Pharmacokinetic Role of P-Glycoprotein in Oral Bioavailability and Intestinal Secretion of Grepafloxacin in Vivo

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ABSTRACT

The purpose of this study was to clarify the contribution of P-glycoprotein to the bioavailability and intestinal secretion of grepafloxacin and levofloxacin in vivo. Plasma concentrations of grepafloxacin and levofloxacin after intravenous and intraintestinal administration were increased by cyclosporin A, a P-glycoprotein inhibitor, in rats. The total body clearance and volume of distribution at steady state of grepafloxacin were significantly decreased to 60% and 63% of the corresponding control values by cyclosporin A. The apparent oral clearance of grepafloxacin was decreased to 33% of the control, and the bioavailability of grepafloxacin was increased to 95% by cyclosporin A from 53% in the controls. Intestinal clearance of grepafloxacin and levofloxacin were decreased to one-half and one-third of the control, respectively, and biliary clearance of grepafloxacin was also decreased to one-third with cyclosporin A in rats. Intestinal secretion of grepafloxacin in mdr1a/1b (−/−) mice, which lack mdr1-type P-glycoproteins, was significantly decreased compared with wild-type mice, although the biliary secretion was similar. Intestinal secretion of grepafloxacin in wild-type mice treated with cyclosporin A was comparable to those in mdr1a/1b (−/−) mice with or without cyclosporin A, indicating that cyclosporin A completely inhibited P-glycoprotein-mediated intestinal transport of grepafloxacin. In conclusion, our results indicated that P-glycoprotein mediated the intestinal secretion of grepafloxacin and limited the bioavailability of this drug in vivo.

Grepafloxacin and levofloxacin are new quinolone antibacterial drugs with potent activities against a broad spectrum of bacteria. These drugs are well absorbed from the intestine and distributed to many tissues (Sörgel et al., 1989a; Wolfson and Hooper, 1989). The bioavailabilities of grepafloxacin and levofloxacin in humans are 72% and approximately 100%, respectively (Efthymiopoulos et al., 1997; Fish and Chow, 1989). The bioavailability of grepafloxacin was increased to 95% by cyclosporin A from 53% in the controls. Intestinal clearance of grepafloxacin and levofloxacin were decreased to one-half and one-third of the control, respectively, and biliary clearance of grepafloxacin was also decreased to one-third with cyclosporin A in rats. Intestinal secretion of grepafloxacin in mdr1a/1b (−/−) mice, which lack mdr1-type P-glycoproteins, was significantly decreased compared with wild-type mice, although the biliary secretion was similar. Intestinal secretion of grepafloxacin in wild-type mice treated with cyclosporin A was comparable to those in mdr1a/1b (−/−) mice with or without cyclosporin A, indicating that cyclosporin A completely inhibited P-glycoprotein-mediated intestinal transport of grepafloxacin. In conclusion, our results indicated that P-glycoprotein mediated the intestinal secretion of grepafloxacin and limited the bioavailability of this drug in vivo.

P-glycoprotein, a product of the mdr1 gene, mediates the active and outward transport of various lipophilic substrates, including vinca alkaloids, antibiotics, steroids, and immunosuppressive drugs (Lum et al., 1993; Raderer and Scheithauer, 1993). We previously demonstrated that the quinolone antibacterial drugs levofloxacin and DU-6859a (sitafloxacin) were substrates for P-glycoprotein by using LLC-GA5-COL150 cell monolayers overexpressing human P-glycoprotein on the apical membrane (Ito et al., 1997). Because P-glycoprotein is found in not only tumor cells but also a variety of normal tissues such as the kidney, intestine, liver, and brain capillaries (Ford and Hait, 1990; Gottesman and Pastan, 1993), we hypothesized that the pharmacokinetics as well as absorption of quinolones was regulated by P-glycoprotein in vivo.

In the present study, we examined the contribution of
P-glycoprotein to the bioavailability and intestinal secretion of grepafloxacin and levofloxacin in vivo, by using rats treated with cyclosporin A, a typical inhibitor of P-glycoprotein-mediated transport, and mdr1a/1b knockout mice lacking mdr1-type P-glycoproteins.

**Experimental Procedures**

**Materials.** Grepafloxacin was kindly supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan), and levofloxacin was a gift from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Cyclosporin A (Sandimmun injection, 50 mg/ml) was obtained from Novartis Pharma KK (Tokyo, Japan). All other chemicals used were of the highest purity available.

**Animals.** Male Wistar rats weighing 200 to 240 g were used. FVB wild-type and mdr1a/1b (−/−) mice obtained from Taconic Farms (Germantown, NY) were used between 10 and 12 weeks of age. Before the experiment, animals were fasted overnight but given free access to water. Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Supplemental doses of pentobarbital were administered as required. Body temperature was maintained with appropriate heating lamps. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University.

**Pharmacokinetic Studies in Rats.** The femoral artery and vein were cannulated with polyethylene tubing (PE-50; BD Biosciences, San Jose, CA) filled with heparin solution (100 U/ml) for blood sampling and drug administration, respectively. Grepafloxacin or levofloxacin was injected intravenously at a dose of 10 mg/kg via the catheterized right femoral vein over a period of 1 min at 5 min after intravenous administration of 30 mg/kg cyclosporin A or saline (control). In a separate experiment for intraintestinal administration of grepafloxacin or levofloxacin, the abdominal cavity of rats was opened via a midline incision, and the upper site of the duodenum was exposed to administer the drug. Grepafloxacin or levofloxacin was injected into the lumen of the duodenum at a dose of 10 mg/kg at 5 min after intravenous administration of 30 mg/kg cyclosporin A or saline (control). Blood samples were collected from the left femoral artery at 5, 15, 30, 45, 60, 90, 120, 240, and 360 min after the end of the injection.

In a separate experiment, the abdominal cavity was opened via a midline incision, and a catheter with a 27-gauge needle was carefully fixed with cyanoacrylate glue into the portal vein. Grepafloxacin (10 mg/kg) was infused over 30 min (2.2 ml/h) via the portal vein by means of an automatic infusion pump at 5 min after intravenous administration of 30 mg/kg cyclosporin A or saline (control). Blood samples were collected at 5, 15, 30, and 60 min after the start of intraportal administration of grepafloxacin.

**Intestinal, Renal, and Biliary Clearance in Rats.** The femoral artery and vein were cannulated as described above for the pharmacokinetic studies. The abdominal cavity of rats was opened via a midline incision to gain access to the small intestine. The common bile duct was cannulated with PE-10 tubing (BD Biosciences) for bile collection. The bladder was cannulated with PE-50 tubing for urine collection. The whole small intestine starting from the Treitz ligament was used to make an intestinal loop. After washing the loop with saline until the efflux was clear, 5 ml of saline was injected into the loop. Grepafloxacin or levofloxacin was injected at a dose of 10 mg/kg intravenously via the catheterized right femoral vein over a period of 1 min at 5 min after intravenous administration of 30 mg/kg cyclosporin A or saline (control). Blood samples were collected at 2, 5, 15, 30, 45, and 60 min after the injection from the left femoral artery. After 60 min, the contents of the loop were withdrawn as completely as possible, and the lumen was washed with saline to give a volume of 30 ml.

**Elimination and Tissue Distribution Studies in mdr1a/1b (−/−) Mice.** After opening the abdominal cavity, the common bile duct was ligated. The gallbladder was then cannulated using PE-10 tubing (BD Biosciences) for bile collection. The whole small intestine starting from the Treitz ligament was used to make an intestinal loop, which was filled with 1 ml of saline. Grepafloxacin (10 mg/kg) was injected into the tail vein at 5 min after intravenous administration of 30 mg/kg cyclosporin A. Control received no injection. After 60 min, the contents of the loop were withdrawn as completely as possible, and the lumen was washed with saline to give a volume of 5 ml. Blood and urinary bladder contents were also collected. At this time, tissues were removed and homogenized with 9 volumes of saline, except for the brain, which was homogenized with 4 volumes of saline.

**Analytical Methods.** The concentrations of grepafloxacin and levofloxacin in plasma, intestinal fluid, urine, bile, and tissue homogenate were measured by high-performance liquid chromatography according to the reported procedures with slight modifications (Akiyama et al., 1995; Ohtomo et al., 1996). The lower limit of the assay for each drug was 0.01 µg/ml.

**Pharmacokinetic Analysis.** A conventional two-compartment model was used to analyze the plasma concentration-time profiles of grepafloxacin and levofloxacin after intravenous administration in rats. The parameters, total body clearance (CL), central volume of the distribution (V₁), intercompartmental clearance (Q), and volume of distribution at steady state (Vss), were calculated by the nonlinear least-squares method.

The apparent oral clearance (CL/F) expressed by the CL and bioavailability (F) after intraintestinal injection was calculated from the dose divided by the area under the plasma concentration-time curve (AUC). The AUC after intraintestinal injection was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the last measurable grepafloxacin or levofloxacin concentration to the mean terminal disposition rate constant after intravenous administration. The F value after intraintestinal injection was calculated from the CL and CL/F values. The AUC from 0 to 30 min after intraportal infusion was calculated using the linear trapezoidal rule. Intestinal, renal, and biliary clearances in rats were calculated by dividing the amount of grepafloxacin or levofloxacin eliminated into intestinal loop, urine, and bile during 60 min by AUC until 60 min, respectively.

**Statistical Analysis.** Values are expressed as means ± S.E. The statistical significance of differences between mean values was analyzed using the nonpaired t test. Multiple comparisons were performed using Scheffé’s test following analysis of variance. Differences were considered significant at P < 0.05.

**Results**

**Pharmacokinetics of Grepafloxacin and Levofloxacin in Rats.** We first examined the effects of cyclosporin A, a P-glycoprotein inhibitor, on plasma concentrations of grepafloxacin and levofloxacin after intravenous and intraintestinal administration. Plasma concentrations of grepafloxacin after both intravenous and intraintestinal administration were significantly increased by predadministration of cyclosporin A (Fig. 1). Plasma concentrations of levofloxacin were also increased (Fig. 2), but the degree of the increase in plasma concentrations of levofloxacin was smaller than that of grepafloxacin. Pharmacokinetic parameters of grepafloxacin and levofloxacin after intravenous and intraintestinal administration are summarized in Tables 1 and 2, respectively. The CL and Vss of grepafloxacin were decreased to 60 and 63% of the respective control values in the presence of cyclosporin A, and these parameters of levofloxacin tended to decrease. The values of V₁ and Q of each quinolone were not changed by cyclosporin A. The CL/F of grepafloxacin and levofloxacin were significantly decreased to 33 and 83% of the
control values, respectively, and the $F$ of grepafloxacin was increased markedly to 95% from 53% in the control, whereas that of levofloxacin was not affected by cyclosporin A. Although the time to peak plasma concentration ($T_{\text{max}}$) values of these quinolones were not significantly changed by cyclosporin A, the peak plasma concentration ($C_{\text{max}}$) value of grepafloxacin was significantly increased (up to 2.8-fold).

**Effects of Cyclosporin A on Hepatic Extraction of Grepafloxacin in Rats.** We next measured the plasma concentration of grepafloxacin after intraportal administration to evaluate the effects of cyclosporin A on hepatic extraction. Figure 3 shows the plasma concentration of grepafloxacin after intraportal infusion in rats. No effects of cyclosporin A were observed at any time point examined. The AUC from 0 to 30 min of grepafloxacin also was not affected by cyclosporin A (46.8 ± 3.9 μg · min/ml in control rats; 47.2 ± 11.4 μg · min/ml in rats with cyclosporin A, mean ± S.E. of five rats).

**Effects of Cyclosporin A on Intestinal, Renal, and Biliary Clearance of Grepafloxacin and Levofloxacin in Rats.** To elucidate the contribution of P-glycoprotein to the elimination mechanisms, the effects of cyclosporin A on excretion of grepafloxacin and levofloxacin into gastrointestinal fluid, urine, and bile were examined. In control rats, intestinal clearance of grepafloxacin was 4-fold greater than that of levofloxacin, and renal clearance of levofloxacin was 3-fold greater than that of grepafloxacin (Fig. 4). Intestinal clearance of grepafloxacin and levofloxacin was decreased to 51 and 30% of the respective control values, and biliary clearance of grepafloxacin was also decreased to 36% of the
control with cyclosporin A (Fig. 4). However, no significant changes were observed in renal clearance of either drug with or without cyclosporin A.

Intestinal, Renal, and Hepatobiliary Elimination of Grepafloxacin in mdr1a/1b (−/−) Mice. Next, we evaluated the secretion of grepafloxacin in wild-type and mdr1a/1b (−/−) mice. Figure 5 shows the intestinal and biliary secretion of grepafloxacin over 60 min in wild-type and mdr1a/1b (−/−) mice with or without cyclosporin A treatment. Intestinal secretion of grepafloxacin in mdr1a/1b (−/−) mice was decreased to 62% of that in wild-type mice.

### Table 1
Pharmacokinetic parameters of grepafloxacin and levofloxacin after intravenous administration in rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (ml/min)</td>
<td>10.8 ± 0.8</td>
<td>6.5 ± 0.5*</td>
</tr>
<tr>
<td>V₁ (liters)</td>
<td>0.84 ± 0.07</td>
<td>0.71 ± 0.08</td>
</tr>
<tr>
<td>Q (ml/min)</td>
<td>29.2 ± 2.9</td>
<td>22.1 ± 1.7</td>
</tr>
<tr>
<td>Vss (liters)</td>
<td>2.35 ± 0.30</td>
<td>1.50 ± 0.17*</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from control.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (ml/min)</td>
<td>20.6 ± 6.2</td>
<td>6.8 ± 0.4*</td>
</tr>
<tr>
<td>T_max (min)</td>
<td>15.0 ± 0.0</td>
<td>15.0 ± 0.0</td>
</tr>
<tr>
<td>C_max (µg/ml)</td>
<td>0.85 ± 0.21</td>
<td>2.34 ± 0.17*</td>
</tr>
<tr>
<td>F (%)</td>
<td>52.6</td>
<td>95.2</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from control.

### Table 2
Pharmacokinetic parameters of grepafloxacin and levofloxacin after intraintestinal administration in rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (ml/min)</td>
<td>20.6 ± 6.2</td>
<td>6.8 ± 0.4*</td>
</tr>
<tr>
<td>T_max (min)</td>
<td>15.0 ± 0.0</td>
<td>15.0 ± 0.0</td>
</tr>
<tr>
<td>C_max (µg/ml)</td>
<td>0.85 ± 0.21</td>
<td>2.34 ± 0.17*</td>
</tr>
<tr>
<td>F (%)</td>
<td>52.6</td>
<td>95.2</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from control.
Limited Bioavailability of Grepafloxacin by P-glycoprotein

Limited Bioavailability of Grepafloxacin by P-glycoprotein

(-/-) mice, cyclosporin A treatment decreased the hepatobiliary elimination of grepafloxacin in both wild-type and mdr1a/1b (-/-) mice to 40 and 42% of the corresponding control levels, respectively. No significant differences in the urinary elimination of grepafloxacin were observed between wild-type and mdr1a/1b (-/-) mice. Brain concentration of grepafloxacin in mdr1a/1b (-/-) mice was significantly higher (2.7-fold) than that in wild-type mice. Cyclosporin A treatment in wild-type mice increased the brain concentration of grepafloxacin up to the same level as in mdr1a/1b (-/-) mice. Other tissue concentrations were not different between wild-type and mdr1a/1b (-/-) mice.

Tissue Distribution of Grepafloxacin in mdr1a/1b (-/-) Mice. Tissue distributions of grepafloxacin examined at the end of the 60 min experiments are shown in Table 3. Brain concentration of grepafloxacin in mdr1a/1b (-/-) mice was significantly higher (2.7-fold) than that in wild-type mice. Cyclosporin A treatment in wild-type mice increased the brain concentration of grepafloxacin up to the same level as in mdr1a/1b (-/-) mice. Other tissue concentrations were not different between wild-type and mdr1a/1b (-/-) mice.

Discussion

Some quinolone antibacterial drugs such as ciprofloxacin and temafloxacin are known to be eliminated from the gastrointestinal tract in humans (Granneman et al., 1991; Sörgel et al., 1989b, 1991). We previously reported that gastrointestinal secretion of grepafloxacin and levofloxacin was mediated by P-glycoprotein and another transport system distinct from organic cation and anion transporters in Caco-2 cells (Yamaguchi et al., 2000). Because P-glycoprotein is found in normal tissues such as the kidney, intestine, liver, and brain capillaries (Ford and Hait, 1990; Gottesman and Pastan, 1993), we hypothesized that P-glycoprotein might be a determinant factor of the absorption, distribution, and elimination of grepafloxacin and levofloxacin in vivo.

Plasma concentrations of grepafloxacin and levofloxacin after intravenous and intraintestinal administration were elevated by preadministration of cyclosporin A (Figs. 1 and 2). We previously reported that P-glycoprotein-mediated transport of levofloxacin was almost completely inhibited by 5 μM cyclosporin A in LLC-GA5-COL150 cell monolayers (Ito et al., 1997). In this study, because the blood concentration of cyclosporin A was 6.3 μM (mean of two rats) at 360 min after administration of quinolones, transport activity of P-glycoprotein was considered to be completely inhibited by cyclo-

Fig. 5. Intestinal (A) and biliary (B) secretion of grepafloxacin over 60 min in wild-type and mdr1a/1b (-/-) mice treated with or without cyclosporin A. Grepafloxacin was injected at a dose of 10 mg/kg at 5 min after intravenous administration of cyclosporin A (30 mg/kg; □) or not (■). Each column represents the mean ± S.E. of four to six mice. *, P < 0.05, significantly different.

Table 3

Tissue distribution of grepafloxacin at 60 min after intravenous administration in wild-type and mdr1a/1b (-/-) mice

Grepafloxacin was injected at a dose of 10 mg/kg at 5 min after intravenous administration of cyclosporin A (30 mg/kg) or not (control). Each value represents the mean ± S.E. of four to six mice.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Wild-Type Mice</th>
<th>+Cyclosporin A</th>
<th>mdr1a/1b (-/-) Mice</th>
<th>+Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (μg/ml for plasma or μg/g for tissue)</td>
<td>Kp</td>
<td>Concentration (μg/ml for plasma or μg/g for tissue)</td>
<td>Kp</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.92 ± 0.10</td>
<td>1.04 ± 0.92</td>
<td>1.04 ± 0.11</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>Brain</td>
<td>0.30 ± 0.02</td>
<td>0.3 ± 0.05</td>
<td>0.3 ± 0.08*</td>
<td>0.3 ± 0.1*</td>
</tr>
<tr>
<td>Lung</td>
<td>15.1 ± 1.1</td>
<td>16.8 ± 1.4</td>
<td>13.9 ± 1.7</td>
<td>14.7 ± 0.6</td>
</tr>
<tr>
<td>Heart</td>
<td>7.53 ± 0.83</td>
<td>8.2 ± 0.6</td>
<td>7.77 ± 0.52</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>13.8 ± 1.4</td>
<td>16.1 ± 2.8</td>
<td>13.3 ± 1.4</td>
<td>13.8 ± 2.1</td>
</tr>
<tr>
<td>Small intestine</td>
<td>9.02 ± 0.95</td>
<td>10.0 ± 1.1</td>
<td>7.18 ± 0.57</td>
<td>8.30 ± 1.20</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.4 ± 1.9</td>
<td>15.0 ± 2.5</td>
<td>20.6 ± 3.4</td>
<td>18.8 ± 2.0</td>
</tr>
<tr>
<td>Testis</td>
<td>1.32 ± 0.32</td>
<td>1.4 ± 0.2</td>
<td>1.33 ± 0.14</td>
<td>0.82 ± 0.08</td>
</tr>
</tbody>
</table>

Kp, tissue-to-plasma concentration ratio.

* P < 0.05, significantly different from corresponding control value of wild-type mice.
sporin A administration. Pharmacokinetic analysis showed that the CL of grepafloxacin was significantly decreased, and that of levofloxacin tended to decrease (Table 1). In addition, apparent oral clearance (CL/F) of both quinolones was significantly decreased, and the $F$ value of grepafloxacin was increased to approximately 100% by cyclosporin A (Table 2). Therefore, P-glycoprotein was considered to function as an absorption barrier as well as in the elimination mechanisms of both quinolones, especially grepafloxacin. We also examined the effects of cyclosporin A on hepatic extraction of grepafloxacin in rats. Our results indicated that cyclosporin A treatment did not change the plasma concentration of grepafloxacin after intraperitoneal infusion (Fig. 3). Therefore, we confirmed that the major mechanism of increased bioavailability of grepafloxacin by cyclosporin A was the increase of intestinal absorption of the drug, but not the decrease of hepatic first-pass metabolism.

To evaluate the contribution of P-glycoprotein to the gastrointestinal secretion of grepafloxacin and levofloxacin, intestinal clearance was estimated in the presence of cyclosporin A in rats. Because P-glycoprotein is found in elimination tissues such as the kidney and liver as well as intestine (Ford and Hait, 1990; Gottesman and Pastan, 1993), we examined urinary and biliary clearance of each quinolone at the same time. Intestinal clearance of grepafloxacin and levofloxacin was decreased to 51 and 30% of the control in the presence of cyclosporin A, indicating that the contribution of P-glycoprotein to the gastrointestinal secretion of these quinolones was one-half and two-thirds, respectively. In Caco-2 cells, the basolateral-to-apical transport of grepafloxacin was mainly explained by P-glycoprotein, and that of levofloxacin was largely mediated by another transport system(s) distinct from organic cation and anion transport systems and MRP2 (Yamaguchi et al., 2000). The reasons for the discrepancy in the contribution of P-glycoprotein to the gastrointestinal elimination between rats and Caco-2 cells remain unknown. Biliary clearance of grepafloxacin was decreased to 36% of the control with cyclosporin A, whereas that of levofloxacin was not affected (Fig. 4). Cyclosporin A was reported to inhibit not only P-glycoprotein but also canalicular multispecific organic anion transporter (MRP2/canalicular multispecific organic anion transporter), a member of the ATP binding cassette transporter family (Chen et al., 1999). Sasabe et al. (1998) demonstrated that a part of grepafloxacin transport and a major part of its glucuronide transport across the bile canalicular membrane were mediated by MRP2. Therefore, it is possible that cyclosporin A inhibited the biliary clearance of grepafloxacin via MRP2 to some extent. On the other hand, no significant differences were observed in renal clearance of each quinolone (Fig. 4). We previously reported that the basolateral-to-apical transcellular transport of levofloxacin and grepafloxacin were mediated by a specific transport system distinct from organic cation and anion transporters and P-glycoprotein in LLC-PK1 cell monolayers (Matsuo et al., 1998). These findings suggested that the contribution of P-glycoprotein to renal tubular secretion of quinolones was relatively small, and that another specific transport system played an important role.

In the next experiment, we used mdr1a1b (−/−) mice to confirm the contribution of P-glycoprotein to the intestinal and biliary secretion of grepafloxacin, and the efficacy and specificity of cyclosporin A in inhibiting P-glycoprotein-mediated transport of grepafloxacin. Intestinal secretion of grepafloxacin over 60 min was decreased in mdr1a1b (−/−) mice compared with wild-type mice (Fig. 5). Cyclosporin A treatment decreased the intestinal secretion of grepafloxacin in wild-type mice to the same level as that in mdr1a1b (−/−) mice, and the intestinal secretion in mdr1a1b (−/−) mice was not changed by cyclosporin A treatment. These results indicated that the intestinal secretion of grepafloxacin was mediated by P-glycoprotein, and that cyclosporin A completely inhibited this transport but did not inhibit other transport systems. The intestinal secretion of grepafloxacin in mdr1a1b (−/−) mice was 62% of that in wild-type mice. We therefore considered that the intestinal secretion of grepafloxacin was mediated by P-glycoprotein and another secretory transport system(s), which was presented by our previous report (Yamaguchi et al., 2000). Recently, Narushashi et al. (2001) reported that in mdr1a1b (−/−) mice, the intestinal secretory clearance was smaller than that in wild-type mice after intravenous administration of grepafloxacin, consistent with our results. On the other hand, as shown in Fig. 5, hepatobiliary secretion of grepafloxacin in mdr1a1b (−/−) mice was identical to that in wild-type mice. When cyclosporin A was preadministered with grepafloxacin, hepatobiliary secretion of the drug was significantly reduced in both wild-type and mdr1a1b (−/−) mice. These observations indicated that hepatobiliary secretion of grepafloxacin was mediated not via P-glycoprotein but via MRP2 and/or another cyclosporin A-inhibitable transport system.

The brain penetration of grepafloxacin in mdr1a1b (−/−) mice was significantly higher than that in wild-type mice (2.7-fold), indicating that P-glycoprotein played an important role in a barrier function in the brain. Previous studies showed that the entry of HSR-903, grepafloxacin and sparfloxacin into the brain was restricted by P-glycoprotein by using brain capillary endothelial cells, rat brain capillary endothelial cells, and mdr1a1b (−/−) mice (Murata et al., 1999; Tamai et al., 2000), consistent with our results. In the tissues other than the brain, no significant differences were observed in the distribution of grepafloxacin between wild-type and mdr1a1b (−/−) mice (Table 3). Quinolone antibacterial drugs have been reported to be recognized by active transport systems on the basolateral membrane in the small intestine, liver, and kidney (Griffiths et al., 1994; Sasabe et al., 1997; Ito et al., 1999). Therefore, the tissue concentration in the small intestine and liver might be regulated by not only P-glycoprotein and/or another transporter(s) across brush-border membranes but also the transport systems on the basolateral membranes in these organs.

In conclusion, we clarified the contribution of P-glycoprotein to the pharmacokinetics of grepafloxacin and levofloxacin in vivo. Our results demonstrated that P-glycoprotein restricted the bioavailability, and contributed to the elimination pathway as intestinal secretion in addition to the low brain distribution of grepafloxacin.

References

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