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Contribution of CYP3A5 to the in Vitro Hepatic Clearance of Tacrolimus

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Background: **Tacrolimus is metabolized predominantly to 13-*O*-demethyltacrolimus in the liver and intestine by cytochrome P450 3A (CYP3A). Patients with high concentrations of CYP3A5, a CYP3A isoenzyme polymorphically produced in these organs, require higher doses of tacrolimus, but the exact mechanism of this association is unknown.**

Methods: **cDNA-expressed CYP3A enzymes and a bank of human liver microsomes with known CYP3A4 and CYP3A5 content were used to investigate the contribution of CYP3A5 to the metabolism of tacrolimus to 13-*O*-demethyltacrolimus as quantified by liquid chromatography–tandem mass spectrometry.**

Results: **Demethylation of tacrolimus to 13-*O*-demethyltacrolimus was the predominant clearance reaction. Calculated K_m and V_{max} values for CYP3A4, CYP3A5, and CYP3A7 cDNA-expressed microsomes were 1.5 μmol/L and 0.72 pmol · (pmol P450)$^{-1}$ · min^{-1}, 1.4 μmol/L and 1.1 pmol · (pmol P450)$^{-1}$ · min^{-1}, and 6 μmol/L and 0.084 pmol · (pmol P450)$^{-1}$ · min^{-1}, respectively. Recombinant CYP3A5 metabolized tacrolimus with a catalytic efficiency (V_{max}/K_m) that was 64% higher than that of CYP3A4. The contribution of CYP3A5 to 13-O-demethylation of tacrolimus in human liver microsomes varied from 1.5% to 40% (median, 18.8%). There was an inverse association between the contribution of CYP3A5 to 13-O-demethylation and the amount of 3A4 protein (r = 0.90; P <0.0001). Mean 13-O-demethylation clearances in CYP3A5 high and low expressers, estimated by the parallel-tube liver model, were 8.6 and 3.57 mL · min^{-1} · (kg of body weight)$^{-1}$, respectively (P = 0.0088).**

Conclusions: **CYP3A5 affects metabolism of tacrolimus, thus explaining the association between CYP3A5 genotype and tacrolimus dosage. The importance of CYP3A5 status for tacrolimus clearance is also dependent on the concomitant CYP3A4 activity.**

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Tacrolimus (FK506), a potent immunosuppressive macrolide lactone *(1)* isolated from the fermentation broth of *Streptomyces tsukubaensis*, is a relatively specific inhibitor of T-lymphocyte proliferation that exerts its immunosuppressive activity mainly through binding to immunophilins (FK-binding proteins). Because of these immunosuppressive properties, tacrolimus is widely used in the prophylaxis of organ rejection after allogeneic solid-organ transplantation. Tacrolimus is subject to substantial intestinal and hepatic first-pass effects, and its bioavailability is individually variable. Thus, tacrolimus elimination, expressed as total body clearance, varies interindividually from 0.041 to 0.36 L · h^{-1} · (kg of body weight)$^{-1}$ *(2)*. Because of this variability in conjunction with its narrow therapeutic index, monitoring of whole-blood concentrations of tacrolimus is essential to achieve optimal efficacy while minimizing the risk of toxicity *(3)*. Tacrolimus undergoes O-demethylation, hydroxylation, and/or oxidative metabolic reactions in the liver and intestine, and it is eliminated mostly with the bile *(4–9)*. Demethylation to 13-*O*-demethyltacrolimus by cytochrome P450 3A (CYP3A)[5] is quantitatively the most important metabolic route *(6, 7, 10, 11)*.

CYP3A concentrations and activity display interindividual variability, which may in part explain some of the variation in the bioavailability of tacrolimus. Historically,

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[5] Nonstandard abbreviations: CYP3A, cytochrome P450 3A; HLM, human liver microsome; LC-MS/MS, liquid chromatography–tandem mass spectrometry; and CL_{p1} and CL_{p2}, predicted pharmacokinetic clearances obtained by use of the well-stirred and the parallel-tube model, respectively.

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tacrolimus has been considered to be a substrate for the most extensively investigated CYP3A isoenzyme, CYP3A4. However, several independent clinical studies recently demonstrated significant associations between blood concentrations of tacrolimus and/or maintenance dose in transplant patients and the CYP3A5 concentration. Unlike CYP3A4, CYP3A5 is expressed polymorphically, with expression controlled by only a few genetic variants *(12, 13)*. In Caucasians, the expression of CYP3A5 appears to be controlled exclusively by only 1 genetic variant (*CYP3A5*1*). CYP3A5 "high expressers" account for ~10% of Caucasian, 33% of Japanese, and 55%–70% of African or African-American populations. Several clinical studies have demonstrated that carriers of *CYP3A5*1* alleles (CYP3A5 high expressers) require higher doses of tacrolimus to achieve target blood concentrations than do homozygous carriers of the *CYP3A5*3* allele (CYP3A5 "low expressers") *(14–21)*.

At present, the potential of *CYP3A5* genotyping in tacrolimus therapies is unclear *(22)*. Target tacrolimus concentrations in plasma can be ultimately achieved in all patients, irrespective of genotype, by means of drug monitoring-based dose adjustments. More important may be the reported impact of the polymorphism during the early period after transplantation. CYP3A5 high expressers had lower mean tacrolimus concentrations during the first week after renal transplantation, and rejection occurred earlier in these patients *(23)*. On the basis of these results, it has been proposed that a *CYP3A5* genotype-based adjusted initial dosing regimen for tacrolimus, subsequently guided by concentration measurements, has the potential to improve transplant survival.

On the other hand, it has been noted that a significant portion of interindividual variability for tacrolimus cannot be explained by the *CYP3A5* polymorphisms. This could reflect the effect of other tacrolimus-metabolizing enzymes (e.g., CYP3A4), transporters (P-glycoprotein), or of CYP3A5 induction *(24)*. Better understanding of the role of CYP3A5 in tacrolimus disposition has been hampered by the paucity of in vitro data on tacrolimus metabolism by CYP3A5. Indeed, although tacrolimus metabolism to 13-*O*-demethyltacrolimus by CYP3A5 has been demonstrated *(25)*, a detailed investigation on the kinetics of this reaction is lacking. Particularly informative would be a direct comparison with CYP3A4. We therefore set out to assess the relative contributions of CYP3A5 and CYP3A4 to the formation of the main tacrolimus metabolite (13-*O*-demethyltacrolimus), using cDNA-expressed enzymes and a bank of human liver microsomes derived from low and high CYP3A5 expressers.

Materials and Methods

Chemicals and reagents

Tacrolimus was a kind gift of Fujisawa (Osaka, Japan). The tacrolimus metabolites 13,15-*O*-didemethyltacrolimus and 13,31-*O*-didemethyltacrolimus were kind gifts of Dade Behring (Schwallbach, Germany). 13-*O*-Demethyltacrolimus, 15-*O*-demethyltacrolimus, and 31-*O*-demethyltacrolimus were kindly provided by Professor Uwe Christians (University of Colorado Health Sciences Center, Denver, CO). NADPH was purchased from Roche. The bank of 15 phenotyped (6β-testosterone hydroxylase activity) liver samples [BD Gentest single donor human liver microsomes (HLMs); cat. nos. HG03, HG64, and HG74 (CYP3A5 low expressers), and HH31, HH54, HH47, HH91, HG95, HH108, HH86, HH3, HH1, HH89, HH48, and HH9 (CYP3A5 high expressers)] and baculovirus-derived microsomes producing CYP1A2/OR (cat. no. P203; lot 21), CYP2A6/OR (cat. no. P204; lot 14), CYP2B6/OR (cat. no. P210; lot 9), CYP2C8/OR/b5 (cat. no. P252; lot 10), CYP2C9*1/OR (cat. no. P218; lot 15), CYP3A4/OR (cat. no. P207; lot 18), CYP3A7/OR/b5 (cat. no. P235; lot 7), and CYP3A5/OR (cat. no. P235; lot 17) were obtained from Natutec. Purified cytochrome b5 (cat. no. P2252; lot 24548B) was purchased from Panvera (now owned by Invitrogen Corporation).

Tacrolimus metabolism by HLM- and baculovirus-expressed cytochrome P450

Stock solutions of tacrolimus were prepared in methanol. Samples were evaporated under N_2, dissolved in 100 mmol/L potassium phosphate buffer (pH 7.4), and supplemented with either HLM- or baculovirus-expressed cytochrome P450. The final incubation volume was 100 μL. The reaction mixture with CYP3A4/OR or CYP3A5/OR was additionally supplemented with purified cytochrome b5 (P450:b5 ratio, 1:1) according to a previously described method *(26)* before the start of the reaction with NADPH. The reaction was initiated through addition of NADPH, was allowed to proceed for 10 min, and then was stopped by addition of a freshly prepared stop solution composed of 0.3 mol/L $ZnSO_4$–methanol (3:7 by volume) containing internal standard (25 μg/L ascomycin). After reaction termination, the incubation mixtures were centrifuged at 10 000*g* for 5 min. Supernatants were transferred to new tubes, and 100 μL was used for the liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. The formation of 13-*O*-demethyltacrolimus was linear with time between 5 and 20 min and with protein over the range from 5 to 50 μg for HLMs and from 1 to 8 pmol for baculovirus-expressed CYP3A. The end concentrations of tacrolimus ranged from 0 to 12.5 μmol/L. The substrate consumption was <15% over the incubation time (10 min).

LC-MS/MS analysis

An on-line extraction method with a column-switching technique combined with analytical LC and electrospray MS/MS was used to quantify 13-*O*-demethyltacrolimus. The supernatants obtained from microsomal incubations were injected with a series 200 autoinjector (Perkin-Elmer). The extraction cartridge used was a security guard C_{18} cartridge (4.0 × 3.0 mm; Phenomenex), and the analytical column was a Nucleosil C_{18} reversed-phase

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column [250 × 2.1 mm (i.d.); 5 μm; MZ-Analysentechnik] maintained at 65 °C with a DuPont column oven. The sample was introduced on the extraction column at a flow rate of 1500 μL/min with deionized water (solvent A) for 0.5 min. After the diverter valve was switched, analytes were eluted at a flow rate of 500 μL/min onto the analytical column with the following stepwise elution protocol: 0.5 to 3 min, 65% solvent B (0.01 g/L formic acid and 2 mmol/L ammonium acetate in methanol); 3 to 4 min, 75% B; 4 to 6 min, 80% B; 6 to 9.5 min, 100% B. The extraction column was then reequilibrated with solvent A for 0.5 min at 1500 μL/min before the next injection. An API 2000 triple-stage quadrupole instrument was used for quantification in the positive-ion mode. Multiple reaction monitoring with argon as collision gas and the following mass transitions (ammonium adducts) was used for quantification: 13-*O*-demethyltacrolimus, *m/z* 807.5→754.5; ascomycin, *m/z* 809.5→756.5. Peak areas for 13-*O*-demethyltacrolimus were linear from 0.5 to 5 μg/L. The limit of quantification for 13-*O*-demethyltacrolimus was 0.25 μg/L. The limit of detection was 0.1 μg/L. The between-run imprecision (n = 20) was 7.0% at 3 μg/L and 2.7% at 5 μg/L 13-*O*-demethyltacrolimus.

CALCULATION OF RELATIVE CONTRIBUTIONS

The relative contribution of each CYP3A isoform (i) to the overall rate of tacrolimus 13-O-demethylation (f_i) was predicted as a function of substrate concentration (s) by use of the relative hepatic abundance (A_i) of each recombinant CYP3A, as determined by quantitative Western blotting using isoenzyme-specific antibodies, and the reaction velocity, V_i(s), based on the recombinant enzyme kinetic parameters (K_m and V_{max}) determined for each enzyme *(27)*:

$$f_i\,(\%) = \frac{A_i V_i(s)}{\sum_{i=1}^{n} A_i V_i(s)} \times 100$$

PREDICTION OF PHARMACOKINETIC CLEARANCE

The kinetic parameters of baculovirus-expressed isoforms were used to estimate the in vitro intrinsic clearance of tacrolimus to 13-*O*-demethyltacrolimus. The intrinsic clearance was calculated as the V_{max}/K_m ratio *(28)*. The net in vitro human liver microsomal intrinsic clearance was calculated for each CYP isoform, weighted by the respective baculovirus relative activity factor estimates, as defined previously *(29)*. This was then scaled up to in vivo intrinsic clearance by use of previously published values of scaling factors: 50 mg microsomal protein/g of liver, and 20 g liver/kg of body weight *(30)*. The resulting estimated intrinsic clearance of tacrolimus via 13-O-demethylation (CL_{int}') was used in conjunction with estimates of human hepatic blood flow (Q; 20 mL $min^{-1} \cdot kg^{-1}$) and the free fraction of tacrolimus in human plasma (f_u; 0.01) *(31)* to predict intravenous clearance via 13-O-demethylation. This was done according to the well-stirred (CL_{p1}) and parallel-tube (CL_{p2}) models *(32, 33)* and the respective, following equations *(34, 35)*:

$$CL_{p1} = \frac{Q \cdot f_u \cdot CL'_{int}}{Q + f_u \cdot CL'_{int}}$$

$$CL_{p2} = Q \cdot \left(1 - e^{\frac{-CL_{int} \cdot f_u}{Q}}\right)$$

DATA ANALYSIS

The enzyme kinetic parameters (K_m and V_{max}) were determined by nonlinear least-squares regression (SigmaPlot 8.0; SPSS). The significance of differences in predicted clearance values between different groups was assessed by the Mann–Whitney *U*-test. The association between 2 variables was assessed by the Spearman rank test in the case of nonparametric data or by the Pearson correlation coefficient (SPSS 12.0) in the case of gaussian-distributed data.

Results

TACROLIMUS METABOLISM BY BACULOVIRUS-EXPRESSED CYTOCHROME P450

We determined the enzyme kinetic parameters for 13-O-demethylation of tacrolimus catalyzed by baculovirus-expressed CYP3A4, CYP3A5, and CYP3A7. The tacrolimus concentration range varied from 0 to 12.5 μmol/L. The K_m and V_{max} values calculated from Michaelis–Menten plots were 1.5 μmol/L and 0.72 pmol · (pmol P450)$^{-1}$ · min^{-1} for CYP3A4 (Fig. 1A) and 1.4 μmol/L and 1.1 pmol · (pmol P450)$^{-1}$ · min^{-1} for CYP3A5 (Fig. 1B). CYP3A7 also exhibited some catalytic activity (data not shown), but its velocity was 10- to 20-fold lower [0.084 pmol · (pmol P450)$^{-1}$ · min^{-1}], whereas the K_m value was 4.3- to 8.3-fold higher (6 μmol/L) than those of CYP3A4 and CYP3A5. The corresponding V_{max}/K_m ratios were 0.48, 0.79, and 0.014 μL · (pmol P450)$^{-1}$ · min^{-1} for CYP3A4, CYP3A5, and CYP3A7, respectively. Other metabolites, such as 15-*O*-demethyltacrolimus, 31-*O*-demethyltacrolimus, 13,15-*O*-didemethyltacrolimus, and 13,31-*O*-didemethyltacrolimus were also produced by CYP3A4 and CYP3A5, but not by CYP3A7. Their amounts were 10- to 20-fold lower than those of 13-*O*-demethyltacrolimus. Several other P450 isoenzymes were also tested and showed extremely low [$<$0.005 pmol · (pmol P450)$^{-1}$ · min^{-1} for CYP2A6, CYP2B6, CYP2C8, and CYP2C9] or no detectable 13-*O*-tacrolimus demethylation activity (CYP1A2; data not shown). An example of LC-MS/MS extracted ion peak chromatograms for 13-*O*-demethyltacrolimus, 15-*O*-demethyltacrolimus, 31-*O*-demethyltacrolimus, 13,31-*O*-didemethyltacrolimus, and 13,15-*O*-didemethyltacrolimus are shown in Fig. 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol51/issue8/.

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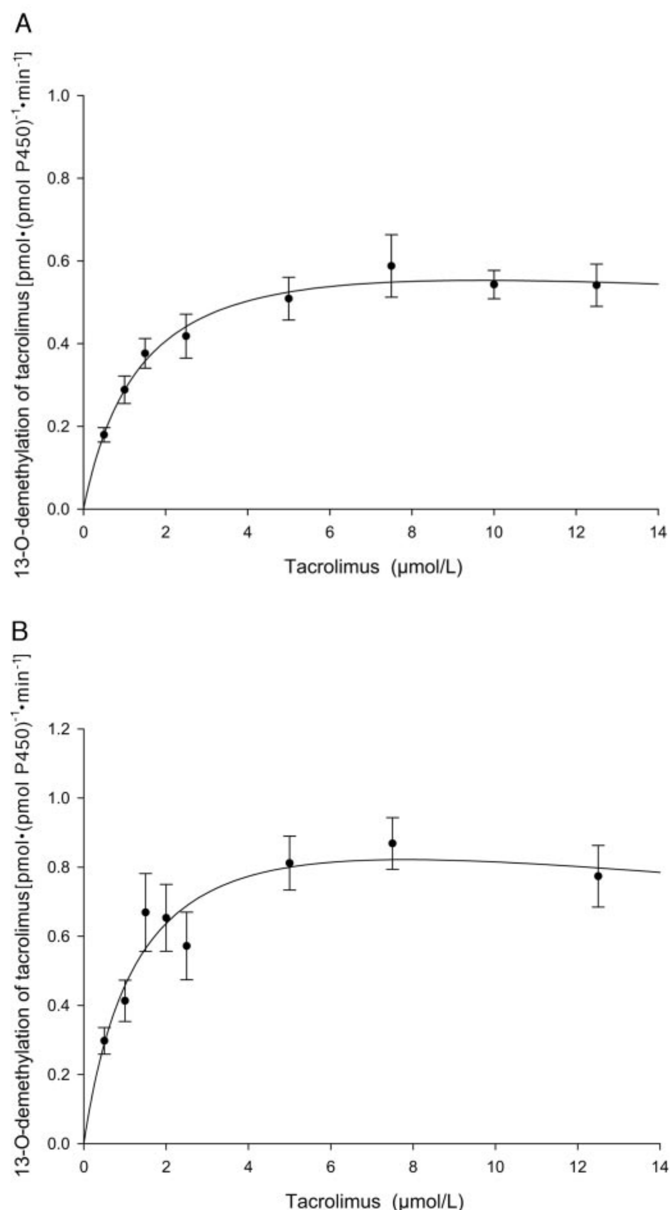

Fig. 1. Kinetic plots of 13-O-demethylation of tacrolimus by cDNA-expressed CYP3A4 (*A*) and CYP3A5 (*B*).

CYP3A4 and CYP3A5 were supplemented with b5. The ratio of 3A/OR to b5 was 1:1.

tacrolimus metabolism by HLMs

We next investigated the tacrolimus 13-O-demethylation activity in a bank of 15 human liver samples, including 12 high expressers and 3 low expressers for CYP3A5. The relevant data are given in Table 1. There was an almost 19-fold variation in the V_{max} for tacrolimus 13-O-demethylation with values ranging from 199.3 $pmol \cdot mg^{-1} \cdot min^{-1}$ to 3746.7 $pmol \cdot mg^{-1} \cdot min^{-1}$ (median, 564.8 $pmol \cdot mg^{-1} \cdot min^{-1}$; Table 1). Variation was smaller for the respective K_m values (median, 0.74 μmol/L; range, 0.38–1.20 μmol/L).

We next compared the V_{max} values obtained for the individual HLMs with corresponding CYP3A4 and CYP3A5 protein content. The correlation coefficient (r) between the V_{max} for tacrolimus 13-O-demethylation and CYP3A5 protein concentration was higher than that for V_{max} and CYP3A4 protein concentration: 0.65 ($P = 0.008$) vs 0.49 ($P = 0.09$; Fig. 2 of the online Data Supplement). We also observed a good correlation ($r = 0.64$; $P = 0.01$; Fig. 2 of the online Data Supplement) between V_{max} values for tacrolimus 13-O-demethylation and those for testosterone 6β-hydroxylation (supplied in the package insert for each HLM). Because the latter reaction is a marker of CYP3A activity *(36)*, this result further confirms that tacrolimus is mostly metabolized by CYP3A. The data in Fig. 2 of the online Data Supplement were also analyzed after omission of an outlier, liver HH31, and correlations between V_{max} and either CYP3A5 protein ($r = 0.56$; $P = 0.03$) or testosterone 6β-hydroxylation ($r = 0.57$; $P = 0.03$) remained significant. We observed no significant correlation between V_{max} and CYP3A4 protein concentration ($r = 0.35$; $P = 0.265$).

relative contribution of cyp3a5 to 13-o-demethylation of tacrolimus

We investigated the contribution of CYP3A5 to 13-O-demethylation of tacrolimus in the liver bank, using the relative hepatic abundance approach and assuming that the enzyme kinetic constants that were determined for the individual recombinant CYP3A isoenzymes were also valid for the hepatic liver microsomes. At 5 μmol/L tacrolimus, a concentration within the range measured in hepatic tissue in vivo, CYP3A5 accounted for 1.5%–40% of 13-O-demethylation of tacrolimus. We found no significant association between the contribution of CYP3A5 to 13-O-demethylation and the amount of CYP 3A5 protein (Fig. 2A; $r = 0.20$; $P = 0.51$), but there was an inverse association with the amount of 3A4 protein (Fig. 2B; $r = 0.90$; $P < 0.0001$). Similar results were obtained at 0.5 and 50 μmol/L tacrolimus (data not shown).

prediction of pharmacokinetic clearance

When we used the relative activity factor approach *(29)* and the parallel-tube model, the predicted tacrolimus 13-O-demethylation clearance values (CL_{p2}) in livers from CYP3A5 high and low expressers were, on average, 8.60 and 3.57 $mL \cdot min^{-1} \cdot (kg\ of\ body\ weight)^{-1}$, respectively ($P = 0.0088$, Mann–Whitney U-test; Fig. 3 of the online Data Supplement). The predicted tacrolimus 13-O-demethylation clearance values (CL_{p1}) obtained with the well-stirred model were also calculated and were similar to the CL_{p2} values (Table 1).

Discussion

Previous investigations using HLMs have indicated that CYP3A4 plays an important role in the metabolism of tacrolimus, the major primary metabolite being 13-*O*-demethyltacrolimus *(6, 9, 10)*. It was, however, suggested that members of the CYP3A protein family other than

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Table 1. Kinetic constants for the conversion of tacrolimus to 13-*O*-demethyl tacrolimus and estimated intrinsic tacrolimus clearances in a bank of human liver samples.

Sample	V_{max}, pmol · mg^{-1} · min^{-1}	K_m, μmol/L	V_{max}/K_m, μL · mg^{-1} · min^{-1}	CL_{p1}, mL · min^{-1} · kg^{-1}	CL_{p2}, mL · min^{-1} · kg^{-1}	CYP3A4 protein,[a] pmol/mg	CYP3A5 protein,[a] pmol/mg	Sex[a]	Age,[a] years	T-6ß-OH activity,[a,b] pmol · mg^{-1} · min^{-1}
HG03	320.7	0.43	745.81	3.63	3.98	95	0.9	F	30	4600
HG64	199.3	0.51	390.78	2.43	2.58	NA	1.2	M	63	1800
HG74	336.4	1.2	280.33	3.78	4.16	NA	1.1	M	32	2600
HH54	671.3	0.56	1198.75	6.35	7.44	87	8.6	F	62	4300
HH31	3746.7	0.54	6938.33	14.44	18.51	300	14	F	51	12 000
HH47	1129.5	0.47	2403.19	8.78	10.85	62	9.3	F	53	4100
HH91	1069.7	0.53	2018.3	8.51	10.46	46	5.7	F	55	2900
HG95	335.8	0.74	453.78	3.77	4.15	29	6.9	F	47	760
HH86	564.8	0.38	1486.32	5.62	6.47	83	7.6	M	57	3500
HH3	835	0.96	869.79	7.33	8.78	76	11	M	38	6500
HH108	448	0.86	520.93	4.74	5.33	27	11	F	27	3700
HH89	453.8	1.1	412.55	4.78	5.39	38	11	M	33	2200
HH48	457.7	0.85	538.47	4.81	5.43	30	9.8	M	62	1700
HH9	943.4	0.89	1060	7.9	9.59	53	12	M	51	5300
HH1	1122.9	1.1	1020.82	8.75	10.81	110	12	F	31	8000
All livers[c] (n = 15)										
Median	564.80	0.74	869.79	5.62	6.47	62.00	9.30		51	3700
Range	199.3–3746.7	0.38–1.12	280.33–6938.33	2.43–14.4	2.58–18.51	27–300	0.9–14		27–63	760–12 000
CYP3A5 LE livers[c] (n = 3)										
Median	320.7	0.51	390.78	3.63	3.98		1.1		32	2600
Range	199.3–336.4	0.43–1.2	280.3–745.81	2.43–3.78	2.45–4.16		0.9–1.2		30–63	1800–4600
CYP3A5 HE livers[c] (n = 12)										
Median	753.15	0.79	1040.41	6.84	8.11	57.5	10.4		51	3900
Range	335.8–3746.7	0.38–1.1	412.55–6938.33	3.77–14.44	4.15–18.51	27–300	5.7–14		27–62	760–12 000

[a] Data supplied by BD Gentest.

[b] T-6ß-OH, 6β-testosterone hydroxylase; LE, low expresser; HE, high expresser.

[c] Summary statistics.

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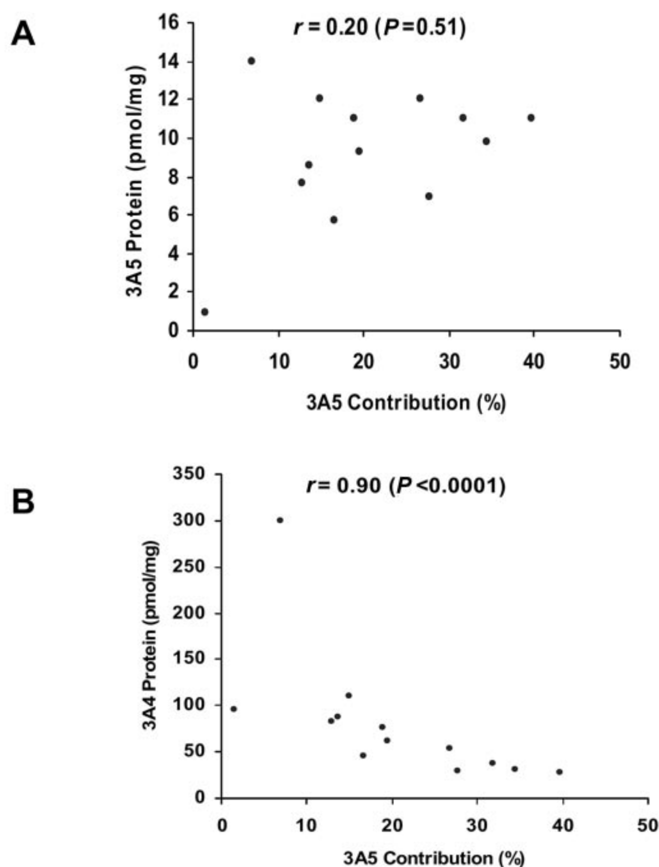

Fig. 2. Correlation between relative contribution of CYP3A5 to the 13-O-demethylation of tacrolimus and CYP3A5 protein (*A*) and CYP3A4 protein (*B*) in the liver bank samples (n = 13).

The 2 livers with no CYP3A4 protein data available were excluded from the analysis. The relative contribution was calculated based on the hepatic abundance approach as described in the *Materials and Methods*. The tacrolimus concentration was 5 μmol/L.

CYP3A4 might be involved in the 13-O-demethylation of tacrolimus *(6)*. More recently, Bader et al. *(25)* established that tacrolimus is indeed metabolized by CYP3A5, but no detailed kinetic data were presented.

Using cDNA-expressed P450 enzymes, we could show that tacrolimus is indeed metabolized predominantly by members of the CYP3A subfamily to 13-*O*-demethyltacrolimus. The metabolites 13,15-*O*-demethyltacrolimus and 13,31-*O*-demethyltacrolimus were also detected, but their concentrations were 10- to 20-fold lower than the corresponding concentrations of 13-*O*-demethyltacrolimus. Recombinant CYP3A5 metabolized tacrolimus with an affinity comparable to that of CYP3A4 but with a catalytic efficiency (V_{max}/K_m) that was 64% higher than that of CYP3A4. This further supports the growing recognition that the specific activities of CYP3A4 and CYP3A5 are comparable for certain substrates, including ethylmorphine, lidocaine, testosterone, and alfentanil *(36–39)*. In contrast, the metabolism of tacrolimus by CYP3A7 was characterized by a much lower substrate affinity and reaction velocity and a catalytic efficiency that was only 29% of that observed with CYP3A4. Contrary to initial observations, CYP3A7 is produced polymorphically in livers and small intestines of ~15% of adult Caucasians *(13, 40)*. The low affinity and capacity of CYP3A7 toward tacrolimus suggest that this P450 will, however, play no role in tacrolimus metabolism in vivo.

The K_m values observed for recombinant CYP3A4 (1.5 μmol/L) and CYP3A5 (1.4 μmol/L) were similar to the K_m values obtained for the transformation of tacrolimus to 13-*O*-demethyltacrolimus in a bank of 15 HLMs (median, 0.74 μmol/L; range, 0.38–1.20 μmol/L). Using the kinetic constants derived from the recombinant enzyme experiments and the concentrations of CYP3A4 and CYP3A5 protein in the respective microsomes, we were able to estimate the contribution of CYP3A5 to the transformation of tacrolimus in each microsomal preparation. The contribution of CYP3A5 to the 13-O-demethylation of tacrolimus in HLMs varied from 1.5% to 40% and was particularly strong in livers with low CYP3A4, whereas it was lower in those with high CYP3A4 and in the 3 CYP3A5 low expressers. These findings support the observations that CYP3A5 is an important source of interindividual variability for CYP3A when CYP3A5 content represents a significant fraction of the total hepatic CYP3A pool *(36, 38, 41)*. Conversely, the share of CYP3A5 in tacrolimus metabolism undergoes "dilution" in individuals with high concentrations of CYP3A4. In other words, the relative contribution of CYP3A5 strongly depends on the concomitant amount of CYP3A4. The predominant role of CYP3A isoforms in the 13-O-demethylation of tacrolimus is in agreement with the good correlation observed between tacrolimus 13-O-demethylation activity and 6β-hydroxylation of testosterone in the liver samples investigated. The latter is widely used as a marker of CYP3A activity *(36, 42)*.

In our hands, the mean predicted tacrolimus pharmacokinetic clearances in CYP3A5 low expressers (3.57 $mL \cdot min^{-1} \cdot kg^{-1}$) and CYP3A5 high expressers (8.60 $mL \cdot min^{-1} \cdot kg^{-1}$) were in general in the same range as in vivo (0.68–6 $mL \cdot min^{-1} \cdot kg^{-1}$) *(2)*. Clearance values >6 $mL \cdot min^{-1} \cdot kg^{-1}$ observed in some liver samples may have been caused by high CYP3A concentrations.

Taken together, our results explain, at least in part, the recently reported positive association between *CYP3A5* genotype and tacrolimus dosage *(14–21)*. Furthermore, our data offer an explanation for the substantial variability in tacrolimus dosage independent of *CYP3A5* genotype, i.e., that observed within the groups of high and low CYP3A5 expressers *(14)*. It is likely that part of this variability is brought about by genotype-independent differences in CYP3A5 concentrations. Indeed, CYP3A5 concentrations varied 2-fold (range, 6.9–14 pmol/mg) among the 12 high-expresser livers. We speculate that an even bigger variability in tacrolimus maintenance dose is caused by differences in CYP3A4 concentrations. For comparison, the expression of this CYP3A isoenzyme varied 11-fold (27–300 pmol/mg) within the same set of

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12 samples. Further variability may be attributable to differential inhibition or induction by exogenous substances *(24, 43, 44)* and by the expression of CYP3A5 and CYP3A4 in the small intestine *(45)*. This is particularly important for corticosteroids because these compounds are often used concomitantly with tacrolimus after organ transplantation and they are substrates, inducers, and inhibitors of CYP3A *(2)*.

In addition to CYP3A, tacrolimus is a substrate for the P-glycoprotein encoded by the *MDR1* gene, and the trough concentration of tacrolimus is indeed affected by the intestinal production of MDR-1 during the first week after transplantation. Subsequently, it is mostly hepatic metabolism that contributes to tacrolimus clearance *(46)*. A polymorphism in the *MDR1* gene has been associated with tacrolimus clearance *(15, 18)*, although these findings have not been confirmed by other investigators *(14, 16, 45)*. Interestingly, *MDR1* and *CYP3A5* (as well as *CYP3A4*) share transcriptional activators such as pregnane X receptor *(24, 47)*. This latter mechanism may explain the intriguing findings by Goto et al. *(46)*, who described an association between an *MDR1* polymorphism and CYP3A4 concentrations.

Tacrolimus emerges as another clinically relevant substrate of CYP3A5. Previously, Katz et al. *(48)* found that in vivo drug disposition of ABT-773, a ketolide antibiotic that is a substrate for both CYP3A and P-glycoprotein, depends on the *CYP3A5*, but not the *MDR1* genotype. CYP3A5 expressers had higher metabolism of lovastatin, simvastatin, and atorvastatin *(49)*, whereas no association was found between the polymorphism and efficacy of statins that are not metabolized by CYP3A5 (fluvastatin and pravastatin).

What are the implications of our findings for the currently discussed *(22)* prospect of *CYP3A5* genotyping in patients treated with tacrolimus? Certainly, *CYP3A5* genotyping will not eliminate the need to monitor blood tacrolimus concentrations because CYP3A4 also contributes to its metabolism, and production of both CYP3A4 and CYP3A5 *(24)* undergoes strong modulation by nongenetic (environmental) factors. However, a *CYP3A5* genotype–based adjusted initial dosing regimen for tacrolimus may allow more rapid and efficient attainment of therapeutic blood concentrations in the early postoperative period. Indeed, CYP3A5 high expressers had lower mean tacrolimus concentrations during the first week after transplantation, and rejection occurred earlier in these patients *(23)*. Prospective studies are now needed to validate this hypothesis.

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