Effect of Cyclosporine and Tacrolimus on Cytochrome P450 Activities in Human Liver Microsomes

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(Received July 20, 2006; Accepted September 30, 2006; Published online October 16, 2006)

The effects of cyclosporine and tacrolimus on cytochrome P450 (CYP) 1A2-mediated 7-ethoxyresorufin O-deethylation, CYP2C9-mediated tolbutamide hydroxylation, CYP2C19-mediated S-mephenytoin 4′-hydroxylation, CYP2D6-mediated debrisoquine 4-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, and CYP3A4-mediated nifedipine oxidation, and CYP3A4-mediated testosterone 6β-hydroxylation activities in human liver microsomes were compared. Cyclosporine and tacrolimus, at concentrations of 0.2 or 2 μM, neither inhibited nor stimulated any of the metabolic activities except for those of CYP3A4. On the other hand, cyclosporine and tacrolimus competitively inhibited CYP3A4-mediated nifedipine oxidation activity, with inhibition constants (Ki) of 1.42 and 0.36 μM, respectively. In addition, 20 μM cyclosporine inhibited CYP2C19 and CYP2D6 activities by 29% and 30%, respectively. These results suggest that tacrolimus would not cause clinically significant interactions with other drugs, which are metabolized by CYPs, via the inhibition of hepatic metabolism and that the reason why cyclosporine, but not tacrolimus, has a pharmacokinetic inhibitory effect might be that the dosage and/or the unbound concentrations around its metabolic enzymes are higher than those of tacrolimus, rather than the differences in the inhibition potential. Obvious substrate-dependent effects on CYP3A4-inhibition potential were not observed.

Key words—cyclosporine; tacrolimus; cytochrome P450; calcineurin inhibitor; human liver microsomes

INTRODUCTION

Cytochrome P450s (CYPs) comprise a superfamily of enzymes that catalyze the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens.1–3 Multiple drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions, and, as a results, many drug-drug interactions involving metabolic inhibition are being reported.4,5 Calcineurin inhibitors, cyclosporin (INN: ciclosporin) and tacrolimus, are the primary immunosuppressants widely used to prevent acute rejection following solid organ transplantation, and both cyclosporine and tacrolimus are metabolized predominantly by CYP3A4.6,7 In most case, the immunosuppressants are co-administered with drugs such as antibiotics, corticosteroids, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and the possibility of interactions between them and other drugs exist. It is well known that the metabolism of cyclosporine and tacrolimus decrease when they are co-administered with drugs that act as substrates and/or inhibitors for CYP3A4.8,9 In addition, cyclosporine enhances the plasma concentrations of several drugs such as atorvastatin and repaglinide, which are metabolized by CYP3A4, whereas tacrolimus has no effect on atorvastatin pharmacokinetics (Table 1).10–16 However, there are few in vitro studies comparing the effects of the immunosuppressants on human hepatic CYP-mediated drug-metabolizing activity under the same experimental conditions.

In this study, the effects of cyclosporine and tacrolimus on specific activities by main CYPs, such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, in human liver microsomes were compared under the same experimental conditions in order to clarify the reason(s) for the differences in the in vivo drug interactions between cyclosporine and tacrolimus.

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Table 1. Drug Interaction of Cyclosporine and Tacrolimus in a Clinical Study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose†</th>
<th>Co-medication</th>
<th>Primary metabolic enzyme or transporter</th>
<th>Pharmacokinetic effect§ (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>2.5 mg/kg/day (bid), 1 day, po</td>
<td>Atorvastatin</td>
<td>CYP3A4</td>
<td>Cmax AUC C\text{\textsubscript{AUC}} t\text{\textsubscript{1/2}}</td>
<td>Lemahieu et al. (2005)††</td>
</tr>
<tr>
<td></td>
<td>100 mg, 2 days, po</td>
<td>Repaglinide</td>
<td>CYP3A4&gt;CYP2C8</td>
<td>—</td>
<td>Kajosaari et al. (2005)†</td>
</tr>
<tr>
<td></td>
<td>2.5 mg/kg/day, po</td>
<td>Diltiazem</td>
<td>CYP3A4</td>
<td>(90) (100) (87)</td>
<td>Asberg et al. (1999)†</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg, 1 day, po</td>
<td>Micafungin</td>
<td>Multiple enzymes</td>
<td>—</td>
<td>Hebert et al. (2005a)††</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.0625 mg/kg/day (bid), 1 day, po</td>
<td>Atorvastatin</td>
<td>CYP3A4</td>
<td>[NI] [NI] [NI]</td>
<td>A prolongation of t\text{\textsubscript{1/2}} for cyclosporine elimination was noted in the patients who received both cyclosporine and tacrolimus.</td>
</tr>
<tr>
<td></td>
<td>5 mg, 1 day, po</td>
<td>Cyclosporine</td>
<td>CYP3A4</td>
<td>—</td>
<td>Fung J.J. et al. (1990)††</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Cyclosporine: 2.84 mg/kg/dose, bid, po</td>
<td>Micafungin</td>
<td>Multiple enzymes</td>
<td>(99) (93)</td>
<td>Hebert et al. (2005b)††</td>
</tr>
<tr>
<td>/Tacrolimus</td>
<td>Tacrolimus: 0.07 mg/kg/dose, bid, po</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney transplant patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Healthy volunteer, except where indicated. †† Values in parentheses show the values calculated from the mean C\text{\textsubscript{max}}, AUC, C\text{\textsubscript{AUC}} or t\text{\textsubscript{1/2}} values described in the paper. NI: No pharmacokinetic interaction. § Ratio of the cyclosporine group: tacrolimus group.

MATERIALS AND METHODS

Materials  Pooled human liver microsomes from 50 individuals (lot no. 0310241) were obtained from XenoTech (Lenexa, KS, U.S.A.). Cyclosporine and tacrolimus were prepared by Astellas Pharma Inc. Resorufin, 7-ethoxyresorufin, tolbutamide, debrisoquine sulfate, chloroxazone, and nifedipine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). S-Mephentoin, 4'-hydroxymephentoin, oxidized nifedipine, and 6β-hydroxytestosterone were purchased from Ultrafine Chemicals (Manchester, UK), and hydroxytolbutamide, 4-hydroxydebrisoquine, and 6-hydroxychloroxazone from Sumitomo Chemical Co., Ltd. (Tokyo, Japan). Testosterone, phenobarbital sodium, and p-hydroxybenzoic acid isopropyl were obtained from Nacalai Tesque (Kyoto, Japan), Wako Pure Chemicals (Osaka, Japan), and Tokyo Chemical Industry (Tokyo, Japan), respectively.

Determination of Human CYP Activity  7-

Ethoxyresorufin O-deethylation activity (CYP1A2), tolbutamide hydroxylation activity (CYP2C9), S-mephentoin 4'-hydroxylation activity (CYP2C19), debrisoquine 4-hydroxylation activity (CYP2D6), chloroxazone 6-hydroxylation activity (CYP2E1), nifedipine oxidation activity (CYP3A4), and testosterone 6β-hydroxylation activity (CYP3A4) in human liver microsomes in the presence or absence of calcineurin inhibitors were determined as described previously. 17,18 Briefly, the incubation mixture consisted of human microsomes, 2 mM NADPH, 10 mM glucose-6-phosphate, 5 mM magnesium chloride, 1 unit/ml of glucose-6-phosphate dehydrogenase, 100 mM phosphate buffer (pH 7.4), and 5 μl of methanol or 0.02—2 mM calcineurin inhibitors dissolved in methanol in a final volume of 500 μl. The microsomal protein concentration in the mixture was 0.05 (for nifedipine concentration), 0.1 (for 7-ethoxyresorufin O-deethylation and testosterone 6β-hydroxylation), 0.2
(for chlorzoxazone 6-hydroxylation), or 0.5 mg/ml
(for tolbutamide hydroxylation, S-mephenytoin
4'-hydroxylation, and debrisoquine 4-hydroxyla-
tion).\textsuperscript{17,18} Because the $K_m$ values for 7-ethoxyresoru-
fin O-deethylation, tolbutamide hydroxylation, S-
mephenytoin 4'-hydroxylation, debrisoquine 4-hy-
droxylation, chlorzoxazone 6-hydroxylation, nifedi-
pine oxidation, and testosterone 6β-hydroxylation by
human liver microsomes were 0.22, 150.8, 27.3, 83.9,
47.7, 12.2, and 50.3 μM,\textsuperscript{17,18} respectively, the concen-
trations of 7-ethoxyresorufin, tolbutamide, S-mephe-
nytoin, debrisoquine, chlorzoxazone, nifedipine,
and testosterone were 0.25, 200, 30, 100, 50, 10,
and 50 μM, respectively, which are around the expected
values for $K_m$. Incubation was carried out at 37°C for
5 min (for testosterone 6β-hydroxylation), 10 min
(for 7-ethoxyresorufin O-deethylation, chlorzoxa-
zone 6-hydroxylation, and nifedipine oxidation), 30
min (for tolbutamide hydroxylation and S-mephe-
nytoin 4'-hydroxylation), or 60 min (for debriso-
quine 4-hydroxylation).\textsuperscript{17,18} In preliminary exper-
iments, the linearity of the reaction with regard to in-
cubation time and protein concentration was con-
firmed for each assay condition.

**Results**

The inhibitory effects of cyclosporine and tacrol-
mus at concentrations of 0.2 and 2 μM on metaboli-
c activities in human liver microsomes are shown in
Fig. 1. Because the maximum blood concentrations
($C_{max}$) of cyclosporine after intravenous or oral dos-
ing are more than 20 times higher than those of tacro-
mus,\textsuperscript{21–25} the inhibitory effects of 20 μM cy-
closporine was also estimated. Cyclosporine and
tacrolimus neither inhibited nor stimulated any of the
metabolic activities except that 20 μM cyclosporine in-
hibited CYP2C19 and CYP2D6 activities by 29% and
30%, respectively, and that both cyclosporine and
tacrolimus inhibited CYP3A4-mediated nifedipine
oxidation and testosterone 6β-hydroxylation activi-
ties. Therefore, the effects on CYP3A4-mediated
nifedipine oxidation activity were investigated in de-
tail (Fig. 2). Cyclosporine and tacrolimus competi-
tively inhibited the CYP3A4 activity, with $K_i$ values
of 1.42 and 0.36 μM, respectively. Because cyclo-
sporine was not soluble in the reaction mixture at concen-
trations over 40 μM, the effects of cyclosporine on
CYP2C19 and CYP2D6 could not be investigated in
detail.

**Discussion**

This study compared the effects of cyclosporine and
tacrolimus on CYP activities under the same ex-
perimental conditions. It was demonstrated that the
only influence cyclosporine or tacrolimus had on any
metabolic activities was that cyclosporine inhibited
CYP2C19 and CYP2D6 activities by 30% at a concen-
tration of 20 μM, and that both drugs competitively
inhibited CYP3A4-mediated nifedipine oxidation activi-
ty with $K_i$ values of 1.42 and 0.36 μM, respec-
tively (Figs. 1 and 2, Table 2).\textsuperscript{26,29} These results
in the effects of tacrolimus on support the results of
the previous study which found that tacrolimus has
no effect on CYP1A2, CYP2C9, CYP2D6, CYP2E1,
and CYP3A4 at concentrations below 1 μM, but it has
a slightly competitive inhibitory effect on CYP3A4 ac-
tivity, with $K_i$ values of 2–3 μM, which suggests
that tacrolimus is unlikely to potentiate the effect of
coadministered drugs through inhibition of hepatic
metabolism.\textsuperscript{30} In addition, the $K_i$ values of cyclo-
sporine and tacrolimus against CYP3A4-mediated es-
tradial 2-hydroxylation are reported to be 0.30 and
0.88 μM, respectively.\textsuperscript{27} Since substrate-dependent
effects on CYP3A4-inhibition potential have been re-
ported previously,\textsuperscript{26,29} this study focused on the es-
timation of the degree of the CYP3A4-inhibition by
the use of other typical CYP3A4-substrates, nifedi-
pine and testosterone. Although we have demon-
strated that the $K_i$ value for cyclosporine against
CYP3A4-mediated nifedipine oxidation activity was
approximately 4 times higher than that of tacrolimus,
the observed $K_i$ and IC₅₀ values of cyclosporine and
tacrolimus reported here and elsewhere were around 1—4 μM. The one exception to this was a report that stated that cyclosporine metabolism and tacrolimus 13-O-demethylation were inhibited by tacrolimus and cyclosporine, respectively, with Ki values of 37 μM (Table 2). That is, obvious substrate-dependent effects on CYP3A4-inhibition potential were not observed.

The initial therapeutic doses of cyclosporine administered by intravenous infusion or oral dosing after organ transplantation in Japan are 3—6 mg/kg/day and 3—8 mg/kg bid, respectively, and those of tacrolimus are 0.03—0.1 mg/kg/day and 0.03—0.15 mg/kg bid, respectively. This indicates that the clinical therapeutic doses of cyclosporine are 30—167 times higher than those of tacrolimus. The clinical maximum blood concentrations (Cmax) after intravenous or oral dosing of cyclosporine and tacrolimus have been reported as 759—1801 ng/ml and 12.7—78 ng/ml, respectively, that is, around 1—2 μM and 0.02—0.1 μM, respectively. No inhibition of human CYPs, except for CYP3A4, by tacrolimus at 2 μM concentration, which is more than 20 times greater than the expected Cmax, was observed. When the substrate concentration was much lower than the Km value, the ratio of intrinsic metabolic clearance (CLint) in the presence and absence of the inhibitor can be expressed by the following equation, independent of the inhibition type, except in the case of uncompetitive inhibition:

$$\frac{CL_{int}(+\text{Inhibitor})}{CL_{int}(-\text{Inhibitor})} = \frac{1}{1 + \frac{I_u}{K_i}}$$

where Iu is the unbound concentration of the inhibitor. Additionally, when the absorption rate is maximum, the maximum concentration of the inhibitor in the blood flowing into the liver (Iu,max) after oral dosing can be expressed as

$$I_{u, max} = I_{b, max} + \left( k_a \cdot \frac{D}{Q_H} \cdot F_a \right)$$
where $I_{\text{max}}$, $k_{\text{a}}$, $D$, $Q_{\text{H}}$, and $F_{\text{u}}$ represent the maximum blood concentration of the inhibitor in the circulation, the absorption rate constant, the dose, the hepatic blood flow rate, and the fraction absorbed from the gastrointestinal tract into the portal vein, respectively. After an oral dose(s) of 90.3 mg cyclosporine or 0.16 mg/kg (body weight: 57.4 kg) tacrolimus, the peak blood concentrations ($I_{\text{max}}$) are expected to be 979 ng/ml (0.81 µM) or 44 ng/ml (0.05 µM), respectively.23,24 The unbound concentrations of $I_{\text{H, max}}$ ($I_{\text{H, max}}$) for cyclosporine and tacrolimus after an oral dosing were calculated to be 78 nM and 0.16 nM, respectively, using the following values: free fraction present in the plasma ($f_{\text{u}}$) = 0.015 and 0.011,35,36 blood to plasma concentration ratio ($R_{\text{b}}$) = 1.05 and 52.9,36,37 respectively, $k_{\text{a}}$ = 0.1 min$^{-1}$, $Q_{\text{H}}$ = 1610 ml/min, and where $F_{\text{u}}$ = 1 to avoid false-negative predictions. In this paper, it was demonstrated that cyclosporine and tacrolimus did not inhibit human CYPs (except CYP3A4) at concentrations of 2 µM or more (Table 1), which is 26- and 12500-fold higher, respectively, than the predicted $I_{\text{H, max}}$ values. In addition, the $1 + I_{\text{H, max}}/K_{i}$ values of cyclosporine and tacrolimus for CYP3A4-mediated nifedipine oxidation were 1.05 and 1.0004, respectively. Based on these estimations, it was speculated that, after intravenous or oral dosing, these calcineurin inhibitors would not cause clinically significant interactions with any other drugs metabolized by CYPs, via the inhibition of hepatic metabolism. It has been shown that the pharmacokinetics of atorvastatin are not affected by tacrolimus.10 Although tacrolimus reportedly prolongs the half-life of cyclosporine elimination,40 there have been few clinical reports that tacrolimus increases the blood concentrations of other CYP-metabolized drugs as a result of its inhibition of metabolism (Table 1). The mechanism of this cyclosporine-tacrolimus interaction is not clear, but it has led to the provision of their concomitant use.

In contrast, cyclosporine enhances the plasma concentrations of CYP3A4 substrates, such as atorvastatin and repaglinide (Table 1).10,11 In addition, the presence of cyclosporine increases the AUC values of lovastatin and pravastatin are 20- and 5-fold, respectively. This suggests that interaction with cyclosporine has a higher impact on lovastatin pharmacokinetics than those of pravastatin. This is because lovastatin is metabolized by CYP3A4, whereas the major pravastatin metabolites are generated by non-CYP-dependent processes.38,39 The AUC of bosentan nearly doubled after seven days of co-administration with cyclosporine, but the AUC values of cyclosporine were the same for doses with and without bosentan.40 Bosentan is metabolized by CYP3A4 and CYP2C9, but it also induces these CYPs.41 The interaction observed between bosentan and cyclosporine in clinical trials also occurs in rats, the major determinant of which seems to be the inhibition of active uptake of bosentan from the blood into the liver (liver transport processes).42 Furthermore, the $C_{\text{max}}$, AUC, and trough concentrations of sirolimus with cyclosporine were 1.42—1.46 times higher than those when combined with tacrolimus.16 Sirolimus, cyclosporine, and tacrolimus are all substrates not only for CYP3A4 but also multidrug resistance protein 1 (MDR1).16 Recent studies in rats have been shown that cyclosporine increases the plasma concentration of cerivastatin by inhibiting transporter-mediated hepatic uptake.43 CYP3A4 exists not only in the liver but also in the gut, where it plays an important role in the first-pass metabolism after oral administration of its substrates.44 For these reasons, CYP3A4’s contribution to the metabolism and the transport process initiated by MDR1 in the gut in addition to
Table 2. \( K_i \) Values of Cyclosporine and Tacrolimus against Human CYP Activities

<table>
<thead>
<tr>
<th>Drug</th>
<th>CYP</th>
<th>Reaction</th>
<th>( K_i ) or IC(50^c ) (( \mu M ))</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>CYP1A2</td>
<td>7-Ethoxysresoruﬁn O-deethylation</td>
<td>NI(^b)</td>
<td>Kenworthy et al. (1999)(^{20})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7-Ethoxysresoruﬁn O-deethylation</td>
<td>NI(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2C9</td>
<td>Tolbutamide hydroxylation</td>
<td>NI(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2C19</td>
<td>S-Mephenytoin 4'-hydroxylation</td>
<td>&gt;20(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>Debrisoquin 4'-hydroxylation</td>
<td>&gt;20(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2E1</td>
<td>Chlorozaxone 6'-hydroxylation</td>
<td>NI(^b)</td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Nifedipine oxidation</td>
<td></td>
<td>1.42 (C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tacrolimus 31-O-demethylation</td>
<td></td>
<td>37 (C)</td>
<td>Lampen et al. (1995)(^{29})</td>
</tr>
<tr>
<td></td>
<td>Estradiol 2-hydroxylation</td>
<td></td>
<td>0.30 (C)</td>
<td>Satoh et al. (2003)(^{27})</td>
</tr>
<tr>
<td></td>
<td>Estradiol 2-hydroxylation</td>
<td></td>
<td>0.06 (S)</td>
<td>Satoh et al. (2000)(^{20})</td>
</tr>
<tr>
<td></td>
<td>Estradiol 4-hydroxylation</td>
<td></td>
<td>0.05 (S)</td>
<td>Satoh et al. (2000)(^{20})</td>
</tr>
<tr>
<td></td>
<td>7-Benzoxysteroruﬁn O-dealkylation</td>
<td></td>
<td>1.2 (S)</td>
<td>Streser et al. (2000)(^{26})</td>
</tr>
<tr>
<td></td>
<td>Fluorescein formation from dibenzyﬂuorescein</td>
<td></td>
<td>3.3 (S)</td>
<td>Streser et al. (2000)(^{26})</td>
</tr>
<tr>
<td></td>
<td>Nifedipine oxidation, testosterone 6β-hydroxylation, midazolam 1'-hydroxylation</td>
<td></td>
<td>—</td>
<td>Cyclosporine (10 ( \mu M )) inhibits the activities by 35--88%.</td>
</tr>
<tr>
<td></td>
<td>Nifedipine oxidation, testosterone 6β-hydroxylation, midazolam 1'-hydroxylation, triazolam 4'-hydroxylation, terfenadine C-hydroxylation, terfenadine N-demethylation, diazepam N-demethylation, dextromethorphan N-demethylation, erythromycin N-demethylation, methoxymorpholinoxorubicin metabolism</td>
<td></td>
<td>—</td>
<td>Cyclosporine (30 ( \mu M )) inhibits the activities by 68--90%.</td>
</tr>
</tbody>
</table>

| Tacrolimus   | CYP1A2                     | 7-Ethoxysresoruﬁn O-deethylation         | NI\(^b\)                                | Lecointre et al. (2002)\(^{13}\) |
|              |                            | 7-Ethoxysresoruﬁn O-deethylation         | NI\(^b\)                                | Matsuda et al. (1996)\(^{32}\) |
|              |                            | 1-Methylxanthine formation from theophylline | NI\(^b\) | Matsuda et al. (1996)\(^{32}\) |
|              |                            | 3-Methylxanthine formation from theophylline | NI\(^b\) |                             |
|              | CYP2A6                     | Coumarin 7-hydroxylation                 | NI\(^b\)                                | Lecointre et al. (2002)\(^{13}\) |
|              | CYP2C9                     | Tolbutamide hydroxylation                | NI\(^b\)                                | Lecointre et al. (2002)\(^{13}\) |
|              | CYP2C19                    | S-Mephenytoin 4'-hydroxylation           | NI\(^b\)                                |                             |
|              | CYP2D6                     | Debrisoquin 4'-hydroxylation             | NI\(^b\)                                | Lecointre et al. (2002)\(^{13}\) |
|              | CYP2E1                     | Chlorozaxone 6'-hydroxylation            | NI\(^b\)                                | Lecointre et al. (2002)\(^{13}\) |
|              |                                | Chlorozaxone 6'-hydroxylation            | NI\(^b\)                                |                             |
|              | CYP3A4                     | Nifedipine oxidation                     | 0.36 (C)                                |                             |
|              | Estradiol 2-hydroxylation  |                                          | 0.88 (N)                                 | Satoh et al. (2003)\(^{27}\) |
|              | Estradiol 2-hydroxylation  |                                          | 0.64 (S)                                 | Satoh et al. (2003)\(^{27}\) |
|              | Midazolam 1'-hydroxylation |                                          | 3.7 (C)                                 | Lecointre et al. (2002)\(^{13}\) |
|              | Midazolam 4'-hydroxylation |                                          | 2 (C)                                   | Lecointre et al. (2002)\(^{13}\) |
|              | Cyclosporine metabolism     |                                          | 37 (C)                                  | Lampen et al. (1996)\(^{26}\) |
|              | Prednisolone 6β-hydroxylation |                                      | —                                        | Tacrolimus (10 \( \mu M \)) inhibits the activity by 68%. |

\( ^a \) C: Competitive inhibition; N: Non-competitive inhibition; \( ^b \) No inhibition was observed at 20 \( \mu M \); \( ^c \) No inhibition was observed at 30 \( \mu M \); \( ^d \) No inhibition was observed at 2 \( \mu M \); \( ^e \) No inhibition was observed at 1 \( \mu M \); \( ^f \) No inhibition was observed at 1--100 \( \mu M \) concentrations.
metabolism by CYPs in the liver should be taken into consideration. Further studies are required to assess the degree of the contribution of these processes.

In conclusion, this study suggests that tacrolimus would not cause any clinically significant interactions with other drugs that are metabolized by CYPs, via inhibition of hepatic metabolism. Results also suggest that the reason why cyclosporine but not tacrolimus has an inhibitory effect on pharmacokinetics might be because of the dosage and/or because the unbound concentrations of cyclosporine around the metabolic enzymes and/or MDR1 is higher than that of tacrolimus, not because of the differences between the inhibition potencies.

REFERENCES
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