Apparent Lack of Effect of P-Glycoprotein on the Gastrointestinal Absorption of a Substrate, Tacrolimus, in Normal Mice

Win L. Chiou,1,2 Sang M. Chung,1 and Ta C. Wu1

RESUL TS AND DISCUSSION

In the reported study (1) the oral bioavailability (F) of tacrolimus was estimated based on the comparison of the total blood concentration vs. time area from time zero to 5 hr obtained after oral and intravenous administration of the same dose. Theoretically speaking (2), F is preferred to be calculated by the total area between time zero to infinity as shown below.

\[
F = \frac{\text{AUC}_{\text{po,0}\rightarrow\infty}}{\text{AUC}_{\text{iv,0}\rightarrow\infty}}
\]

Assuming that oral absorption ceased at 5 hr after dosing, the oral AUC from 5 hr to infinity can be best approximated by the mean blood concentration at 5 hr (Fig. 5 in reference 1) divided by the mean terminal first-order rate constant obtained after intravenous administration (i.e., A2 in Table 1 of reference 1). The mean oral AUC from time zero to 5 hr was estimated from their Fig. 5 (1) using the linear (during the ascending period) and log (during the descending period) trapezoidal rule method recommended earlier (3). The AUC_{0 \rightarrow \infty} was estimated by the integration method based on the reported (1) disposition function. Since tacrolimus is known to be significantly excreted in urine as unchanged drug after intravenous administration (4), the hepatic extraction ratio (Eh) can be estimated by (2,5)

\[
E_h = \frac{\text{CL}_{\text{total}}}{Q_h}
\]

where \(\text{CL}_{\text{total}}\) is the reported (1) mean total blood clearance obtained after intravenous administration and \(Q_h\) is the mean hepatic blood flow in mice, 72.5 ml/min/kg (6). The predicted bioavailability (\(F_{\text{pred}}\)) is estimated by one minus \(E_h\). The mean bioavailability rate of tacrolimus in normal and knockout mice after oral administration was calculated by a method reported earlier by Chiu (7), and a cumulative bioavailability profile was subsequently obtained.

RESULTS AND DISCUSSION

The results of our analysis are summarized in Table I. Our calculated mean oral bioavailabilities of tacrolimus in the two different groups of mice are all higher than those reported previously (0.22 vs. 0.17 in normal mice and 0.72 vs. 0.59 in knockout mice). The reported lower F values are attributed to the truncated method used in the early (1) analysis. The reported oral sustained blood-level profiles (1) are indicative of continuing absorption for at least up to 5 hr as shown in (Fig.1). The amount absorbed after 5 hr was probably relatively small in view of the short small intestinal transit time in rodents (8). Therefore, the F values estimated using Eq. 1 in the present activity as well as efflux transport of P-glycoprotein that may potentially serve as an absorption barrier in normal mice (1). The authors (1) also concluded that they could not determine whether the P-glycoprotein contributes to the intestinal permeability. The purpose of this communication is to report the result of our findings suggesting an apparent lack of significant effect of P-glycoprotein on the intestinal permeability and on the rate and extent of oral absorption of tacrolimus in normal mice.

METHODS

In an excellent study published recently (1) on the P-glycoprotein-dependent distribution kinetics of tacrolimus, it was shown that, compared to normal mice, the \(mdr1a\) knockout mice showed a 3-fold reduction in total clearance, a 10-fold increase in maximum brain concentration and a 3.5-fold difference (0.59 vs. 0.17) increase in oral bioavailability. Based on their results the authors (1) suggested that the absorption of tacrolimus (a known substrate of P-glycoprotein) from the gastrointestinal tract is limited in part by P-glycoprotein. The increase in oral bioavailability in knockout mice may be attributed to the variation of first-pass effect due to hepatic and/or intestinal metabolic activity as well as efflux transport of P-glycoprotein that may potentially serve as an absorption barrier in normal mice (1). The authors (1) also concluded that they could not determine whether the P-glycoprotein contributes to the intestinal permeability. The purpose of this communication is to report the result of our findings suggesting an apparent lack of significant effect of P-glycoprotein on the intestinal permeability and on the rate and extent of oral absorption of tacrolimus in normal mice.

INTRODUCTION

In an excellent study published recently (1) on the P-glycoprotein-dependent distribution kinetics of tacrolimus, it was shown that, compared to normal mice, the \(mdr1a\) knockout mice showed a 3-fold reduction in total clearance, a 10-fold increase in maximum brain concentration and a 3.5-fold (0.59 vs. 0.17) increase in oral bioavailability. Based on their results the authors (1) suggested that the absorption of tacrolimus (a known substrate of P-glycoprotein) from the gastrointestinal tract is limited in part by P-glycoprotein. The increase in oral bioavailability in knockout mice may be attributed to the variation of first-pass effect due to hepatic and/or intestinal metabolic

\[1\] Department of Pharmaceutics and Pharmacodynamics (M/C 865), College of Pharmacy, University of Illinois at Chicago, Illinois 60612.

\[2\] To whom correspondence should be addressed. (e-mail: chiou@uic.edu)
Table I. Summary of Kinetic Data of Tacrolimus in Normal and Knockout Mice Following 2 mg/kg of Intravenous or Oral Dose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal mice</th>
<th>Knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{po, 0 → 5hr}</td>
<td>7745</td>
<td>75620</td>
</tr>
<tr>
<td>AUC_{iv, 0 → 5hr}</td>
<td>35900</td>
<td>104000</td>
</tr>
<tr>
<td>CL_{oral} (ml/hr/kg)</td>
<td>55.7</td>
<td>19.2</td>
</tr>
<tr>
<td>CL_{int, app} (ml/hr/kg)</td>
<td>258</td>
<td>26.4</td>
</tr>
<tr>
<td>Fર</td>
<td>0.22</td>
<td>0.72</td>
</tr>
<tr>
<td>F西安市</td>
<td>0.77</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Based on Eq. 1. Based on Eq. 2. 1 - F西安市

The very early bioavailability peaks (Fig. 1) are indicative of high permeability or absorptive clearance (10) of tacrolimus in these mice. The prolonged oral absorption (Fig. 1) observed could be mainly attributed to the precipitation from the administered solution and the subsequent slow in vivo dissolution of this highly water insoluble (<100 ng/ml) drug (11). The above reasoning is consistent with the slow and poor absorption of the tacrolimus crystalline powder (in micron range) in beagle dogs reported earlier (11).

Based on the above data and discussions, one may conclude that although P-glycoprotein can markedly affect the brain uptake, systemic clearance and oral bioavailability of tacrolimus in mice, its effect on the intestinal permeability or the rate and extent of gastrointestinal absorption of the drug is probably minimal. It should be emphasized that the present analysis is not inconsistent with the notion that, mechanistically, P-glycoprotein may still be involved in the tacrolimus absorption as an efflux transporter (1). However, apparently because of its high permeability, the secreted molecules of tacrolimus could be rapidly reabsorbed back into the enterocytes for potential transport into the mesenteric blood and the absorption barrier effect of P-glycoprotein would then become not noticeable. In other words, the impact of P-glycoprotein on the rate and extent of oral absorption may be expected to be more pronounced for those more slowly or incompletely (12,13) absorbed drugs (i.e., drugs with relatively low permeabilities). This seems to be supported by the absorption data of talinolol enantiomers, P-glycoprotein substrates, in humans following oral administration (14). As expected (14), the extent of absorption as measured by dose normalized AUC was increased and the first peak time (T_{max}) was reduced when oral dose was increased. Data for S-(-)-talinolol are shown in Fig. 2. It should be noted that the terminal half-life and thus plasma clearance of the drug were not changed with dose (14).