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. Wu[1]

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Purpose. To study the contribution of P-glycoprotein (P-gp) to the oral absorption of a substrate, tacrolimus, by comparing the extent and rate of bioavailability in normal and *mdr*1a knockout mice.

Methods. Intravenous and oral (2 mg/kg) blood concentration data of tacrolimus in normal and knockout mice were obtained from a study by K. Yokogawa et al. in Pharm. Res. 16:1213–1218 (1999). Mean bioavailability (F), mean hepatic first-pass extraction ratio (F_h), mean bioavailability rates, mean oral clearance, and mean total hepatic intrinsic clearance were calculated using standard pharmacokinetic methods.

Results. The mean F of tacrolimus (an apparently highly permeable compound) was increased from 0.22 in normal mice to 0.72 in knockout mice. These values were consistent with mean predicted E_h (based on intravenous data) of 0.77 and 0.27 in normal and knockout mice, respectively. Great similarity in the relative bioavailability profile (such as short T_{max}) between normal and knockout mice was also found. Mean oral clearance and mean total or unbound hepatic intrinsic clearance of tacrolimus in knockout mice were found to be about 10 times lower compared to those in normal mice.

Conclusions. The above results suggest an apparent lack of effect of P-gp on the gastrointestinal absorption of tacrolimus in normal mice under the study condition. It is postulated that the effect of P-gp on the rate and extent of oral absorption should be more pronounced for those more slowly or incompletely absorbed drugs (i.e., drugs with relatively low permeabilities) as illustrated by talinolol in humans. The clearance data also suggest a very dominant role of P-glycoprotein in controlling the rate of hepatic metabolism of tacrolimus in normal mice, and P-glycoprotein may serve as an effective efflux pump for direct transport of metabolites formed in hepatocytes into the blood circulation.

KEY WORDS: tacrolimus; P-glycoprotein; bioavailability; drug absorption; 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 *mdr*1a 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 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 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.

$$F = \frac{AUC_{po,0\rightarrow\infty}}{AUC_{iv,0\rightarrow\infty}} \quad (1)$$

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., λ_2 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 $AUC_{iv,\,0\rightarrow\infty}$ was estimated by the integration method based on the reported (1) disposition function. Since tacrolimus is known to be insignificantly excreted in urine as unchanged drug after intravenous administration (4), the hepatic extraction ratio (E_h) can be estimated by (2,5)

$$E_h = \frac{CL_{total}}{Q_h} \quad (2)$$

where CL_{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_{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 Chiou (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

[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)

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Table I. Summary of Kinetic Data of Tacrolimus in Normal and Knockout Mice Following 2 mg/kg of Intravenous or Oral Dose

Parameter	Normal mice	Knockout mice
$AUC_{po,\ 0 \rightarrow 5hr}$ (hr · ng/ml)	5700	46800
$AUC_{po,\ 0 \rightarrow \infty}$ (hr · ng/ml)	7745	75620
$AUC_{iv,\ 0 \rightarrow \infty}$ (hr · ng/ml)	35900	104000
CL_{total} (ml/hr/kg)	55.7	19.2
CL_{oral} (ml/hr/kg)	258	26.4
$CL_{int,\ app}$ (ml/hr/kg)	258	26.4
F^a	0.22	0.72
$E_h{}^b$	0.77	0.27
$F_{pred}{}^c$	0.23	0.73

[a] Based on Eq. 1.
[b] Based on Eq. 2.
[c] $1 - E_h$.

study should be reasonably accurate. Interestingly, these estimates are essentially identical (0.22 vs. 0.23 for normal mice and 0.72 vs. 0.73 for knockout mice) to the predicted bioavailability values based on Eq. 2 (Table I). The above results strongly suggest that the marked difference in oral bioavailability between normal mice and knockout mice was most likely due to the difference in hepatic first-pass extraction caused by a drastic reduction in hepatic blood clearance in the knockout mice. The implication of the liver in causing the bioavailability difference may also be supported by the reported (1) significant increase of liver/blood ratio of tacrolimus in the knockout mice when compared to the normal mice (3.20 vs. 1.84). An oral F of 0.72 or greater (if there was a continued absorption after 5 hr) in the knockout mice estimated in the present study also indicates a virtually complete oral absorption of this drug in mice. The oral bioavailability estimated here or earlier (1) in normal mice is similar to the reported mean bioavailability of 16% in humans (9).

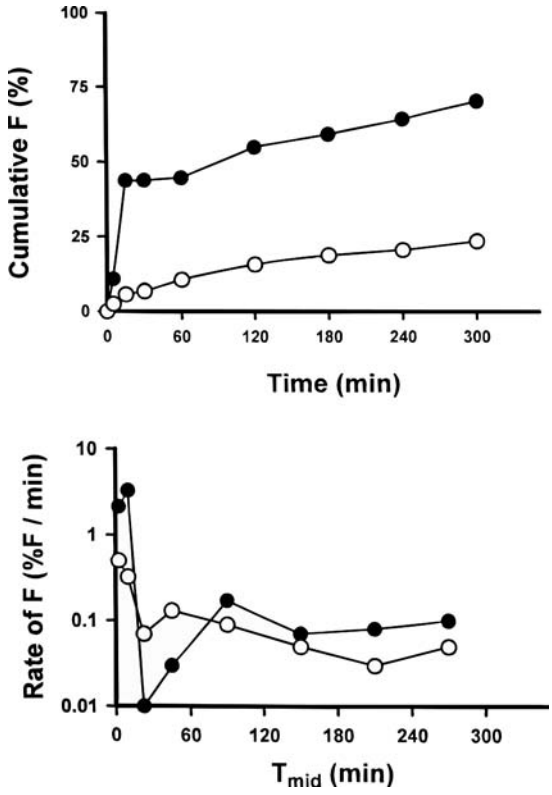

Fig. 1. The time course of cumulative bioavailability (top) and rate of bioavailability (bottom) of tacrolimus after oral administration (2 mg/kg) to normal (○) and knockout (●) mice.

Another important evidence to support the apparent lack of effect of the P-glycoprotein on the gastrointestinal permeability or absorption is shown by great similarity in the relative bioavailability rate profile or cumulative bioavailability profile between the normal and knockout mice (Fig. 1). The mean peak rate of bioavailability was found to occur during the first blood sampling period (0 to 5 min) in the normal mice while this occurred during the second blood sampling period (5 to 15 min) in the knockout mice. If the P-glycoprotein present in normal mice would have had a significant barrier effect on the absorption of tacrolimus due to its ability to pump the absorbed drug from enterocytes into the intestinal lumen, one may then expect a delay in the peak time for the bioavailability rate when compared to the knockout mice. Since this didn't occur (an opposite phenomenon seemed to have occurred), its effect on the absorption seems insignificant. The apparent lack of the P-glycoprotein effect on absorption is also supported by the reported (1) virtually identical ratios (4.08 vs. 3.99) of the gut/blood tacrolimus concentration at 5 hr after intravenous dosing between normal and knockout mice.

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).

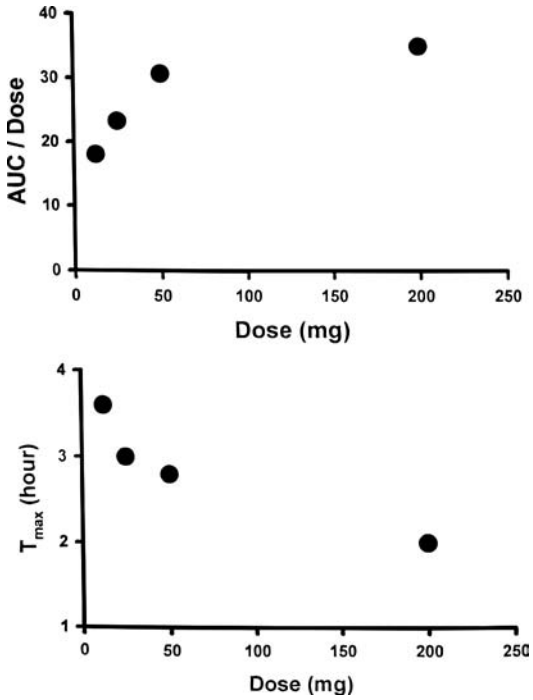

Fig. 2. Relationship between the mean AUC/dose (top) or mean first T_{max} (bottom) and the dose for S-(−)-talinolol in 12 normal human subjects following oral administration of various doses of racemic talinolol. Data were obtained from reference 14.

One complication in the present or earlier (1) study is the fact tacrolimus has been shown to be a potent inhibitor of P-glycoprotein *in vitro* (15). It is possible that the oral dose (2 mg/kg) used in the mouse study (1) may be sufficient to create a 'chemical knockout' in the normal mice thus rendering the intestinal P-glycoprotein inactive (the potential inhibitory effects on the blood-brain barrier and hepatic elimination were apparently not complete in the present study) or to provide enough luminal concentrations to completely inhibit the effect of the intestinal P-glycoprotein during absorption. It is of interest to note that in the *in vitro* inhibition study (15), measured tacrolimus potencies for P-glycoprotein inhibition varied greatly, up to 7,800 times, with the sources of tumor cells and also with the substrates of P-glycoprotein employed (15). Tacrolimus concentrations as low as 3 μM have been shown to completely inhibit the P-glycoprotein activity in P388/VCR cell line (15). Therefore, the inhibition activity of tacrolimus for normal intestinal P-glycoprotein may need to be studied in order to fully understand the mechanisms of the observed effects.

There is a dramatic difference in the mean oral clearance (CL_{oral}) of tacrolimus calculated by dose/F (5), between the normal and knockout mice with the latter being 9.7 times lower (Table I). Since the drug is virtually not excreted unchanged in urine (4), the CL_{oral} calculated is equal to the (apparent) total hepatic intrinsic clearance ($CL_{int,app}$) (5). It is known that the only major difference between the *mdr*1a knockout and normal mice is the lack of P-glycoprotein in the knockout mice (16). Hepatic blood flow rate, plasma protein binding, hepatic P450 enzymes and other physiological/biochemical properties should be the same between them (16). In this regard, the intrinsic hepatic clearance (CL_{int}) based on unbound drug was also reduced 10 times in the knockout mice. Thus, the above suggests that P-glycoprotein not only can affect drug transport/excretion across cell membranes in various parts of the body as being widely recognized, it can also affect drug metabolism in the liver apparently in a very significant or dominant manner. The exact mechanism of this is unknown. However, one may speculate that absence of P-glycoprotein in the liver (16) may result in a marked hepatic accumulation of drug and metabolite(s) formed in the liver. Under such conditions, the accumulated drug may cause partial saturation of the enzyme(s) and the accumulated metabolite(s) may competitively inhibit the metabolism of the drug (17), leading to a drastic reduction in $CL_{int,app}$ of tacrolimus. The significant accumulation of tacrolimus in the liver was demonstrated by the higher (73%) mean ratio of the liver/blood concentration in the knockout mice than in the normal mice (1). The above discussions may suggest that P-glycoprotein could also function as an effective transporter to pump the more hydrophilic metabolite(s) out of hepatocytes directly into the blood circulation once it is formed. This may explain why some metabolites often appear in high concentrations in systemic blood very shortly (within a few to 30 minutes) after intravenous administration. Whether the present finding of the very dominant role of P-glycoprotein in tacrolimus metabolism in mice can also be found in other species or for other drugs remains to be studied.

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