tr>
<td>Straub tail</td>
<td>0/12 0</td>
<td>0/12 0</td>
<td>0/12 0</td>
<td>2/12 2</td>
</tr>
<tr>
<td>Pilocerection</td>
<td>0/12 0</td>
<td>4/12 1</td>
<td>7/12 2</td>
<td>3/12 1</td>
</tr>
<tr>
<td>Shallow breathing</td>
<td>0/12 0</td>
<td>5/12 2</td>
<td>2/12 1</td>
<td>9/12 3</td>
</tr>
<tr>
<td>Eyes</td>
<td>0/12 0</td>
<td>0/12 2</td>
<td>0/12 0</td>
<td>5/12 2</td>
</tr>
<tr>
<td>Eye protrusion</td>
<td>0/12 0</td>
<td>26</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

N/n, number of rats displaying these clinical signs/number of rats per group (n = 12).

References


TABLE 4

Opioid-induced clinical signs

The degrees of the clinical signs are scored as 0 = none, 1 = mild, 2 = moderate and 3 = severe. Values shown in the degree columns are the mean degree score for each symptom. Total score = total sum of N x degree. Rats that displayed a score of 1 or higher on 3 or more signs were considered to display opioid-induced behavior.


Address correspondence to: Rita Nieto Montesinos, Laboratory of Pharmaceutical Engineering, University of Franche-Comté, 19, rue Ambroise Paré, 25000 Besançon, France. E-mail address: milynm@gmail.com