Limited Role of P-Glycoprotein in the Intestinal Absorption of Cyclosporin A

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The contribution of P-glycoprotein (P-gp) to the intestinal absorption of cyclosporin A (CsA) was investigated by comparing the in vivo pharmacokinetics of CsA in P-gp knockout mice versus wild-type mice following both oral and intravenous administration and by examining the transport of CsA across Caco-2 cell monolayers. The apparent oral bioavailability of CsA in P-gp knockout mice was 1.55-fold larger than in wild-type mice, leading to an apparent absolute bioavailability of 41.8%. A concentration dependent efflux transport of CsA across Caco-2 cell monolayers was found, which exhibited saturation at a CsA concentration of 1 µg. These results suggest that the involvement of P-gp in the intestinal absorption of CsA is not as profound as was previously thought.

Key words cyclosporin A; P-glycoprotein; absorption; Caco-2 cell; barrier

Cyclosporin A (CsA), a powerful immunosuppressive agent, is orally administered to prevent rejection in new organ transplants and to treat a variety of autoimmune diseases. The intestinal absorption of CsA following oral administration is poor (typically 20—50%) and varies greatly among patients, owing to its relatively high molecular weight (1202.6), poor solubility in aqueous fluids, site-specific absorption in the small intestine, and intestinal first-pass metabolism by the Cyp450 system. Furthermore, P-glycoprotein (P-gp), an ATP-dependent efflux transporter, has been proposed as a factor in the poor absorption of CsA. The area under the blood CsA concentration from time 0 to 24 h after administration (AUC0–24) was determined by comparing the AUC0–24 versus wild-type mice following intravenous administration. The systemic clearance (CL) and volume of distribution (Vd) of CsA following intravenous administration were calculated by a standard noncompartment method (WINNONLIN version 3.1, Pharsight Corporation, NC, U.S.A.). The two-sided unpaired Student’s t-test and or 2-way ANOVA were used for the statistical analysis of results.

Transport of CsA in Caco-2 Cell System Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.), grown in 75 cm² tissue culture flasks and seeded in Transwell® (0.4 µm pore size, 1 cm²; Corning Costar Corp., Cambridge, MA, U.S.A.) to obtain monolayers. The apical side chamber of the Transwell® was filled with HBSS/HEPES buffer containing 50% (w/v) human plasma. The addition of human plasma was needed to prevent adsorption of the drug to the well surface of the Transwell® and to simulate extracellular protein binding. The apical side chamber was loaded with a CsA solution (0.05, 0.1, 0.2, 0.5, 1, 2, 5 µM) and aliquots were removed from the basolateral side at 30 min intervals for 2 h, and quantified for CsA by scintillation counting (LS 6000SE; Beckman Coulter, Fullerton, CA, U.S.A.).

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known to remain intact after transport across Caco-2 cell monolayers.\textsuperscript{13)}

CsA transport was expressed as $P_{\text{app}}$, expressed in centimeters per second according to the following equation\textsuperscript{19)}:

$$P_{\text{app}} = \frac{1}{A} \frac{dQ}{dt}$$  \hspace{1cm} (1)

where $dQ/dt$ is the rate of appearance of drugs on the basolateral side (flux, pmol/hour), $C_0$ is the initial CsA concentration on the apical side ($\mu$m), and $A$ is the surface area of the monolayer (cm$^2$).

RESULTS

Blood Concentration–Time Profiles and Tissue Distribution of CsA in Wild-Type and P-gp Knockout Mice

Blood concentration versus time profiles for total $^3$H-CsA after both intravenous and oral administration to wild-type and P-gp knockout mice at a dose of 1 mg/kg are shown in Figs. 1A and B, respectively, and the pharmacokinetic parameters following each administration are summarized in Table 1. All parameters examined in the wild-type mice were similar with previous findings reported for rats.\textsuperscript{20)} In the intravenous administration study, no significant differences were observed for the blood concentration–time profiles (Fig. 1A, 2-way ANOVA) or the pharmacokinetic parameters between P-gp knockout mice and wild-type mice (Table 1). However, a 14.6-fold increase in the distribution of CsA to the brain was observed in the P-gp knockout mice compared to wild-type mice (i.e., $4.24 \pm 0.94$ to $0.29 \pm 0.05$, mean$\pm$S.D., $n=3-4$, $p<0.01$, when expressed as the ratio of the amount of CsA in 1 g brain tissue/1 ml blood). In the oral administration study, significantly ($p<0.05$, 2-way ANOVA) higher blood concentrations were obtained for P-gp knockout mice compared to wild-type mice at each time point (Fig. 1B), leading to significantly larger $AUC_{0-\infty}$, peak blood concentrations ($C_{\text{max}}$) and, consequently, the apparent absolute oral bioavailability of CsA for the knockout mice ($F_{\text{app}}$, $p<0.01$). As a result, the apparent oral bioavailability of CsA could be elevated up to 41.8% in P-gp knockout mice from 27.0% in wild-type mice (1.55-fold increase).

Concentration Dependency of CsA Transport across Caco-2 Cell System

The time-dependent apical to basal CsA concentration range of 0.05—1 $\mu$m, but was linear for CsA concentrations of over 1 $\mu$m, consistent with the previous polarized and carrier-mediated efflux of CsA by P-gp.\textsuperscript{11,21)}

DISCUSSION

In the intravenous administration study, blood concentrations (Fig. 1) and pharmacokinetic parameters (Table 1) for P-gp knockout mice and wild-type mice were similar. However, an increased distribution of CsA to the brain was observed in the P-gp knockout mice compared to wild-type mice. It is consistent with the expected role of P-gp as a blood-brain barrier in wild-type mice.\textsuperscript{28)} The unchanged blood

Table 1. Pharmacokinetic Analysis of CsA after the Intravenous or Oral Administration to P-gp Knockout and Wild-Type Mice at a CsA Dose of 1 mg/kg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-type</th>
<th>P-gp knockout</th>
<th>Wild-type</th>
<th>P-gp knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-\infty}$, ng·h/ml</td>
<td>4850±560</td>
<td>4930±500</td>
<td>1310±230</td>
<td>2060±370**</td>
</tr>
<tr>
<td>$Cl$, l/h/kg</td>
<td>0.197±0.028</td>
<td>0.184±0.014</td>
<td>0.870±0.067</td>
<td>0.768±0.044</td>
</tr>
<tr>
<td>$V_{\text{f}}$, l/kg</td>
<td>1.45±0.17</td>
<td>1.74±0.25</td>
<td>0.823±0.137</td>
<td>0.714±0.092</td>
</tr>
<tr>
<td>Half-life, h</td>
<td>5.72±1.40</td>
<td>7.68±1.29</td>
<td>4.24±0.94</td>
<td>0.29±0.05</td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/ml</td>
<td>144±26</td>
<td>222±59**</td>
<td>27.0%</td>
<td>41.8%</td>
</tr>
</tbody>
</table>

$n=3-7$, mean$\pm$S.D. **$p<0.01$, unpaired $t$-test.
pharmacokinetics despite the increased brain distribution suggests that the contribution of altered tissue distribution, such as brain distribution, to the blood profiles is negligible in terms of the amount of CsA. Another possible explanation is the counterbalance effect between biliary excretion and tissue distribution, i.e., a decreased elimination of CsA via the biliary route, a major route of CsA elimination which is considered the CsA dose in the present study (1 mg/kg) and the range.11,21) A low saturation concentration of CsA has also been reported for vecuronium.22,23) Irrespective of the mechanism, the constant blood pharmacokinetics for intravenously administered CsA in P-gp knockout mice seems favorable in analyzing the blood pharmacokinetics of orally administered CsA to P-gp knockout mice could be attributed solely to differences in absorption kinetics. Therefore, the increased blood levels (Fig. 1B) and bioavailability parameters (Table 1) for P-gp knockout mice following oral administration are consistent with the increased intestinal absorption of CsA, which might be accomplished by the absence of a P-gp mediated efflux of CsA in these mice.

The difference in the apparent oral bioavailability of CsA between the P-gp knockout mice and wild-type mice is smaller (1.55-fold) compared with cases for the other P-gp substrates such as taxol (3.14-fold)25) and saquinavir (6.5-fold).25) This indicates that although the role of intestinal P-gp as an absorption barrier of CsA is distinct, its contribution of the role to overall bioavailability is not so profound, consistent with the report on the permeability of CsA across the in situ intestine in mdrla knockout mice25) and the clinical study to show the moderate correlation of P-gp expression with the variability of oral CsA clearance.13)

The limited contribution of P-gp to intestinal absorption may be associated with the transport characteristics of the P-gp substrates administered.26) In the present study, the A-B flux for CsA across Caco-2 cell monolayers became linear at an apical CsA concentration of around 1 μM (inset of Fig. 2), implying a loss of contribution of P-gp in this concentration range.11,21) A low saturation concentration of CsA has also been reported for human MDR1 (i.e., \(K_m = 0.17 \mu M\)).27) Considering the CsA dose in the present study (1 mg/kg) and the reported average mouse gut volume (1.5 ml/0.02 kg),28) the CsA concentration in the intestinal lumen of mice is calculated to be in the 10 μM range, similar to the CsA concentration in the human intestine lumen for a typical CsA dose.2,28)

Interestingly, saturated efflux of CsA was already reported in rat blood-brain barrier; Low brain/blood concentration ratios of about 0.1 were found in the unbound CsA blood concentration range of 0.002—0.036 μM, followed by an increase up to 0.28 at 0.30 μM and 1.1 at 0.87 μM.20,29) Since CsA efflux, probably by P-gp, may be already saturated at an apical CsA concentration of 0.28 μM (in the rodent) or 1 μM (in the human), the contribution of P-gp efflux to the overall intestinal absorption of CsA should have been significantly limited, when an oral dose of CsA yielded a much higher intestinal concentration than 10 μM, as in the present study.

In this study, total radioactivity was used as an index of CsA level for the characterization of CsA pharmacokinetics, despite the known metabolism by CYP3A.31) Under this experimental design, the bioavailability found in the P-gp knockout animal was approximately 41.8% (Table 1). In the present study, intact CsA in the serum could not be determined due to its limited sensitivity. However, the bioavailability estimated using intact CsA would have been lower than 41.8%. Therefore, the involvement of P-gp in the barrier function of the intestine for intact CsA may be even lower than that suggested in this study. In addition, an interplay between P-gp and CYP3A, which was reported to be involved in the potentiation of the barrier function of the intestine for CsA,30) is not likely to affect the conclusion of this study by the identical reasoning.

Therefore, a conclusion could be made based on the total radioactivity data for in vivo experiments as follows: the limited contribution of P-gp in the intestinal absorption of CsA is proposed, and the saturation of the efflux system at the usual dose of CsA appears to be the one of probable mechanism.

REFERENCES
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