

# Role of intestinal P-glycoprotein (*mdr1*) in interpatient variation in the oral bioavailability of cyclosporine

Interpatient differences in the oral clearance of cyclosporine (INN, ciclosporin) have been partially attributed to variation in the activity of a single liver enzyme termed CYP3A4. Recently it has been shown that small bowel also contains CYP3A4, as well as P-glycoprotein, a protein able to transport cyclosporine. To assess the importance of these intestinal proteins, the oral pharmacokinetics of cyclosporine were measured in 25 kidney transplant recipients who each had their liver CYP3A4 activity quantitated by the intravenous [ $^{14}\text{C}$ -*N*-methyl]-erythromycin breath test and who underwent small bowel biopsy for measurement of CYP3A4 and P-glycoprotein. Forward multiple regression revealed that 56% (i.e.,  $r^2 = 0.56$ ) and 17% of the variability in apparent oral clearance [ $\log(\text{dose}/\text{area under the curve})$ ] were accounted for by variation in liver CYP3A4 activity ( $p < 0.0001$ ) and intestinal P-glycoprotein concentration ( $p = 0.0059$ ), respectively. For peak blood concentration, liver CYP3A4 activity accounted for 32% ( $p = 0.0002$ ) and P-glycoprotein accounted for an additional 30% ( $p = 0.0024$ ) of the variability. Intestinal levels of CYP3A4, which varied tenfold, did not appear to influence any cyclosporine pharmacokinetic parameter examined. We conclude that intestinal P-glycoprotein plays a significant role in the first-pass elimination of cyclosporine, presumably by being a rate-limiting step in absorption. Drug interactions with cyclosporine previously ascribed to intestinal CYP3A4 may instead be mediated by interactions with intestinal P-glycoprotein. (Clin Pharmacol Ther 1997;62:248-60.)

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Cyclosporine (INN, ciclosporin) is the primary immunosuppressant used in solid organ transplantation. Its use has greatly improved allograft survival by decreasing the frequency of both acute T-cell-mediated cellular rejection and chronic rejection.<sup>1-3</sup> However, its clinical use is complicated by its narrow therapeutic index; the daily dose of cyclosporine required to suppress organ rejection in some patients can cause renal and neurologic toxicity in others. The clinical difficulties presented by this narrow therapeutic index are compounded by the existence of large interpatient differences in the oral bioavailability of cyclosporine.<sup>4,5</sup> This is reflected in

the observation that the daily dose of cyclosporine required to suppress rejection while avoiding toxicity varies at least tenfold among transplant recipients.<sup>6</sup>

Recently considerable insight has been obtained regarding the mechanisms that may underlie the marked variability in the oral pharmacokinetics of cyclosporine. The enzyme principally responsible for cyclosporine metabolism is a cytochrome P450 termed CYP3A4.<sup>7-9</sup> This is the major cytochrome P450 enzyme present in the liver.<sup>10</sup> There are marked interindividual differences in the concentration and activity of hepatic CYP3A4.<sup>10-13</sup> Thummel et al.<sup>14</sup> showed that variation in liver content of CYP3A4 in liver transplant recipients accounted for 66% of the interpatient variation in the clearance of intravenously administered cyclosporine.

In contrast, variation in liver CYP3A4 activity does not appear to account for a majority of the interpatient variability in pharmacokinetics when cyclosporine is administered orally. In a previous study we showed that variation in liver CYP3A4 activity, as measured by the intravenous <sup>14</sup>C-erythromycin breath test (ERMBT),<sup>15</sup> predicted only about one third of the interpatient variability in the oral clearance (CL/F) of cyclosporine in kidney transplant patients.<sup>16</sup> The basis for the remaining variation in CL/F is unknown. However, one possible explanation may be related to the abundant expression of CYP3A4 in small bowel epithelial cells (enterocytes).<sup>17,18</sup> Several studies have indicated that intestinal CYP3A4 is responsible for significant first-pass metabolism of orally administered cyclosporine.<sup>19-22</sup>

We have found that the enterocyte content of CYP3A4 protein correlates with its catalytic activity (midazolam 1'-hydroxylation) and varies up to tenfold among patients.<sup>13</sup> Furthermore, intestinal and liver CYP3A4 expression do not appear to be coordinately regulated.<sup>13</sup> Hence it is possible for an individual to have relatively high liver CYP3A4 activity while having relatively low intestinal CYP3A4 activity or to have relatively low liver CYP3A4 activity while having relatively high intestinal CYP3A4 activity. If the extent of intestinal first-pass metabolism is proportional to the intestinal content of CYP3A4, it would seem to be likely that variation in intestinal CYP3A4 activity could account for a portion of the unexplained variation in the oral pharmacokinetics of cyclosporine.

Another potential source of variation in oral cyclosporine pharmacokinetics may be related to the expression of P-glycoprotein, the *mdr1* gene prod-

uct,<sup>23</sup> in the intestine. P-glycoprotein is a versatile transporter that is able to pump a wide variety of xenobiotics including cyclosporine.<sup>24-28</sup> In the intestine, P-glycoprotein is located almost exclusively within the brush border on the apical (luminal) surface of the enterocyte where it pumps xenobiotics from the cytoplasm to the exterior of the cell (i.e., from the enterocyte back into the intestinal lumen).<sup>29-32</sup> We recently showed that, as with CYP3A4, there is significant interindividual variation in the intestinal expression of P-glycoprotein.<sup>33</sup> Given its transport function, high intestinal levels of P-glycoprotein may interfere with drug absorption and contribute to the variation in cyclosporine oral pharmacokinetics.

To directly test the hypothesis that the levels of intestinal CYP3A4 and P-glycoprotein expression are important determinants of oral cyclosporine bioavailability, we measured the pharmacokinetics of orally administered cyclosporine in stable kidney transplant recipients. Each patient had his or her liver CYP3A4 activity measured with the ERMBT and underwent endoscopy to obtain small bowel biopsy specimens for determination of the enterocyte content of CYP3A4 and P-glycoprotein.

## METHODS

### Subjects

Statistical power analysis indicated that 20 patients at steady state would be needed to provide an 85% chance of detecting a significant correlation between cyclosporine pharmacokinetics and intestinal CYP3A4 or P-glycoprotein, if the true respective correlation coefficients were 0.6 or greater. In our previous study of cyclosporine pharmacokinetics,<sup>16</sup> 20% (4 of 20) of the subjects entered in the study were not at steady state, when steady state was defined as having initial and 24-hour cyclosporine (trough) blood levels that differed by less than 25%. For our current study we therefore recruited 25 subjects from among the stable kidney transplant recipients monitored in the University of Michigan Medical Center nephrology clinic.

To be included in the study, patients had to be at least 18 years old and be considered medically stable. Transplantation must have occurred at least 6 months before the study. In addition, each patient had to be taking cyclosporine as a single daily dose and, to increase the likelihood of recruiting patients at steady state, must not have had an adjustment in cyclosporine dose within 30 days of study participation. All patients were receiving the Sandimmune

**Table I. Patient characteristics**

Patient No.	Kidney disease	Age	Sex	Cyclosporine dose (mg/kg/day)	ERMBT (% <sup>14</sup> C/hr)	Enterocyte CYP3A4*	Enterocyte P-glycoprotein*
1	Diabetes	67	Male	2.58	2.77	90 ± 27	89 ± 35
2	Immunoglobulin A nephropathy	34	Male	7.85	2.64	92 ± 37	131 ± 42
3	Diabetes	58	Male	2.67	1.69	96 ± 26	153 ± 10
4	Chronic pyelonephritis	36	Male	3.18	3.80	87 ± 22	31 ± 10
5	Chronic pyelonephritis	38	Female	5.98	3.00	58 ± 13	81 ± 23
6	Lupus	38	Female	6.04	2.78	162 ± 25	263 ± 75
7†	Diabetes	64	Male	6.22	4.39	94 ± 18	100 ± 28
8	Chronic glomerulonephritis	30	Male	5.35	2.67	104 ± 31	103 ± 33
9	Unknown	54	Male	3.48	3.08	66 ± 11	40 ± 13
10‡	Membranoproliferative glomerulonephritis	64	Male	4.34	3.36	89 ± 15	45 ± 7
11	Lupus	21	Female	5.73	4.26	69 ± 17	63 ± 14
12	Chronic glomerulonephritis	24	Female	6.28	2.59	145 ± 24	88 ± 18
13	Polycystic kidney disease	60	Male	4.38	2.78	101 ± 25	94 ± 29
14	Chronic pyelonephritis	42	Male	4.79	2.46	90 ± 36	117 ± 23
15‡	Obstruction	24	Male	4.48	1.68	127 ± 23	72 ± 13
16	Amyloidosis	45	Male	4.83	2.64	151 ± 42	114 ± 20
17‡	Polycystic kidney disease	63	Male	2.10	1.93	78 ± 23	47 ± 20
18	Diabetes	34	Female	6.09	3.39	37 ± 13	55 ± 11
19	Chronic pyelonephritis	47	Male	4.06	1.68	32 ± 13	64 ± 22
20	Polycystic kidney disease	64	Male	2.35	1.66	302 ± 123	70 ± 6
21	Chronic glomerulonephritis	45	Male	5.24	3.04	72 ± 26	43 ± 12
22	Diabetes	48	Male	4.62	2.25	70 ± 15	57 ± 14
23‡	Chronic pyelonephritis	46	Female	3.55	4.72	111 ± 42	65 ± 26
24‡	Diabetes	38	Female	2.49	2.08	119 ± 20	48 ± 8
25	Chronic pyelonephritis	23	Male	7.34	2.50	56 ± 14	117 ± 14
Mean (all patients, <i>n</i> = 24)		43 ± 13	—	4.57 ± 1.59	2.73 ± 0.79	100 ± 54	85 ± 50
Mean (patients analyzed, <i>n</i> = 19)		43 ± 13	—	4.89 ± 1.57	2.72 ± 0.66	99 ± 60	93 ± 53

Data are mean values ± SD.

\*Enterocyte CYP3A4 and P-glycoprotein are expressed as the ratio of the computer-determined densitometric value of the protein of interest relative to villin (see Methods).

†Patient excluded from study because he was taking phenytoin, a known potent CYP3A4 inducer.

‡Patients excluded from analysis because they were not at steady state.

(Sandoz Pharmaceuticals, East Hanover, N.J.) formulation of cyclosporine. At the time this study was performed, the microemulsion formulation of cyclosporine, Neoral, was not in use at our institution. Patients were also excluded from consideration if they were pregnant, were allergic to erythromycin, had active peptic ulcer disease, had an episode of rejection within 30 days of participation in the study, or were taking medications known to be potent inducers or inhibitors of CYP3A4 activity. No consideration was given to the primary kidney disease, race, or gender of the patients.

A summary of patient characteristics is shown in Table I. Three of the patients (patients 6, 20, and 21) had participated in our earlier study.<sup>16</sup> Most of the patients were receiving prednisone, azathioprine, and cyclosporine for immunosuppression.

Many were also receiving multiple concomitant medications (medication information is available from the authors on request). Written informed consent was obtained from all subjects. This study was approved by the Institutional Review Board of the University of Michigan.

### Experimental design

Patients were admitted to the University of Michigan General Clinical Research Center (GCRC) for the duration of their 3-day involvement in the study. To maintain individuals at steady state, every effort was made to continue patients on their usual diets. The GCRC dietary staff planned menus to match the subjects' usual dietary content of fat, protein, and carbohydrate determined from computer analysis (Food Processor II program, Esha Research,

Salem, Ore.) of detailed diet diaries filled out by each subject for the 3 days before admission.

Patients were admitted to the GCRC the evening before the start of the study. The following morning the subjects took their routine dose of cyclosporine in their usual manner (i.e., at the same time of day, with the same liquid, and in the same relationship to breakfast). Blood samples (10 ml) were obtained at 0, 1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 5, 6, 8, 10, 12, 18, and 24 hours after cyclosporine administration and were frozen. After completion of the study, the samples were thawed and the whole blood was analyzed for parent cyclosporine by HPLC.<sup>21</sup>

Immediately after the 24-hour blood sample was collected, each subject was given the ERMBT. After finishing the breath test, each subject again took his or her usual cyclosporine dose in his or her customary manner and ate breakfast; thereafter, no additional oral intake was allowed. Four hours after breakfast, each subject underwent upper endoscopy to obtain small bowel biopsy specimens for determination of intestinal CYP3A4 and P-glycoprotein content. After they completely recovered from the sedation for the endoscopy, patients were discharged from the GCRC.

#### Pharmacokinetic analysis

Subjects whose 0-hour and 24-hour cyclosporine levels varied by more than 25% were not considered to be at steady state and were excluded from analysis. The total area under the observed blood concentration–time curve (AUC) was calculated by use of the linear and logarithmic trapezoidal method in the rising and declining phases of cyclosporine concentrations, respectively. The apparent CL/F was calculated by dividing the cyclosporine dose by the AUC. The drug half-life ( $t_{1/2}$ ) was determined by the slope of the log-linear terminal phase of the curve. The peak blood concentration ( $C_{max}$ ), time to peak ( $t_{max}$ ), and trough blood level ( $C_{min}$ ) were determined directly from the blood concentration versus time data.

We have shown previously that the ERMBT result best correlates with the body weight–corrected pharmacokinetic parameters of a drug.<sup>13</sup> All pharmacokinetic parameters were therefore normalized to the dose of cyclosporine, expressed in milligrams per kilogram.

#### Erythromycin breath test

The *in vivo* catalytic activity of hepatic CYP3A4 can be conveniently and noninvasively estimated as

the rate of  $^{14}\text{CO}_2$  exhaled after an intravenous test dose of [ $^{14}\text{C}$ -*N*-methyl]-erythromycin. This test is based on the observation that CYP3A4 exclusively catalyzes the *N*-demethylation of erythromycin in liver microsomes<sup>34</sup> and that the carbon atom in the resulting formaldehyde should largely appear in the breath as carbon dioxide.<sup>35</sup> We have shown that the ERMBT result correlates well with the liver content of CYP3A4 in patients undergoing liver transplant and does not at all correlate with the liver content of five other major liver P450s.<sup>36</sup>

The ERMBT was administered as described previously.<sup>34</sup> Breath test results were expressed as the percentage of administered  $^{14}\text{C}$  that was exhaled during the first hour after the injection of erythromycin<sup>34</sup> estimated from the rate of radiolabel exhalation at 20 minutes, as described previously.<sup>37</sup>

#### Intestinal CYP3A4 and P-glycoprotein analysis

**Endoscopy.** Each patient fasted for 4 hours after breakfast and then underwent upper intestinal endoscopy (5 hours after the ERMBT). Patients were sedated with intravenous midazolam (Roche Pharmaceuticals, Nutley, N.J.) and meperidine (INN, pethidine) (Sanofi Winthrop Pharmaceuticals, New York, N.Y.), and a fiberoptic endoscope was passed into the small intestine (distal duodenum) where five mucosal biopsy specimens (approximately 5 mg wet weight each) were obtained from the second portion of the duodenum. The biopsy specimens were placed directly in ice cold solution D [0.05 mol/L Tris hydrochloride, 20% glycerol, 2 mmol/L ethylenediaminetetraacetic acid, and 1 mmol/L phenylmethylsulfonyl fluoride<sup>38</sup>], immediately homogenized in a glass tissue grinder, and snap frozen in liquid nitrogen. Samples were stored at  $-80^\circ\text{C}$  until analyzed.

**CYP3A4 and P-glycoprotein immunoblotting.** Immunoblots were performed as described previously with minor modifications.<sup>13</sup> Fifteen micrograms of whole biopsy homogenate was electrophoresed and transferred to nitrocellulose. We have found that, compared with whole homogenate, more than 80% of CYP3A4 immunoreactive protein was lost during preparation of the S9 fraction used in our previous study (Lown et al.,<sup>13</sup> and data not shown). In addition, use of whole homogenate allowed us to sequentially probe a single blot for CYP3A4 (a microsomal protein), P-glycoprotein (a membrane bound protein), and villin (a cytosolic protein).

The blots were sequentially incubated with 13-7-10, a mouse monoclonal antibody specific for human

CYP3A proteins<sup>17,39,40</sup>; a mouse monoclonal antibody raised against chick villin that crossreacts with human villin (Chemicon International, Temecula, Calif.); and MDR-Ab1, a rabbit polyclonal antibody to human P-glycoprotein (Oncogene Science, Uniondale, N.Y.).

The 13-7-10 antibody reacts with all CYP3A proteins. However, because CYP3A4 is the major CYP3A protein expressed in the small intestine,<sup>13,18</sup> the amount of antibody bound on the immunoblots will primarily reflect CYP3A4 protein levels. Essentially no *mdr2* is expressed in human small intestine,<sup>33,41,42</sup> so protein detected by the MDR-Ab1 antibody on the immunoblots corresponds to the *mdr1* gene product, P-glycoprotein.

The blots were developed with a chemiluminescence kit (Amersham, Arlington Heights, Ill.) and exposed to Hyperfilm ECL (Amersham) for different durations to obtain appropriate exposures for CYP3A4 (approximately 3 seconds), villin (approximately 15 seconds), and P-glycoprotein (approximately 3 minutes).

Immunoblot protein concentrations were determined by computer-aided densitometry. Optical densities were performed on a Macintosh computer (Apple Computer, Inc., Cupertino, Calif.) with use of the public domain program NIH Image (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). Individual exposures were scanned into binary images with a Scan Jet IIC color scanner (Hewlett-Packard, Greeley, Colo.). Optical densities were converted to quantitative numbers by comparison with slot blots of serial dilutions of purified CYP3A4 protein that had been processed simultaneously with the immunoblots and exposed on the same films and for the same duration as the protein being quantitated. This allowed for correction of the nonlinearity of ECL light output and ECL Hyperfilm.

**Correction for interbiopsy variation of enterocyte content.** CYP3A4 and P-glycoprotein are expressed exclusively in mature enterocytes in the intestine.<sup>17,18,29</sup> These enterocytes represent only a small fraction of the cells obtained in an intestinal biopsy specimen. The content of mature enterocytes varies widely between biopsy specimens, even among biopsy specimens obtained from a single individual.<sup>13</sup> For example, a deep mucosal biopsy specimen would contain a relatively low proportion of enterocytes per milligram of biopsy homogenate and after blotting would appear to have a lower concentration of P-glycoprotein and CYP3A4 protein per milli-

gram of protein than a shallow biopsy specimen obtained from the same individual.<sup>13</sup>

We have found that the enterocyte content of villin, a constitutively expressed, enterocyte-specific protein,<sup>43</sup> may be used to control for the variation in biopsy content of mature enterocytes.<sup>13,44</sup> Therefore enterocyte levels of CYP3A4 and P-glycoprotein were expressed as a ratio with the villin content of the same sample (i.e., CYP3A4/villin and P-glycoprotein/villin). These villin-corrected values provide a relative measure of enterocyte concentration and, for the sake of simplicity, have been termed *enterocyte concentration* in the text.

Immunoblots of P-glycoprotein and CYP3A4 were repeated a total of four and seven times, respectively. Final values were calculated from the arithmetic mean of all of the runs, with any outlying points (greater than 2 standard deviations from the mean for that sample) omitted. Only seven of the 140 CYP3A4 measurements and five of the 80 P-glycoprotein measurements were discarded.

#### **Additional laboratory studies**

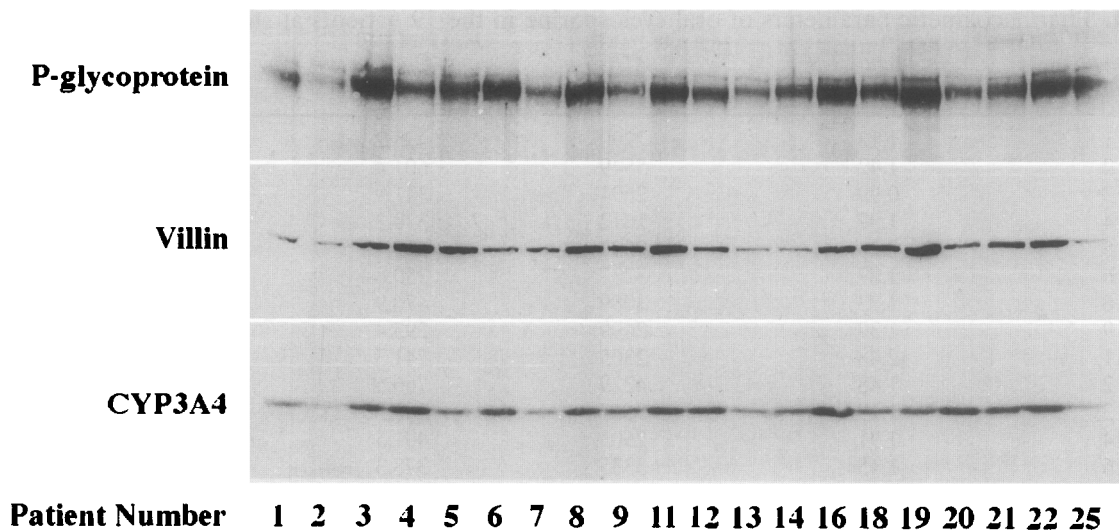
Blood hematocrit, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, total cholesterol, and albumin were determined from blood drawn at the time of the patient's admission to the GCRC.

#### **Statistical analysis**

Statistical analysis of the data was performed with use of StatView 4.5 (Abacus Concepts Inc., Berkeley, Calif.) and SAS (SAS Institute Inc., Cary, N.C.). All values are expressed as mean  $\pm$  SD. Values for cyclosporine clearance,  $C_{max}$ ,  $C_{min}$ , and dose were log-transformed to normalize their distribution for statistical analysis. Values for CYP3A4/villin and P-glycoprotein/villin were log-transformed to normalize their variances. It should be noted that use of nontransformed data in our analyses did not alter any of our findings; all *p* values remained significant. Independent variables that significantly influenced pharmacokinetic parameters were identified by use of linear regression with forward variable selection. Comparisons of means were determined with use of two-tailed *t* tests. Results were considered to be significant when *p* < 0.05.

#### **RESULTS**

All 25 of the stable kidney transplant patients enrolled in the study completed the protocol. Data



**Fig. 1.** Representative immunoblot of intestinal biopsy protein from the 19 subjects included in the analysis and the patient excluded because of treatment with phenytoin (subject 7, Table I). After sequential incubation with the three primary antibodies (see Methods), the blot was exposed to ECL Hyperfilm for different durations to obtain appropriate exposure for CYP3A4 (3 seconds), villin (15 seconds), and P-glycoprotein (3 minutes). Protein levels were quantitated by comparison to serial dilutions of purified CYP3A4 developed simultaneously and exposed for identical time intervals.

on these patients are summarized in Table I. After the completion of the study, we discovered that one of the patients (patient 7) had been taking phenytoin, a potent inducer of liver CYP3A4.<sup>45</sup> This patient therefore did not meet our original entry criteria, so he was excluded from all analyses. However, inclusion or exclusion of this patient did not significantly alter any of the observations made from the data set (not shown). Of the remaining 24 patients, five patients had 0-hour and 24-hour blood concentrations (trough levels) that differed by more than 25% (range, 29% to 683%). These patients did not meet our predetermined definition of steady state (which would cause their cyclosporine pharmacokinetic parameters to be inaccurate) and were therefore excluded from the pharmacokinetic analyses.

The enterocyte concentration of CYP3A4 (expressed as the CYP3A4 to villin ratio) varied almost tenfold among both the 24 subjects who met the study entrance criteria and the final 19 patients (Table I and Fig. 1). There was no significant correlation between the levels of enterocyte CYP3A4 concentration and liver CYP3A4 activity as measured by the ERMBT ( $r = 0.17$ ,  $p = 0.43$ ), confirming our previous observation that liver and intestinal

CYP3A4 are not coordinately expressed.<sup>13</sup> Enterocyte CYP3A4 levels were also not significantly correlated with age ( $p = 0.44$ ) and were similar among men and women (mean, 100.6 and 94.2, respectively).

There was also substantial interpatient variation in intestinal P-glycoprotein expression. The enterocyte concentration of P-glycoprotein (P-glycoprotein/villin ratio) was found to vary more than eightfold among both the 24 subjects who met the study entrance criteria and the final 19 patients (Table I and Fig. 1). There was no significant correlation between the level of enterocyte concentration of P-glycoprotein and either intestinal or liver CYP3A4 levels ( $r = 0.30$ ,  $p = 0.15$  and  $r = -0.26$ ,  $p = 0.23$ , respectively). Enterocyte P-glycoprotein levels were also not significantly correlated with age ( $p = 0.67$ ) and were similar between men and women (mean, 87.4 and 109.9, respectively).

There were large (fourfold to sevenfold) differences in the AUC,  $C_{max}$ ,  $t_{max}$ , and CL/F of cyclosporine among the 19 patients at steady state. The values of these pharmacokinetic parameters are summarized in Table II. We found a highly significant correlation between the log-transformed apparent CL/F of cyclosporine [ $\log(\text{dose}/\text{AUC})$ , in li-

**Table II.** Pharmacokinetic parameters of oral cyclosporine in the 19 patients at steady state

Patient No.	Apparent CL/F (L/hr/kg)	AUC ( $\mu\text{g} \cdot \text{hr/L}$ )	$C_{\text{max}}$ (ng/ml)	$C_{\text{min}}$ (ng/ml)	$t_{\text{max}}$ (hr)
1	1.13	2290	475.2	36.7	2
2	1.42	5549	601.4	105.8	3.5
3	0.99	2691	341.9	63.0	5
4	1.97	1612	339.3	32.6	1.5
5	2.36	2537	355.4	49.8	6
6	1.87	3237	350.8	67.0	5
8	1.73	3089	476.9	52.5	2
9	1.39	2499	293.4	52.2	6
11	2.48	2305	241.5	37.9	12
12	1.48	4229	766.6	50.3	1.5
13	1.19	3662	492.3	75.7	5
14	1.91	2503	401.5	44.2	5
16	1.45	3335	378.3	59.6	5
18	1.26	4826	1055.6	64.9	1
19	0.57	7186	849.6	64.4	2
20	0.64	3706	550.9	57.9	2
21	1.27	4117	624.4	52.8	4
22	1.16	3996	655.6	68.4	1.5
25	1.45	5059	704.2	52.2	5
Mean	1.46	3602	523.9	57.3	3.9
SD	0.51	1360	212.7	16.4	2.6

CL/F, Apparent oral clearance, dose/AUC; AUC, area under the blood concentration-time curve;  $C_{\text{max}}$ , peak blood concentration;  $C_{\text{min}}$ , trough blood level;  $t_{\text{max}}$ , time to reach  $C_{\text{max}}$ .

ters per hour per kilogram] and the erythromycin breath test result in the expected direction ( $r = 0.75$ ,  $p = 0.0003$ ). That is, patients with the highest liver CYP3A4 activity, as measured by the erythromycin breath test, tended to have the highest apparent CL/F values. Interpatient variation in the erythromycin breath test accounted for 56% (i.e.,  $r^2 = 0.56$ ) of the variability in CL/F. Age and total cholesterol were also significantly correlated with the log of cyclosporine CL/F ( $r = -0.63$ ,  $p = 0.0042$  and  $r = -0.48$ ,  $p = 0.038$ , respectively). No significant correlation was observed between cyclosporine CL/F and any of the other variables examined (intestinal CYP3A4 or P-glycoprotein, blood hematocrit, serum creatinine, AST, ALT, alkaline phosphatase, total bilirubin, and albumin).

A stepwise forward regression analysis was performed with the log-transformed cyclosporine CL/F as the dependent variable and the erythromycin breath test result, log of the intestinal biopsy concentrations of CYP3A4 and P-glycoprotein, blood hematocrit, serum creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total cholesterol, and age as the independent variables. In the first step of the regression, as expected, the ERMBT result was selected as most predictive of variation in cyclospor-

ine CL/F. Once the ERMBT result was incorporated into the model, the enterocyte content of P-glycoprotein (log P-glycoprotein/villin) was selected as most predictive of the variation in CL/F. P-glycoprotein was positively correlated with CL/F, i.e. the higher the intestinal P-glycoprotein level, the higher the CL/F. The addition of intestinal P-glycoprotein concentration improved the predictiveness of the model from an  $r^2$  of 0.56 (for ERMBT values alone) to an  $r^2$  of 0.73. Variation in intestinal P-glycoprotein therefore accounted for approximately 17% of the observed variation in CL/F. Once the erythromycin breath test and enterocyte P-glycoprotein level were incorporated into the model, none of the other independent variables examined, including enterocyte CYP3A4 levels, were significantly predictive. In fact, the enterocyte CYP3A4 levels failed to approach significance in any step of the forward regression analysis. Both the ERMBT result ( $p < 0.0001$ ) and intestinal P-glycoprotein expression ( $p = 0.0059$ ) were highly significant in the final model that predicted CL/F ( $r = 0.85$ ;  $p < 0.0001$ ):

$$\log(\text{CL/F}) = 0.231 (\text{ERMBT}) + 0.337 (\log \text{P-glycoprotein/villin}) - 1.137$$

A graph showing the observed clearance values versus those predicted by the above model is shown in Fig. 2, A.

We next determined those variables that correlated with the  $C_{\max}$  corrected for the administered cyclosporine dose ( $C_{\max}/\text{dose}$ ). There was a significant inverse correlation between the log of  $C_{\max}/\text{dose}$  ratio and the ERMBT result in the expected direction ( $r = -0.56, p = 0.012$ ). Patients with the highest hepatic CYP3A4 activity values tended to have the lowest maximal levels of cyclosporine. Age and cholesterol also significantly correlated with log  $C_{\max}/\text{dose}$  levels ( $r = 0.56, p = 0.013$  and  $r = 0.50, p = 0.028$ , respectively). There was no significant correlation between  $C_{\max}/\text{dose}$  and any other variable examined, including enterocyte content of CYP3A4 and P-glycoprotein.

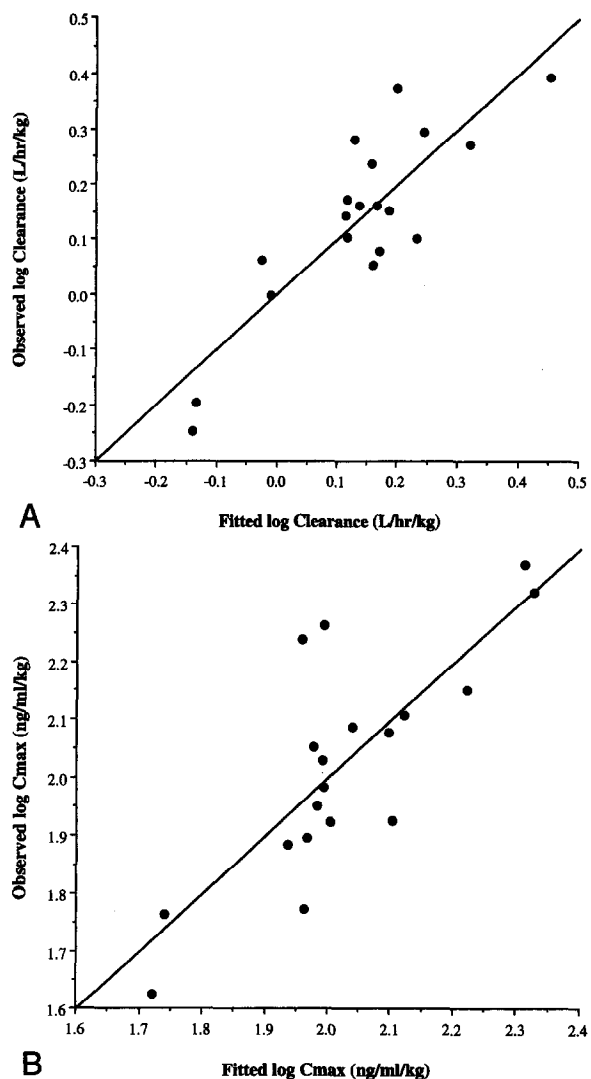
Stepwise forward regression analysis again selected the ERMBT result as the most significant independent predictor of peak cyclosporine levels. The next step of the regression incorporated the log of the enterocyte content of P-glycoprotein into the model. No other independent variables, including enterocyte CYP3A4 levels, were selected as significant in the regression analysis. The addition of enterocyte P-glycoprotein into the model accounted for an additional 30% of the variability in  $C_{\max}$  because the  $r^2$  of the model improved from 0.318 to 0.624. The ERMBT result ( $p = 0.0002$ ) and enterocyte P-glycoprotein expression ( $p = 0.0024$ ) were highly significant in the final model that predicted  $C_{\max}$  ( $r = 0.79, p = 0.0004$ ):

$$\log(C_{\max}/\text{dose}) = -0.236(\text{ERMBT}) - 0.533(\log \text{P-glycoprotein}) + 3.686$$

The relationship between the observed log  $C_{\max}/\text{dose}$  values versus those predicted by the above model is shown in Fig. 2, B.

When the dose adjusted cyclosporine trough values were analyzed, we found a significant inverse correlation between the log-transformed trough levels (log trough/dose) and the ERMBT result ( $r = -0.61, p = 0.0053$ ). Hence, as expected, the higher the ERMBT result was, the lower the cyclosporine trough level was for a given dose. Intestinal CYP3A4 and P-glycoprotein content did not significantly predict cyclosporine trough levels.

The  $t_{1/2}$  was not significantly correlated with the ERMBT result ( $r = 0.12, p = 0.64$ ), intestinal CYP3A4 levels ( $r = 0.21, p = 0.40$ ), or intestinal P-glycoprotein levels ( $r = 0.35, p = 0.14$ ). We also found no significant correlation between the time to



**Fig. 2.** Comparisons of the observed versus predicted values from the multiple regression equations for cyclosporine (INN, ciclosporin) clearance (A) and peak blood concentration ( $C_{\max}$ ) (B). Both regression models were derived from the erythromycin breath test results and intestinal P-glycoprotein measurements. See Results section for the specific predictive equations.

maximal blood cyclosporine level ( $t_{\max}$ ) and any of the variables examined.

## DISCUSSION

The results of this study confirm the important contribution of hepatic CYP3A4 to interpatient differences in cyclosporine pharmacokinetics. The ERMBT result was highly correlated with the apparent CL/F of cyclosporine and accounted for 56%



of the variation in CL/F we observed. The correlation with CL/F was in a positive direction, consistent with the expectation that patients with higher hepatic CYP3A4 activity values have greater rates of cyclosporine metabolism and hence higher cyclosporine clearance values. The ERMBT result was also highly correlated with the dose-adjusted  $C_{\max}$  and trough blood levels of cyclosporine, accounting for 32% and 37% of the variation in  $C_{\max}$  and trough level, respectively. In this case the correlations were inverse, as expected, given that higher levels of hepatic CYP3A4 should lead to lower blood levels.

The primary goal of this study was to determine whether interpatient differences in intestinal CYP3A4 or P-glycoprotein expression also contribute to variability in cyclosporine oral pharmacokinetics. Given the recent evidence indicating that a significant fraction of orally administered cyclosporine is metabolized in the intestine,<sup>19-22</sup> we had anticipated that variation in intestinal CYP3A4 would account for a significant portion of the variation in oral cyclosporine pharmacokinetics. However, we did not find this to be the case. It is possible that a true correlation exists with intestinal CYP3A4 that was not detected by our study. This could have occurred if the level of CYP3A4 we measured in the proximal small bowel did not correlate with the total mass of intestinal CYP3A4. It is possible that proximal intestinal CYP3A4 (and P-glycoprotein levels) do not correlate with levels in the distal intestine, where the majority of cyclosporine absorption occurs. Alternatively, it is possible that intestinal CYP3A4 activity might be incorporated into the ERMBT result, in which case the presence of the ERMBT in the regression model may have prevented the detection of a true correlation with intestinal CYP3A4 levels (i.e., the enterocyte CYP3A4 measurements would not have been an independent variable). This idea is supported by the recent observation that intravenously administered midazolam is significantly metabolized in the intestine.<sup>46</sup> However, we found no correlation between the ERMBT result and intestinal CYP3A4 levels. In addition, we have shown that there is an approximately 80% decrease in the ERMBT result during the anhepatic phase of a liver transplant operation,<sup>47</sup> suggesting that any intestinal contribution to the ERMBT result should be small. Finally, in a recent study similar in design to this one, we found that interindividual variation in  $C_{\max}$  observed after the oral administration of felodipine did correlate

significantly with the enterocyte concentration of CYP3A4 measured in small bowel biopsy specimens.<sup>44</sup>

The above considerations notwithstanding, our observation that the tenfold variation in enterocyte content of CYP3A4 had no clear effect on oral cyclosporine pharmacokinetics does not support our previous hypothesis that induction or inhibition of intestinal CYP3A4 largely accounts for drug interactions involving cyclosporine.<sup>18,19</sup> What did emerge from our analyses was a highly significant correlation between enterocyte P-glycoprotein content and cyclosporine oral pharmacokinetics in stepwise multiple regression analyses. There were no significant direct correlations between enterocyte P-glycoprotein levels and any of the cyclosporine pharmacokinetic parameters examined. However, this appeared to be the result of the large contribution of variation in hepatic CYP3A4 (as measured by the ERMBT). Enterocyte P-glycoprotein levels became highly significant in predicting variations in CL/F once variation in the ERMBT results had been taken into account in a stepwise forward regression analysis (Fig. 2, A). The correlation between P-glycoprotein levels and CL/F was in the expected direction: the higher the enterocyte P-glycoprotein content, the higher the apparent CL/F of cyclosporine. The final model that incorporated the ERMBT result and the intestinal biopsy measurements of P-glycoprotein levels accounted for 73% of the variability in CL/F observed in our stable kidney transplant population. Enterocyte levels of P-glycoprotein alone were able to account for 17% of dose-adjusted CL/F variation. Viewed another way, intestinal P-glycoprotein expression was able to account for almost 40% of the interpatient variation in CL/F not explained by variation in hepatic CYP3A4 expression.

The correlation with P-glycoprotein was even more substantial when we examined the relative contribution of hepatic and intestinal parameters to variation in cyclosporine  $C_{\max}$ . In a stepwise regression model, the ERMBT results were able to explain only 32% of the variation in  $C_{\max}$ , whereas the addition of enterocyte P-glycoprotein content increased the overall predictiveness of  $C_{\max}$  to 62% (Fig. 2, B). Hence, the contribution of P-glycoprotein expression to variation in  $C_{\max}$  (30%) was essentially equal to that of liver CYP3A4 (32%). The variation in enterocyte P-glycoprotein was able to explain 44% of the variability not attributable to hepatic CYP3A4. The greater dependence of  $C_{\max}$

than CL/F on P-glycoprotein is logical because  $C_{max}$  largely reflects the first-pass effects of the intestine and liver. Clearance is more dependent than  $C_{max}$  on the elimination of cyclosporine after it has entered the systemic circulation, a process less likely to reflect the activity of P-glycoprotein on the luminal surface of the intestine.

Enterocyte P-glycoprotein levels were not at all predictive of the observed trough blood levels of cyclosporine. This is also consistent with the effect of P-glycoprotein being primarily on first-pass parameters (i.e., most influencing  $C_{max}$ , with diminishing influence on blood concentrations thereafter).

The highly significant correlations we found between intestinal P-glycoprotein levels and cyclosporine pharmacokinetic parameters do not, in and of themselves, prove a causal relationship; other possible explanations will need to be examined in future studies. For example, P-glycoprotein is also present and has variable expression in the liver.<sup>48</sup> An important role for hepatic P-glycoprotein is supported by the observation that the administration of P-glycoprotein inhibitors alters the pharmacokinetics of intravenously administered P-glycoprotein substrates in patients.<sup>49-51</sup> If there is a strong correlation between intestinal and hepatic levels of P-glycoprotein in patients, then the potential contribution of liver P-glycoprotein to oral pharmacokinetics would have been incorporated into our intestinal P-glycoprotein measurements. However, the fact that intestinal P-glycoprotein levels correlated only with first-pass parameters makes a substantial liver component to our measurements less likely. In addition, there are recent animal data that offer direct evidence to support our conclusion that intestinal P-glycoprotein plays an important role in the oral absorption of P-glycoprotein substrates. Leu et al.<sup>52</sup> showed that inhibition of intestinal P-glycoprotein with C219 (a P-glycoprotein monoclonal antibody), 5'-adenylylimidodiphosphate (a non-hydrolyzable adenosine triphosphate analog), or quinidine (a competitive P-glycoprotein inhibitor) increased the absorption of etoposide from everted gut sacs in rats.

We presume that P-glycoprotein functions to keep cyclosporine out of the body by keeping it within the lumen of the small bowel. This explanation would be consistent with the long-held belief that poor and variable absorption of cyclosporine is a major reason for intersubject kinetic differences.<sup>6</sup> The idea that P-glycoprotein and not CYP3A4 is the major intestinal variable in determining oral cyclo-

sporine kinetics could account for the results of another recent study. Gomez et al.<sup>21</sup> noted that treatment with ketoconazole, a potent inhibitor of CYP3A4, resulted in a dramatic increase in the oral bioavailability of cyclosporine, estimated to exceed 65% in healthy volunteers<sup>21</sup> and 75% in kidney transplant recipients.<sup>22</sup> These investigators assumed that the only effect of ketoconazole was to inhibit CYP3A4 and concluded that what had been assumed to be poor absorption of the original commercial formulation of cyclosporine in fact reflected metabolism of cyclosporine in the intestinal wall. On the basis of the results of our current study, we expand the hypothesis for the effect of ketoconazole on improving the bioavailability of cyclosporine to include the inhibition of P-glycoprotein function. Ketoconazole has been shown to be a potent inhibitor of P-glycoprotein in a highly drug-resistant cancer cell line.<sup>53</sup>

One should not conclude from our study that intestinal metabolism of cyclosporine by CYP3A4 is insignificant. There is now considerable data to support the occurrence of substantial first-pass metabolism of cyclosporine in human intestine.<sup>19-22</sup> However, our data suggests that the extent of intestinal metabolism of cyclosporine does not depend primarily on the level of intestinal CYP3A4. Gan et al.<sup>54</sup> recently found evidence in cultures of the human intestinal cell line Caco-2 that P-glycoprotein may control the enterocyte "residence time" of cyclosporine and concluded that this might influence the extent of first-pass metabolism of cyclosporine in the intestine. Thus there may be complex interactions between P-glycoprotein and CYP3A4 in the intestine that were not evaluated in this study.

We were unable to account for approximately one fourth of the variation in CL/F of cyclosporine by the inclusion of the ERMBT result and enterocyte P-glycoprotein levels into the multiple regression model. It seems likely that other clinical variables, such as variation in gastric mixing and emptying, lipoproteins, cholesterol, age, general health status, and absorption of cyclosporine (independent of P-glycoprotein) may account for the remaining variability.

It should also be noted that we found no significant correlation between enterocyte P-glycoprotein and CYP3A4, suggesting that levels of these two proteins do not vary coordinately, as had been previously suggested in some cancers.<sup>55</sup>

The demonstration of the potential role of P-glycoprotein in determining the CL/F of cyclospor-

ine may have several important clinical implications. First, it seems reasonable to assume that inhibition of P-glycoprotein may improve the oral availability of cyclosporine. Moreover, this strategy holds the potential to reduce intersubject variability in cyclosporine clearance by removing the second most important variable (after liver CYP3A4 activity) from our predictive models. Second, it now seems to be possible that drug interactions involving cyclosporine previously ascribed exclusively to CYP3A4 inhibition (i.e., the elevation of cyclosporine blood levels observed when transplant patients are treated with imidazole antimycotic drugs or macrolide antibiotics) could, at least in part, result from inhibition of P-glycoprotein activity. MDR-1 messenger ribonucleic acid has also recently been shown to be inducible by some xenobiotics in a human intestinal cancer cell line.<sup>56</sup> If future studies show P-glycoprotein to be inducible in normal intestine as well, induction of P-glycoprotein could potentially account for cyclosporine drug interactions previously attributed to induction of intestinal or hepatic CYP3A4.

In summary, our data suggest that approximately three-fourths of interpatient variability in the CL/F of cyclosporine can be attributed to just two factors: variation in liver CYP3A4 activity, as measured by the ERMBT, and variation in expression of P-glycoprotein in small bowel enterocytes. Whether our findings are applicable to the oral pharmacokinetics of other P-glycoprotein substrates is an important question for future studies.

## References

- Pallardó LM, Sánchez P, Sánchez J, García J, Beneyto I, Orero E, et al. Analysis of the risk factors of late failure in renal transplantation under cyclosporine immunosuppression. *Transplant Proc* 1994;26:2536-7.
- Ponticelli C, Tarantino A, Montagnino G. Controlled trials with cyclosporine in kidney transplantation. *Transplant Proc* 1994;26:2490-2.
- Thiel G, Bock A, Spöndlin M, Brunner FP, Mihatsch M, Ruffli T, et al. Long-term benefits and risks of cyclosporin A (sandimmun)—an analysis at 10 years. *Transplant Proc* 1994;26:2493-8.
- Lindholm A, Henricsson S, Lind M, Dahlqvist R. Intraindividual variability in the relative systemic availability of cyclosporin after oral dosing. *Eur J Clin Pharmacol* 1988;34:461-4.
- Lemaire M, Fahr A, Maurer G. Pharmacokinetics of cyclosporine: inter- and intra-individual variations and metabolic pathways. *Transplant Proc* 1990;22:1110-2.
- Kahan BD. Cyclosporine. *N Engl J Med* 1989;321:1725-38.
- Kronbach T, Fischer V, Meyer UA. Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. *Clin Pharmacol Ther* 1988;43:630-5.
- Combalbert J, Fabre I, Fabre G, Dalet I, Derancourt J, Cano JP, et al. Metabolism of cyclosporin A; IV: purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab Dispos* 1989;17:197-207.
- Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fischer V, et al. Cytochrome P-450 hPCN3, a novel cytochrome P450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J Biol Chem* 1989;264:10388-95.
- Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270:414-23.
- Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* 1986;261:5051-60.
- Wrighton SA, Thomas PE, Willis P, Maines SL, Watkins PB, Levin W, et al. Purification of a human liver cytochrome P-450 immunochemically related to several cytochromes P-450 purified from untreated rats. *J Clin Invest* 1987;80:1017-22.
- Lown KS, Kolars JC, Thummel KE, Barnett JL, Kunze KL, Wrighton SA, et al. Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel: lack of prediction by the erythromycin breath test [published erratum appears in *Drug Metab Dispos* 1995;23:following table of contents]. *Drug Metab Dispos* 1994;22:947-55.
- Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, et al. Use of midazolam as a human cytochrome P450 3A probe; I: in vitro-in vivo correlations in liver transplant patients. *J Pharmacol Exp Ther* 1994;271:549-56.
- Watkins PB. Noninvasive tests of CYP3A enzymes. *Pharmacogenetics* 1994;4:171-84.
- Turgeon DK, Normolle DP, Leichtman AB, Annesley TM, Smith DE, Watkins PB. Erythromycin breath test

- predicts oral clearance of cyclosporine in kidney transplant recipients. *Clin Pharmacol Ther* 1992;52:471-8.
17. Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS. Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J Clin Invest* 1987;80:1029-36.
  18. Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450III<sub>A4</sub> (CYP3A<sub>4</sub>) in human small bowel enterocytes. *J Clin Invest* 1992;90:1871-8.
  19. Kolars JC, Awni WM, Merion RM, Watkins PB. First-pass metabolism of cyclosporin by the gut. *Lancet* 1991;338:1488-90.
  20. Hebert MF, Roberts JP, Prueksaritanont T, Benet LZ. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin Pharmacol Ther* 1992;52:453-7.
  21. Gomez DY, Wachter VJ, Tomlanovich SJ, Hebert MF, Benet LZ. The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. *Clin Pharmacol Ther* 1995;58:15-9.
  22. Wu CY, Benet LZ, Hebert MF, Gupta SK, Rowland M, Gomez DY, et al. Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporine. *Clin Pharmacol Ther* 1995;58:492-7.
  23. Chen C, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, et al. Internal duplication and homology with bacterial transport proteins in the *mdr1* (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* 1986;47:381-9.
  24. Roninson IB. From amplification to function: the case of the *MDR1* gene. *Mutat Res* 1992;276:151-61.
  25. Schinkel AH, Borst P. Multidrug resistance mediated by P-glycoproteins. *Sem Cancer Biol* 1991;2:213-26.
  26. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993;62:385-427.
  27. Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T. Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* 1993;268:6077-80.
  28. Tsuji A, Tamai I, Sakata A, Tenda Y, Terasaki T. Restricted transport of cyclosporin A across the blood-brain barrier by a multidrug transporter, P-glycoprotein. *Biochem Pharmacol* 1993;46:1096-9.
  29. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987;84:7735-8.
  30. Hsing S, Gatmaitan Z, Arias IM. The function of Gp170, the multidrug-resistance gene product, in the brush border of rat intestinal mucosa. *Gastroenterology* 1992;102:879-85.
  31. Penny JI, Campbell FC. Active transport of benzo[a]pyrene in apical membrane vesicles from normal human intestinal epithelium. *Biochim Biophys Acta* 1994;1226:232-6.
  32. Saitoh H, Aungst BJ. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine. *Pharm Res* 1995;12:1304-10.
  33. Lown KS, Fontana RJ, Schmiedlin-Ren P, Turgeon DK, Watkins PB. Interindividual variation in intestinal *mdr1*: lack of short term diet effects [abstract]. *Gastroenterology* 1995;108:A737.
  34. Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450: studies in rats and patients. *J Clin Invest* 1989;83:688-97.
  35. Baker AL, Kotake AN, Schoeller DA. Clinical utility of breath tests for the assessment of hepatic function. *Semin Liver Dis* 1983;3:318-29.
  36. Lown K, Kolars J, Turgeon K, Merion R, Wrighton SA, Watkins PB. The erythromycin breath test selectively measures P450III<sub>A</sub> in patients with severe liver disease. *Clin Pharmacol Ther* 1992;51:229-38.
  37. Turgeon DK, Leichtman AB, Lown KS, Normolle DP, Deeb GM, Merion RM, et al. P450 3A activity and cyclosporine dosing in kidney and heart transplant recipients. *Clin Pharmacol Ther* 1994;56:253-60.
  38. Bonkovsky HL, Hauri HP, Marti U, Gasser R, Meyer UA. Cytochrome P450 of small intestinal epithelial cells. Immunochemical characterization of the increase in cytochrome P450 caused by phenobarbital. *Gastroenterology* 1985;88:458-67.
  39. Beaune P, Kremers P, Letawe-Goujon F, Gielen JE. Monoclonal antibodies against human liver cytochrome P-450. *Biochem Pharmacol* 1985;34:3547-52.
  40. Wrighton SA, Brian WR, Sari MA, Iwasaki M, Guengerich FP, Raucy JL, et al. Studies on the expression and metabolic capabilities of human liver cytochrome P450III<sub>A5</sub> (HLp3). *Mol Pharmacol* 1990;38:207-13.
  41. Chin JE, Soffir R, Noonan KE, Choi K, Roninson IB. Structure and expression of the human MDR (P-glycoprotein) gene family. *Mol Cell Biol* 1989;9:3808-20.
  42. Smit JJ, Schinkel AH, Mol CA, Majoor D, Mooi WJ, Jongsma AP, et al. Tissue distribution of the human MDR3 P-glycoprotein [see comments] [published erratum appears in *Lab Invest* 1995;72:following table of contents]. *Lab Invest* 1994;71:638-49.
  43. West AB, Isaac CA, Carboni JM, Morrow JS, Mooseker MS, Barwick KW. Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum and colon and in colonic neoplasms. *Gastroenterology* 1988;94:343-52.
  44. Lown KS, Bailey DG, Fontana RJ, et al. Grapefruit

- juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1977;99:2545-53.
45. Pichard L, Fabre I, Fabre G, Domergue J, Saint Aubert B, Mourad G, et al. Cyclosporin A drug interactions: screening for inducers and inhibitors of cytochrome P450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab Dispos* 1990;18:595-606.
  46. Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, et al. First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* 1996;60:14-24.
  47. Watkins PB, Turgeon DK, Saenger P, Lown KS, Kolars JC, Hamilton T, et al. Comparison of urinary 6- $\beta$ -cortisol and the erythromycin breath test as measures of hepatic P450III<sub>A</sub> (CYP3A) activity. *Clin Pharmacol Ther* 1992;52:265-73.
  48. Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* 1995;275:1011-8.
  49. Lum BL, Kaubisch S, Yahanda AM, Adler KM, Jew L, Ehsan MN, et al. Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. *J Clin Oncol* 1992;10:1635-42.
  50. Wilson WH, Jamis-Dow C, Bryant G, Balis FM, Klecker RW, Bates SE, et al. Phase I and pharmacokinetic study of the multidrug resistance modulator dexverapamil with EPOCH chemotherapy. *J Clin Oncol* 1995;13:1985-94.
  51. Boote DJ, Dennis IF, Twentyman PR, Osborne RJ, Laburte C, Hensel S, et al. Phase I study of etoposide with SDZ PSC 833 as a modulator of multidrug resistance in patients with cancer. *J Clin Oncol* 1996;14:610-8.
  52. Leu BL, Huang J. Inhibition of intestinal P-glycoprotein and effects on etoposide absorption. *Cancer Chemother Pharmacol* 1995;35:432-6.
  53. Siegmund MJ, Cardarelli C, Aksentijevich I, Sugimoto Y, Pastan I, Gottesman MM. Ketoconazole effectively reverses multidrug resistance in highly resistant KB cells. *J Urol* 1994; 151:485-91.
  54. Gan LS, Moseley MA, Khosla B, Augustijns PF, Bradshaw TP, Hendren RW, et al. CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. *Drug Metab Dispos* 1996;24:344-9.
  55. Wachter VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* 1995;13:129-34.
  56. Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* 1996; 49:311-8.