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 collies 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 after oral administration were statistically significant. This result suggests that P-gp limits the intestinal absorption of fexofenadine in dogs. Collies with the Mdr1 mutation will be useful for examining the effect of P-gp on the oral availability of drugs.

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 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 in brain capillary endothelial cells. A number of studies, particularly using $Mdr1a^{-/-}$ and $Mdr1b^{-/-}$ 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, $Mdr1a^{-/-}$ 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). 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, whereas 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 though they are P-gp substrates (Lin and Yamazaki, 2003).

A subpopulation of collies 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-base-pair deletion, which causes a frame-shift mutation generating several stop codons that result in a severely truncated, nonfunctional protein (Mealey et al., 2001). The frequency of the mutant allele is about as high as 60% in collies (Hugnet et al., 2004; Neff et al., 2004; Kawabata et al., 2005). Since dogs (Canis familiaris) have one Mdr1 gene corresponding to human MDR1, collies 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.

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ABBREVIATIONS: ABC, ATP-binding cassette; MDR/Mdr, multidrug resistance; P-gp, P-glycoprotein; AUC, area under the concentration-time curve; AUMC, area under the first-moment curve; MRT, mean residence time.
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 collies. Three therapeutic agents, fexofenadine, quinidine, and loperamide (Fig. 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.

Materials and Methods

Animals. Eleven collies 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-h fast, each animal received 0.5 mg/kg famotidine (Progogue for injection, 20 mg; Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan) intramuscularly to reduce the interindividual variability of the gastrointestinal tract by controlling gastric acid secretion. One hour after famotidine administration, the dogs were orally given a capsule filled with a pulverized intestinal tract by controlling gastric acid secretion. One hour after famotidine administration, the dogs were orally given a capsule filled with a pulverized

The high-performance liquid chromatography separation involved a Waters Alliance 2695 Separation Module (Waters, Milford, MA) with an L-column ODS (2.1 × 150 mm, 5 μm; 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, m/z 502.5, 325.3, and 477.4, respectively.

Pharmacokinetic Analysis. Pharmacokinetic parameters were calculated using noncompartamental 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 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 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 ($AUC_{\text{inf}}$) using the elimination rate constant. The area under the first-moment curve ($AUMC_{0–8}$) and mean residence time (MRT) were calculated using the following equations:

$$\text{AUC}_{0–8} = \text{AUC}_{0–8} + C_{\text{p,inf}}/k_e$$

$$\text{AUMC}_{0–8} = \sum_{t=0}^{n} \left( t_{i+1}C_{i+1} + t_iC_i \right) \cdot (t_{i+1} - t_i)$$

$$\text{MRT} = \frac{\text{AUMC}_{0–8}}{\text{AUC}_{0–8}}$$

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

Statistical Analysis. All data represent five or six experiments and are expressed as the mean ± S.E. Any statistical significance in the differences of
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Tahara et al., 2005), the increase in the plasma concentration of fexofenadine from the systemic circulation is minimal in mice but fexofenadine showed a significant increase at 4 to 8 h following oral administration are shown in Fig. 2. P-gp deficient mutant collies 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 (approximately 0.7 mg/kg) (Tachibana T, Kato M, Sugiyama Y, unpublished observations). 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 doses for elucidating the functional importance of P-gp in the small intestine and its clinical relevance.

The present study highlights the usefulness of collies 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 3300 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 closer sequence similarity between dog and human MRD1 (90% identity at the amino acid level), dog studies will provide clues for how to evaluate the impact of P-gp on the disposition of test compounds in humans. However, we must pay attention to the species difference when the results of dog studies are extrapolated to humans, since it is true that some P-gp substrates were outliers of the correlation of the transport activities by human and dog P-gp (Takeuchi et al., 2006).

In addition to the intestinal absorption and hepatobiliary and urinary excretion, P-gp plays an indispensable role in limiting brain penetration in the blood-brain barrier. Recently, the P-gp function at the blood-brain barrier was evaluated in monkeys using a specific P-gp inhibitor (PSC833) by a positron emission tomography technique (Lee et al., 2006). However, the magnitude of the increase of fexofenadine in the mutant collies may be ascribed to the impaired intestinal efflux. A similar elimination half-life in normal and mutant collies also supports the absence of the effect of functional impairment of P-gp on the systemic elimination of fexofenadine. The details of pharmacokinetic analyses are described in Materials and Methods. Each value represents the mean ± S.E. of five or six independent experiments. P values were obtained by a two-tailed Student’s t test.

**FIG. 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 collies. 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) collies by a single oral administration. Each point represents the mean ± S.E. (n = 5 or 6). *, P < 0.05, significantly different from wild-type dogs.

**Results and Discussion**

A 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 Fig. 2. P-gp deficient mutant collies 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 (Fig. 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

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Wild Type (n = 5)</th>
<th>Mutant (n = 6)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fexofenadine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>53.9 ± 13.1</td>
<td>90.7 ± 23.1</td>
<td>0.224</td>
</tr>
<tr>
<td>AUC_{0–8} (ng/ml)</td>
<td>263 ± 62</td>
<td>435 ± 95</td>
<td>0.186</td>
</tr>
<tr>
<td>AUC_{t} (ng/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>t_{1/2} (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>
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<td></td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>16.5 ± 3.4</td>
<td>20.0 ± 7.9</td>
<td>0.720</td>
</tr>
<tr>
<td>AUC_{0–8} (ng/ml)</td>
<td>54.5 ± 11.5</td>
<td>75.7 ± 21.8</td>
<td>0.441</td>
</tr>
<tr>
<td>AUC_{t} (ng/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>t_{1/2} (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>
<td></td>
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<tr>
<td>C_{max} (pg/ml)</td>
<td>80.8 ± 9.0</td>
<td>101 ± 15</td>
<td>0.312</td>
</tr>
<tr>
<td>AUC_{0–8} (pg/ml)</td>
<td>467 ± 85</td>
<td>556 ± 91</td>
<td>0.501</td>
</tr>
</tbody>
</table>

The in vivo correlation of transport activities of P-gp substrates by human and dog P-gp exhibits a good linear correlation, suggesting that they exhibit similar substrate specificities (Feng et al., 2007). Considering closer sequence similarity between dog and human MRD1 (90% identity at the amino acid level), dog studies will provide clues for how to evaluate the impact of P-gp on the disposition of test compounds in humans. However, we must pay attention to the species difference when the results of dog studies are extrapolated to humans, since it is true that some P-gp substrates were outliers of the correlation of the transport activities by human and dog P-gp (Takeuchi et al., 2006).

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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 collies 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. Collies 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


Takahara 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.


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