Modest effect of impaired P-glycoprotein on the plasma concentrations of fexofenadine, quinidine, and loperamide following oral administration in Collie dogs

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Running title:

Plasma PK profile of drugs in P-gp mutated Collie dogs

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Abbreviations: ABC, ATP-binding cassette; BBB, blood-brain barrier; MDR/Mdr, multidrug resistance; P-gp, P-glycoprotein
Abstract

P-glycoprotein (P-gp), encoded by the multidrug resistance 1 gene (MDR1/ABCB1), exhibits very broad substrate specificity, and plays important roles in drug disposition. The purpose of the present study was to examine the effect of impaired P-gp activity on the plasma pharmacokinetics of P-gp substrates in Collie dogs with or without homozygous mutant alleles producing truncated P-gp. Three therapeutic agents, fexofenadine (0.1 mg/kg), quinidine (0.1 mg/kg), and loperamide (0.01 mg/kg), were simultaneously given orally, and their plasma concentration–time profiles were determined. The plasma concentrations of these drugs tended to be higher in dogs with the homozygous mutated allele. The $C_{\text{max}}$ was 53.9 ± 13.1 and 90.7 ± 23.1 ng/mL for fexofenadine, 16.5 ± 3.4 and 20.0 ± 7.9 ng/mL for quinidine, and 80.8 ± 9.0 and 101 ± 15 pg/mL for loperamide, and the $AUC_{0-8}$ was 263 ± 62 and 435 ± 95 ng·h/mL for fexofenadine, 54.5 ± 11.5 and 75.7 ± 21.8 ng·h/mL for quinidine, and 467 ± 85 and 556 ± 91 pg·h/mL for loperamide, in homozygous wild type and homozygous mutated dogs, respectively. Only the plasma concentration differences of fexofenadine at 4 to 8 h following oral administration were statistically significant. This result suggests that P-gp limits the intestinal absorption of fexofenadine in dogs. Collie dogs with the Mdr1 mutation will be useful for examining the effect of P-gp on the oral availability of drugs.
Introduction

The multidrug resistance 1 gene (MDR1/ABCB1) encodes a 170 kDa transmembrane protein pump, called P-glycoprotein (P-gp), belonging to the ATP-binding cassette (ABC) superfamily of membrane transporters. An increasing number of studies have shown that P-gp exhibits a broad substrate specificity, and a number of structurally unrelated drugs are substrates for P-gp (Choudhuri and Klaassen, 2006). P-gp is expressed in the apical membrane of the epithelial cells in the liver, intestine, and kidney, and brain capillary endothelial cells. A number of studies, particularly using Mdr1a-/- and Mdr1a/1b-/- mice, have demonstrated that P-gp limits oral availability and penetration into the brain, and mediates biliary and urinary excretion by actively extruding xenobiotics into the adjacent luminal space (Chen et al., 2003; Mizuno et al., 2003). For instance, Mdr1a/1b-/- mice exhibited greater plasma concentrations than wild-type mice after oral administration of paclitaxel (Sparreboom et al., 1997), fexofenadine (Tahara et al., 2005), cyclosporin A (Lee et al., 2005), etoposide (Allen et al., 2003), and vinblastine (Ogihara et al., 2006). As for daunomycin, loperamide, quinidine, ritonavir, and verapamil are concerned, the permeability-surface area product in the small intestine increased more than 3-times in Mdr1a/1b-/- mice (Adachi et al., 2003). In humans, P-gp has been suggested to limit the oral availability of some drugs. Co-administration of quinidine increased the plasma concentration of digoxin following oral administration (Pedersen et al., 1983; Fromm et al., 1999). Repeated rifampicin treatment decreased the plasma levels of digoxin and talinolol (Greiner et al., 1999; Westphal et al., 2000). A single dose of St John’s wort increased the plasma concentration of fexofenadine, while long-term treatment reversed the changes in fexofenadine disposition (Wang et al., 2002; Dresser et al., 2003). These changes have been accounted for by modulation of P-gp activity in the small intestine, inhibition or induction. Apart from these drugs, the effect of P-gp on drug absorption in humans remains under question since some clinically important drugs have been developed as oral formulations even although they are
P-gp substrates (Lin and Yamazaki, 2003).

A subpopulation of Collie dogs is extremely sensitive to ivermectin, which is used extensively in veterinary medicine to treat and control infections caused by nematode and arthropod parasites. Sequence analysis of Mdr1 cDNA from ivermectin-sensitive dogs identified a 4-bp deletion, which causes a frame-shift mutation generating several stop codons that result in a severely truncated, non-functional protein (Mealey et al., 2001). The frequency of the mutant allele is about as high as 60% in Collie dogs (Hugnet et al., 2004; Neff et al., 2004; Kawabata et al., 2005). Since dogs (Canis familiaris) have one Mdr1 gene corresponding to human MDR1, Collie dogs with homozygous mutant alleles would be a good animal model for examining the importance of P-gp in the pharmacokinetics of drugs in relatively large animals. In particular, in pharmaceutical companies, dogs are commonly used preclinically as a convenient animal species for testing oral dosage forms because of their anatomical similarity to humans (Lin, 1995), their ability to ingest human-scale dosage forms, and because they are easy to handle.

The purpose of the present study was to examine the effect of impaired P-gp activity on the plasma pharmacokinetics of P-gp substrates in Collie dogs. Three therapeutic agents, fexofenadine, quinidine, and loperamide (Figure 1), were selected. Fexofenadine exhibited a 6-fold increase in oral availability (Tahara et al., 2005), and quinidine and loperamide exhibited an 8- and 3-fold increase in the permeability-surface area product determined by in situ intestinal perfusion in Mdr1a/1b−/− mice (Adachi et al., 2003). The three drugs were simultaneously administered orally, and the plasma concentration–time profiles were determined.
Material and Methods

Animals

Eleven Collie dogs were used in this study (Table 1). The animals were aged between 14 and 68 months and had a body weight of between 18 and 28 kg. Of the 11 dogs, 5 (1 male and 4 females) were homozygous for the wild type Mdr1 allele, and 6 (2 males and 4 females) were homozygous for the mutant allele.

Study design

After a 12-hour fast, each animal received 0.5 mg/kg famotidine (Progogue for Injection 20mg; Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan) intramuscularly to reduce the inter-individual variability of the gastrointestinal tract by controlling gastric acid secretion. One hour after famotidine administration, the dogs were given orally a capsule filled with a pulverized commercial preparation of fexofenadine (allegra 60 mg Tablets; sanofi-aventis K.K., Tokyo, Japan), quinidine sulfate (Quinidine Sulfate Tab.“Hoei”; Merck Seiyaku Ltd., Osaka Japan), and loperamide (LOPEMIN Capsules 1mg; Janssen Pharmaceutical K.K., Tokyo, Japan) at a dose of 0.1 mg/kg, 0.1 mg/kg, and 0.01 mg/kg, respectively. Venous blood samples (2 mL each) were collected in tubes containing EDTA-2K at 0.5, 1, 2, 4, 6, and 8 h after oral administration. Plasma samples were separated by centrifugation and stored at –30°C until analysis.

In this study, three drugs were simultaneously given orally to the dogs. If the concentration of these drugs in the enterocytes exceeds the $K_i$ value for P-gp inhibition, a drug-drug interaction may reduce P-gp function. A prediction criterion for intestinal enzyme-mediated drug-drug interactions was proposed based on the fact that if $\text{Dose}/K_i$ is below 2.76 L, there have been no reports of an obvious drug-drug interaction in humans (Tachibana et al., Prediction of intestinal enzyme mediated drug-drug interaction and nonlinear intestinal first-pass metabolism, 21st JSSX...
Considering that the \textit{in vitro} IC_{50} values for inhibition of digoxin transport in Caco-2 cells are above 100 \(\mu\)M for fexofenadine (Cvetkovic et al., 1999), 2.2 \(\mu\)M for quinidine (Choo et al., 2000), and 2.5 \(\mu\)M for loperamide (Wandel et al., 2002), the \textit{Dose/Ki} values were calculated to be below 0.04 L, 2.8 L, and 0.17 L, respectively, taking the IC_{50} values as the \textit{Ki} values. This suggests that the doses used in this study are low enough to avoid any P-gp based drug-drug interaction in the intestine, although species differences between dogs and humans cannot be excluded. Following the same logic, auto-saturation of intestinal P-gp activity may not take place.

**Quantification of drug concentrations in plasma**

For fexofenadine and quinidine quantification, a 10-\(\mu\)L plasma sample was mixed vigorously with 20 \(\mu\)L acetonitrile and deproteinized by centrifugation. Then, 25 \(\mu\)L supernatant was added to 100 \(\mu\)L water and subjected to liquid chromatography–mass spectrometry. To determine the loperamide concentration, a 500-\(\mu\)L plasma sample was added to a mixture of 0.5 M Na_{2}CO_{3} (50 \(\mu\)L) and ethyl acetate (1000 \(\mu\)L) and mixed vigorously. After centrifugation, 800 \(\mu\)L of the organic layer was collected, dried in a centrifugal concentrator (TOMY, Tokyo, Japan), and dissolved in 40 \(\mu\)L dimethylsulfoxide. Then, 10-\(\mu\)L aliquots were subjected to liquid chromatography–mass spectrometry.

The HPLC separation involved a Waters Alliance 2695 Separation Module (Waters, Milford, MA) with an L-column ODS (2.1 \(\times\) 150 mm, 5 \(\mu\)m; Chemicals Evaluation and Research Institute, Tokyo, Japan). The composition of the mobile phase was acetonitrile/0.05\% formic acid (26:74, 7:93, and 29:71 for fexofenadine, quinidine, and loperamide, respectively). The flow rate was 0.3 mL/min. Mass spectra were determined using a Micromass ZQ2000 mass spectrometer (Waters) with an electrospray ionization interface in the selected ion-monitoring mode using positive ions,
Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated using non-compartmental analysis. The peak plasma concentration ($C_{\text{max}}$) was determined by inspection of individual plasma concentration–time curves. The elimination half-life ($t_{1/2}$) was calculated using the following equation:

$$t_{1/2} = \ln(2) / k_e$$

where $k_e$ is the elimination rate constant calculated from the slope of the terminal portion of the log-transformed plasma concentration–time curve. For fexofenadine and quinidine, the last 3 points, which gave a determination coefficient ($r^2$) > 0.8 were considered as the terminal phase. However, in the case of loperamide, we could not determine $k_e$ because of lack of clarity in its terminal phase.

The area under the concentration–time curve was calculated by the linear trapezoidal rule up to 8 h ($AUC_{0-8}$) and then extrapolated to infinity ($AUC_{0-\infty}$) using the elimination rate constant. The area under the first moment curve ($AUMC_{0-\infty}$) and mean residence time ($MRT$) were calculated using the following equations:

$$AUC_{0-\infty} = AUC_{0-8} + C_{p,8h} / k_e$$

$$AUMC_{0-8} = \sum_{i=0}^{t_e} \frac{(t_{i+1}C_{i+1} + t_iC_i)(t_{i+1} - t_i)}{2}$$

$$AUMC_{0-\infty} = AUMC_{0-8} + 8 \cdot C_{p,8h} / k_e + C_{p,8h} / k_e^2$$

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

where $C_{p,8h}$ is the plasma concentration 8 h after oral administration.

Statistical analysis
All data represent five or six experiments and are expressed as the mean ± SE. Any statistical significance in the differences of the means was assessed using a two-tailed Student’s t test. P < 0.05 was considered statistically significant.

Results and Discussion

The dog has one Mdr1 gene, and the homozygous mutant alleles of the Mdr1 gene totally impair P-gp activity in the body. The present study examined the effect of impaired P-gp activity on the oral availability of P-gp substrates. The plasma concentration–time profiles of fexofenadine, quinidine, and loperamide following simultaneous oral administration are shown in Figure 2. P-gp deficient mutant Collie dogs generally exhibited a higher plasma concentration than normal dogs. However, the difference was marginal for quinidine and loperamide, but fexofenadine showed a significant increase at 4 to 8 h following administration (Figure 2) although such increase was not great enough to produce a statistically significant change in the pharmacokinetic parameters (Table 2). Since the contribution of P-gp to the elimination of fexofenadine from the systemic circulation is minimal in mice (Tahara et al., 2005), the increase in the plasma concentration of fexofenadine in the mutant Collie dogs may be ascribed to the impaired intestinal efflux. A similar elimination half-life in normal and mutant Collie dogs also supports the absence of the effect of functional impairment of P-gp on the systemic elimination of fexofenadine. Comparison of the plasma concentration–time profile following oral and intravenous administration will be required to confirm definitely that the higher plasma levels of fexofenadine in mutant dogs are strictly accounted for by increased intestinal absorption. On average, the change was at most 2-fold, which was smaller than that observed in mice (Tahara et al., 2005). It is possible that the effect of P-gp on drug absorption is not as important as that observed in mice. It should be noted that three drugs were simultaneously administered to dogs in this study. We selected the doses of the drugs tested to be
much smaller than the clinical doses considering the fact that drug-drug interaction involving P-gp does not occur in clinical studies when the inhibitor dose is smaller than 50 mg/body weight (about 0.7 mg/kg) (Tachibana et al., unpublished observation). If these doses are still enough to saturate P-gp mediated efflux, the effect of functional impairment of P-gp will be underestimated. Further studies are necessary to elucidate the importance of P-gp in limiting oral availability of drugs at linear dose ranges and clinical does for elucidating the functional importance of P-gp in the small intestine, and its clinical relevance.

The present study highlights the usefulness of Collie dogs with a hereditary deficiency in P-gp for examining the effect of P-gp on the oral absorption of drugs. Exhaustive comparison of transport activities of 3,300 P-gp substrates by human and mouse P-gp exhibits a good linear correlation, suggesting that they exhibit similar substrate specificities (Feng et al., 2007). Considering more close sequence similarity between dog and human MDR1 (90% identity at the amino acid level), dog studies will give a clue to evaluate the impact of P-gp on the disposition of test compounds in human. However, we must pay attention on the species difference when the results of dog studies are extrapolated to humans since it is true that some P-gp substrates was outlier of the correlation of the transport activities by human and dog P-gp (Takeuchi et al., 2006).

In addition to the intestinal absorption, hepatobiliary and urinary excretion, P-gp plays indispensable role in limiting brain penetration in the blood-brain barrier (BBB). Recently, the P-gp function at the BBB was evaluated in monkey using specific P-gp inhibitor (PSC833) by positron emission tomography (PET) technique (Lee et al., 2006). However, the magnitude of the increase observed in PSC833-treated monkeys was not as high as that observed in P-gp knockout mice. This may be explained by incomplete inhibition of P-gp activity by PSC833 (Kusuhara et al., 1997). Mdr1 mutated Collie dogs will be useful for in vivo evaluation of the role of P-gp in the brain penetration of test compounds in relatively large animals.
In conclusion, this result suggests that P-gp limits the intestinal absorption of fexofenadine in dogs. Collie dogs with the Mdr1 mutation will be useful for examining the effect of P-gp on the oral availability of drugs.

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References


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Tahara H, Kusuhara H, Fuse E and Sugiyama Y (2005) P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role in its biliary excretion. *Drug Metab Dispos* **33**:963-968.


alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. *Anesthesiology* 96:913-920.


Figure legends

Figure 1. Structures of fexofenadine, quinidine, and loperamide.

Figure 2. Time profiles of the plasma concentrations of fexofenadine (A), quinidine (B), and loperamide (C) after a single oral administration in wild-type and Mdr1 mutated Collie dogs.

Fexofenadine (0.1 mg/kg), quinidine (0.1 mg/kg), and loperamide (0.01 mg/kg) were given simultaneously to wild-type (closed symbols) and Mdr1 mutated (open symbols) Collie dogs by a single oral administration. Each point represents the mean ± SE (n = 5 or 6). * P < 0.05, significantly different from wild-type dogs.
Table 1. Information of Collie dogs used in this study.

<table>
<thead>
<tr>
<th></th>
<th>sex</th>
<th>body weight (kg)</th>
<th>age (months)</th>
</tr>
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<tbody>
<tr>
<td>wild-1</td>
<td>F</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>wild-2</td>
<td>F</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>wild-3</td>
<td>F</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>wild-4</td>
<td>F</td>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>wild-5</td>
<td>M</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>mutant-1</td>
<td>F</td>
<td>24</td>
<td>52</td>
</tr>
<tr>
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<td>F</td>
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<td>17</td>
</tr>
<tr>
<td>mutant-3</td>
<td>F</td>
<td>25</td>
<td>68</td>
</tr>
<tr>
<td>mutant-4</td>
<td>M</td>
<td>27</td>
<td>56</td>
</tr>
<tr>
<td>mutant-5</td>
<td>M</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>mutant-6</td>
<td>F</td>
<td>24</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 2. Pharmacokinetic parameters of fexofenadine, quinidine, and loperamide after a single oral administration in wild-type and Mdr1 mutated Collie dogs.

The details of pharmacokinetic analyses are described in Materials and Methods. Each value represents the mean ± SE of five or six independent experiments. *p*-values were obtained by two-tailed Student’s *t* test.

<table>
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<tr>
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<th>wild type (n=5)</th>
<th>mutant (n=6)</th>
<th><em>p</em>-value</th>
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<tbody>
<tr>
<td><strong>Fexofenadine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; ng/mL</td>
<td>53.9 ± 13.1</td>
<td>90.7 ± 23.1</td>
<td>0.224</td>
</tr>
<tr>
<td><em>AUC</em>&lt;sub&gt;0-8&lt;/sub&gt; ng·h/mL</td>
<td>263 ± 62</td>
<td>435 ± 95</td>
<td>0.186</td>
</tr>
<tr>
<td><em>AUC</em>&lt;sub&gt;0-∞&lt;/sub&gt; ng·h/mL</td>
<td>392 ± 77</td>
<td>881 ± 249</td>
<td>0.118</td>
</tr>
<tr>
<td>MRT h</td>
<td>8.21 ± 1.38</td>
<td>11.1 ± 1.9</td>
<td>0.260</td>
</tr>
<tr>
<td><em>t</em>&lt;sub&gt;1/2&lt;/sub&gt; h</td>
<td>5.21 ± 0.77</td>
<td>6.86 ± 1.35</td>
<td>0.343</td>
</tr>
<tr>
<td><strong>Quinidine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; ng/mL</td>
<td>16.5 ± 3.4</td>
<td>20.0 ± 7.9</td>
<td>0.720</td>
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<tr>
<td><em>AUC</em>&lt;sub&gt;0-8&lt;/sub&gt; ng·h/mL</td>
<td>54.5 ± 11.5</td>
<td>75.7 ± 21.8</td>
<td>0.441</td>
</tr>
<tr>
<td><em>AUC</em>&lt;sub&gt;0-∞&lt;/sub&gt; ng·h/mL</td>
<td>58.8 ± 12.8</td>
<td>89.3 ± 21.8</td>
<td>0.284</td>
</tr>
<tr>
<td>MRT h</td>
<td>3.17 ± 0.57</td>
<td>5.14 ± 0.75</td>
<td>0.073</td>
</tr>
<tr>
<td><em>t</em>&lt;sub&gt;1/2&lt;/sub&gt; h</td>
<td>1.95 ± 0.17</td>
<td>2.65 ± 0.36</td>
<td>0.136</td>
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<tr>
<td><strong>Loperamide</strong></td>
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<tr>
<td><em>C</em>&lt;sub&gt;max&lt;/sub&gt; pg/mL</td>
<td>80.8 ± 9.0</td>
<td>101 ± 15</td>
<td>0.312</td>
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<tr>
<td><em>AUC</em>&lt;sub&gt;0-8&lt;/sub&gt; pg·h/mL</td>
<td>467 ± 85</td>
<td>556 ± 91</td>
<td>0.501</td>
</tr>
</tbody>
</table>
Fig1

fexofenadine

quinidine

loperamide
Fig2

A

Plasma Fexofenadine (ng/mL)

Time (h)

B

Plasma Quinidine (ng/mL)

Time (h)

C

Plasma Loperamide (pg/mL)

Time (h)