Cyclosporine markedly raises the plasma concentrations of repaglinide

Background and Objective: **Repaglinide is an antidiabetic drug metabolized by cytochrome P450 (CYP) 2C8 and 3A4, and it appears to be a substrate of the hepatic uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1). We studied the effects of cyclosporine (INN, ciclosporin), an inhibitor of CYP3A4 and OATP1B1, on the pharmacokinetics and pharmacodynamics of repaglinide.**

Methods: **In a randomized crossover study, 12 healthy volunteers took 100 mg cyclosporine or placebo orally at 8 PM on day 1 and at 8 AM on day 2. At 9 AM on day 2, they ingested a single 0.25-mg dose of repaglinide. Concentrations of plasma and urine repaglinide and its metabolites (M), blood cyclosporine, and blood glucose were measured for 12 hours. The subjects were genotyped for single-nucleotide polymorphisms in *CYP2C8, CYP3A5, SLCO1B1* (encoding OATP1B1), and *ABCB1* (P-glycoprotein). The effect of cyclosporine on repaglinide metabolism was studied in human liver microsomes in vitro.**

Results: **During the cyclosporine phase, the mean peak repaglinide plasma concentration was 175% (range, 56%-365%; $P = .013$) and the total area under the plasma concentration–time curve [AUC(0-∞)] was 244% (range, 119%-533%; $P < .001$) of that in the placebo phase. The amount of unchanged repaglinide and its metabolites M2 and M4 excreted in urine were raised 2.7-fold, 7.5-fold, and 5.0-fold, respectively, by cyclosporine ($P < .001$). The amount of M1 excreted in urine remained unchanged, but cyclosporine reduced the ratio of M1 to repaglinide by 62% ($P < .001$). Cyclosporine had no significant effect on the elimination half-life or renal clearance of repaglinide. Although the mean blood glucose–lowering effect of repaglinide was unaffected in this low-dose study with frequent carbohydrate intake, the subject with the greatest pharmacokinetic interaction had the greatest increase in blood glucose–lowering effect. The effect of cyclosporine on repaglinide AUC(0-∞) was 42% lower in subjects with the *SLCO1B1* 521TC genotype than in subjects with the 521TT (reference) genotype ($P = .047$). In vitro, cyclosporine inhibited the formation of M1 (IC_{50} [concentration of inhibitor to cause 50% inhibition of original enzyme activity], 0.2 μmol/L) and M2 (IC_{50}, 4.3 μmol/L) but had no effect on M4.**

Conclusions: **Cyclosporine raised the plasma concentrations of repaglinide, probably by inhibiting its CYP3A4-catalyzed biotransformation and OATP1B1-mediated hepatic uptake. Coadministration of cyclosporine may enhance the blood glucose–lowering effect of repaglinide and increase the risk of hypoglycemia. (Clin Pharmacol Ther 2005;78:388-99.)**

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Repaglinide is a short-acting meglitinide analog antidiabetic drug used to reduce postprandial glucose levels in patients with type 2 diabetes.[1,2] It lowers blood glucose concentrations by enhancing glucose-stimulated insulin release. Repaglinide undergoes first-pass metabolism, resulting in an oral bioavailability of about 60%.[3] Cytochrome P450 (CYP) 3A4 and CYP2C8 are the main enzymes that participate in its oxidative biotransformation.[4,5] Repaglinide is not a substrate of P-glycoprotein, but it is extensively metabolized to inactive metabolites, which are excreted primarily into feces.[6-8] The area under the concentration-time curve (AUC) of repaglinide is markedly increased

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Table I. Characteristics of subjects, pharmacokinetic data for cyclosporine, and *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* genotypes of subjects

			Cyclosporine		*SLCO1B1*		*ABCB1*			
	Age (y)	*Weight (kg)*	*C_{max} (ng/mL)*	*AUC(0-∞) (ng · h/mL)*	*−11187G>A*	*521T>C*	*2677G>T/A*	*3435C>T*	*CYP2C8*	*CYP3A5*
Subject No.										
1	25	93	492	1402	GG	TT	GG	CC	*1/*4	*1/*3
2	23	67	660	1890	GG	TC	GT	CT	*1/*1	*3/*3
3	24	70	716	2611	GA	TT	GT	CT	*1/*1	*3/*3
4	22	84	423	1167	GA	TC	GT	TT	*1/*1	*3/*3
5	25	63	798	3030	GG	TT	TT	TT	*1/*1	*3/*3
6	24	70	846	2694	GG	TT	TT	TT	*1/*1	*3/*3
7	19	77	663	1746	GA	TT	GA	CC	*1/*3	*3/*3
8	22	68	535	1497	GA	TC	GT	CT	*1/*1	*3/*3
9	22	56	956	2631	GG	TT	GT	CC	*1/*1	*3/*3
10	24	72	650	2350	GG	TT	TT	CT	*1/*1	*3/*3
11	20	76	724	1684	GG	TC	GT	CT	*1/*1	*1/*3
12	23	100	499	1270	GG	TT	GT	TT	*1/*1	*3/*3
Mean±SD	23 ± 2	75 ± 13	664 ± 158	1998 ± 636						

C_{max}, Peak concentration in blood; AUC(0-∞), area under blood concentration–time curve from time 0 to infinity.

in homozygous carriers of the *SLCO1B1* 521T>C (Val174Ala) single-nucleotide polymorphism (SNP), strongly suggesting that repaglinide is a substrate of the *SLCO1B1*-encoded hepatic uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1; previously known as LST-1, OATP2, and OATP-C).[7]

The plasma concentrations of repaglinide are moderately raised by drugs that inhibit either CYP2C8 or CYP3A4.[8-11] However, gemfibrozil, a lipid-lowering drug of the fibrate class, has caused, on average, an 8-fold increase in the AUC from time 0 to infinity [AUC(0-∞)] of repaglinide, greatly increasing its glucose-lowering effect.[9] Parent gemfibrozil does not inhibit CYP3A4[12] and is only a moderate inhibitor of CYP2C8.[13] It was recently shown that a glucuronide conjugate of gemfibrozil is a relatively potent inhibitor of CYP2C8 and OATP1B1 but does not inhibit CYP3A4.[14] Combined inhibition of the hepatic uptake and CYP2C8-mediated biotransformation of repaglinide may explain the observed in vivo interaction between gemfibrozil and repaglinide.[9]

Cyclosporine (INN, ciclosporin) is an immunosuppressive drug used in organ transplant patients and in the treatment of chronic inflammatory diseases. Cyclosporine is metabolized in the intestine and liver, and the metabolites are excreted mainly via bile into feces. The peak concentration of cyclosporine in blood is reached approximately 2 hours after its oral administration, and its elimination half-life ($t_{½}$) is about 12 hours.[15] In vitro, cyclosporine potently inhibits the transporter proteins P-glycoprotein and OATP1B1, as well as CYP3A4.[16-19] Cyclosporine raises the plasma concentrations of several statins, at least partially by inhibiting their OATP1B1-mediated hepatic uptake.[16,20,21] For example, the AUC of pravastatin, a statin with no significant CYP-mediated biotransformation, is raised more than 10-fold by cyclosporine.[22,23]

Because cyclosporine markedly increases the plasma concentrations of several OATP1B1 substrates[16,20-22,24] and because polymorphism in the *SLCO1B1* gene encoding for OATP1B1 is a major determinant of the pharmacokinetics of repaglinide,[7] we hypothesized that cyclosporine might interact with repaglinide. Therefore we have investigated the effects of cyclosporine on the pharmacokinetics and pharmacodynamics of repaglinide in healthy subjects.

METHODS

Subjects. Twelve healthy nonsmoking male volunteers (age range, 19-25 years; weight range, 56-100 kg) participated in the study after giving written informed consent (Table I). They were ascertained to be healthy by a medical history, physical examination, and routine laboratory tests. None of the subjects used any continuous medication, and use of grapefruit juice or any pharmaceuticals was not allowed for 2 weeks before the study days. The subjects had previously been genotyped for the −11187G>A SNP in the promoter region and the 521T>C SNP (Val174Ala) in exon 5 of the *SLCO1B1* gene, for the 2677G>T/A SNP (Ala893Ser/

Thr) in exon 21 and the 3435C>T SNP (synonymous) in exon 26 of the *ABCB1* gene, for the *CYP2C8*3* (416G>A, 1196A>G [Arg139Lys, Lys399Arg]) and *CYP2C8*4* (792C>G [Ile264Met]) alleles, and for the *CYP3A5*3* (6986A>G, nonexpressor) allele (Table I).[7]

Study design. The study protocol was approved by the Ethics Committee for Studies in Healthy Subjects and Primary Care of the Hospital District of Helsinki and Uusimaa and the National Agency for Medicines. A randomized crossover study with 2 phases and a washout period of 4 weeks was carried out. The subjects took 100 mg cyclosporine (1 Sandimmun Neoral 100-mg capsule; Novartis Pharma SA, Huningue, France) or placebo orally at 8 PM on day 1 and at 8 AM on day 2. At 9 AM on day 2, after an overnight fast and 1 hour after the second pretreatment dose, they ingested a single 0.25-mg dose of repaglinide (one half of a NovoNorm 0.5-mg tablet; Novo Nordisk A/S, Bagsvaerd, Denmark) with 150 mL water. The volunteers remained seated for 3 hours after the administration of repaglinide. The timing of repaglinide administration was chosen to ensure adequate absorption of cyclosporine before repaglinide ingestion to maximize the extent of possible interaction. For safety reasons, a subtherapeutic repaglinide dose and short-term cyclosporine pretreatment were used.

Food intake on day 2 was identical in both phases. The volunteers received a standardized light breakfast precisely 15 minutes after repaglinide administration, a standardized snack rich in carbohydrates at precisely 1 hour and 2 hours after repaglinide, a standardized warm meal after 3 hours and 7 hours, and a standardized light meal after 11 hours. The breakfast was eaten within 10 minutes and the snacks within 5 minutes. The breakfast contained approximately 1550 kJ energy, 70 g carbohydrates, 8 g protein, and 6 g fat. The snacks were identical and contained about 840 kJ energy, 45 g carbohydrates, 2 g protein, and 1 g fat each. During the days of repaglinide administration, the subjects were under direct medical supervision and blood glucose levels were monitored throughout the day. Additional carbohydrates, glucose solution for intravenous use, and glucagon for intramuscular use were available, but they were not needed. For safety reasons, the blood pressure and heart rate of the subjects were also measured before and at 3, 6, and 12 hours after repaglinide administration. The measurement was done in a sitting position with an automatic oscillometric blood pressure monitor (HEM-711; Omron Healthcare, Hamburg, Germany).

Sampling and determination of blood glucose concentrations. On the days of repaglinide administration, timed blood samples (5 mL each) were drawn from a cannulated forearm vein before and at 20, 40, 60, 80, and 100 minutes and 2, 2.5, 3, 4, 5, 7, 9, and 12 hours after the administration of repaglinide. Blood samples were collected into tubes containing ethylenediaminetetraacetic acid. Blood glucose concentrations were measured immediately after each blood sampling by the glucose oxidase method (Precision G Blood Glucose Testing System; Medisense, Bedford, Mass). Plasma was separated within 30 minutes after blood sampling and stored at −70°C until analysis. Whole-blood cyclosporine concentrations were measured from additional blood samples (3 mL each), drawn before and at 20 and 60 minutes and 2, 3, 5, 7, and 12 hours after the administration of repaglinide. Urine was collected from 0 to 12 hours after the administration of repaglinide.

Determination of drug concentrations. Concentrations of plasma repaglinide and urine repaglinide and its metabolites M1, M2, and M4 (Fig 1) were measured by use of an API 3000 liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS Inc, Toronto, Ontario, Canada). Reversed-phase chromatographic separation was achieved on a Symmetry C_8 column (150 × 2.1 mm internal diameter, 3.5 μm particle size) (Waters, Milford, Mass) by use of gradient elution. The mobile phase consisted of 10-mmol/L ammonium formate (pH 3.5, adjusted with 99% formic acid) and acetonitrile. An aliquot (15 μL) was injected at a flow rate of 180 μL/min to give a total chromatographic run time of 24 minutes. Clopidogrel served as an internal standard. The mass spectrometer was operated in positive TurboIonSpray mode, and the samples were analyzed via selected reaction monitoring by use of the transition of the $[M+H]^+$ precursor ion to product ion for each analyte and internal standard. The selected reaction monitoring ion transitions were mass-to-charge ratio (*m/z*) 453 to *m/z* 230 for repaglinide, *m/z* 385 to *m/z* 162 for M1, *m/z* 485 to *m/z* 230 for M2, *m/z* 469 to *m/z* 246 for M4, and *m/z* 421 to *m/z* 212 for clopidogrel. The limit of quantification for repaglinide was 0.02 ng/mL, and the day-to-day coefficients of variation were 13.7% at 0.05 ng/mL, 8.7% at 0.1 ng/mL, and 6.9% at 2.0 ng/mL (n = 20). Because authentic reference compounds were not available, repaglinide metabolite concentrations are given in arbitrary units (units per milliliter) relative to the ratio of the peak height of each metabolite to that of the internal standard in the chromatogram.

Whole-blood cyclosporine concentrations were measured with a commercially available radioimmunoassay method (CYCLO-Trac; DiaSorin, Stillwater, Minn). The quantification limit was 30 ng/mL. The day-to-day

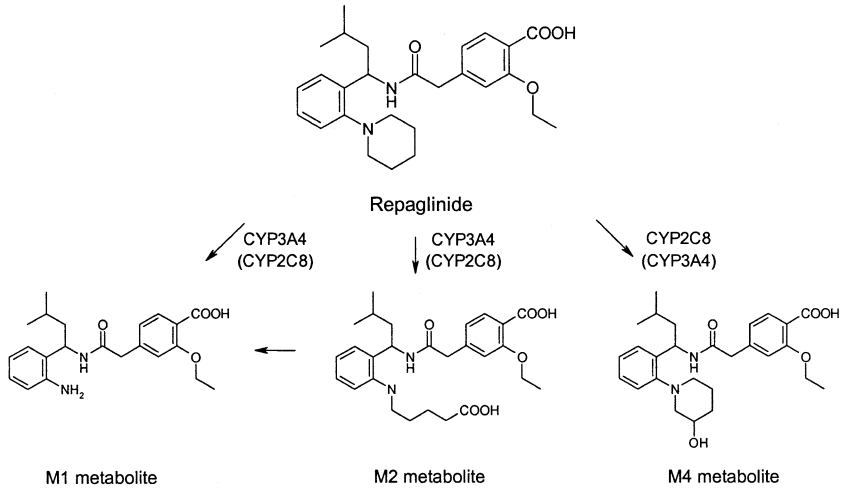

Fig 1. Chemical structures of repaglinide and its M1, M2, and M4 metabolites, as well as enzymes catalyzing reactions in vitro, as described by Bidstrup et al.[5]

coefficients of variation were 4.6% at 100 ng/mL and 3.0% at 350 ng/mL.

Pharmacokinetics. The pharmacokinetics of repaglinide was characterized by the peak concentration (C_{max}) in plasma, time to C_{max} (t_{max}), AUC(0-∞), and $t_{½}$. The C_{max} and t_{max} values were taken directly from original data. The terminal log-linear part of each concentration-time curve was identified visually, and the elimination rate constant (k_e) was determined from log-transformed data by use of linear regression analysis. The $t_{½}$ was calculated by the following equation: $t_{½} = \ln 2/k_e$. The AUC values were calculated by use of the linear trapezoidal rule for the rising phase of the plasma repaglinide concentration–time curve and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by k_e. The renal clearance of repaglinide was calculated by dividing the amount excreted into urine within 12 hours by the repaglinide AUC from 0 to 12 hours. The pharmacokinetics of cyclosporine was characterized by C_{max} in blood and AUC(0-∞). All pharmacokinetic calculations were performed with the program MK-Model, version 5.0 (Biosoft, Cambridge, United Kingdom).

Pharmacodynamics. The pharmacodynamics of repaglinide was characterized by mean change, maximum increase, and maximum decrease in blood glucose concentration. The mean change was calculated by dividing the net area under the blood glucose concentration–time curve from 0 to 3 hours and 0 to 12 hours by the corresponding time interval. The mean blood pressure and mean heart rate were calculated by dividing the area under the blood pressure– or heart rate–time curve from 0 to 12 hours by the corresponding time interval.

Statistical analysis. Results are expressed as mean values ± SD in the text and tables. The pharmacokinetic and pharmacodynamic variables between the placebo and cyclosporine phases were compared by use of repeated-measures ANOVA. The t_{max} values were compared with the Wilcoxon signed rank test. The geometric mean ratio and its 95% confidence interval were calculated for all pharmacokinetic variables, except t_{max}. The Pearson correlation coefficient was used to investigate possible relationships between cyclosporine pharmacokinetic variables, repaglinide blood glucose–lowering response, and the extent of interaction between cyclosporine and repaglinide. Possible associations of *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* SNPs with the degree of interaction between cyclosporine and repaglinide were investigated by use of ANOVA, followed by a posteriori testing with the Tukey test. The analysis was performed with the statistical programs Systat for Windows, version 6.0.1, and SPSS 11.0 for Windows (SPSS, Chicago, Ill). Differences were considered statistically significant at $P < .05$.

In vitro study. Pooled human liver microsomes (HLMs) (new catalog No. 452161, lot 26) and human recombinant (Supersomes) CYP2C8 + b_5 (new catalog No. 456252, lot 15) and CYP3A4 + b_5 (new catalog No. 456202, lot 55) were purchased from Gentest (Woburn, Mass). Repaglinide (Boehringer Ingelheim, Ingelheim, Germany), β-nicotinamide adenine dinucle-

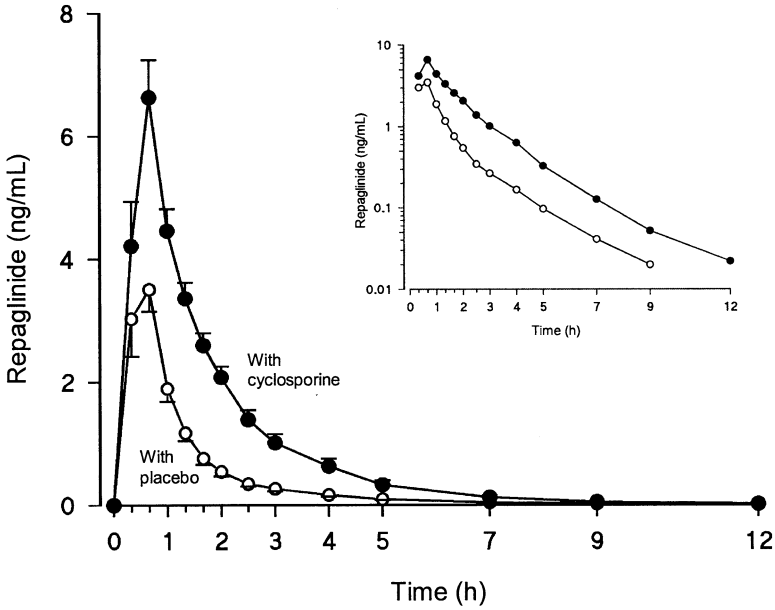

Fig 2. Mean (±SEM) plasma concentrations of repaglinide in 12 healthy volunteers after single oral dose of repaglinide (0.25 mg) during placebo phase (*open circles*) or cyclosporine phase (*solid circles*). *Inset* depicts same data on a semilogarithmic scale.

otide phosphate reduced (β-NADPH), and cyclosporine (Sigma-Aldrich, St Louis, Mo) were used in this study. Methanol, acetonitrile, and 2-propanol were obtained from Rathburn Chemicals (Walkerburn, Scotland); other chemicals were from Merck (Darmstadt, Germany).

All incubations were conducted in duplicate in a shaking water bath at 37°C, and the incubation times and microsomal protein concentrations were within the linear range for reaction velocity. The incubations were carried out in 0.1-mol/L sodium phosphate buffer (pH 7.4), containing 5.0-mmol/L magnesium chloride. The stock solutions of repaglinide and cyclosporine were prepared in methanol (final concentration, 1% [vol/vol] in the incubation mixture). The drug(s), buffer, and HLMs or recombinant enzymes were premixed, and incubations were commenced by the addition of β-NADPH (final concentration, 1.0 mmol/L). The reaction was stopped at 15 minutes from the start of the incubation by the addition of phosphoric acid, and metabolite (M1, M2, and M4) concentrations were measured by liquid chromatography–tandem mass spectrometry.

The formation rates of the M1, M2, and M4 metabolites of repaglinide (2 μmol/L) were compared in recombinant CYP2C8 and CYP3A4. The effect of cyclosporine on the metabolism of repaglinide was studied by coincubating repaglinide (2 μmol/L) with cyclosporine (0-30 μmol/L) in HLMs. The concentrations of HLMs, recombinant CYP2C8, and recombinant CYP3A4 were 0.1 mg/mL, 5 pmol/mL, and 5 pmol/mL, respectively. The conversion factors are as follows: repaglinide, 1 μmol/L equals 453 ng/mL; cyclosporine, 1 μmol/L equals 1203 ng/mL.

Reaction velocity was determined for each incubation by dividing the amount of metabolite formed (arbitrary units) by the respective time interval and enzyme concentration. IC_{50} values (concentration of inhibitor to cause 50% inhibition of original enzyme activity) were estimated from reaction velocity data by use of nonlinear regression analysis with the FigP program (version 6.0; Biosoft).

RESULTS

Repaglinide pharmacokinetics. The plasma concentrations of repaglinide were significantly raised by cyclosporine (Fig 2 and Table II). Cyclosporine raised the mean C_{max} and AUC(0-∞) of repaglinide to 175% ($P = .013$) and 244% ($P < .001$) of control values, respectively. An increase in the AUC(0-∞) was seen in every subject (range, 119%-533% of control). There was no statistically significant change in the t_{max} or $t_{½}$ of repaglinide.

Table II. Pharmacokinetic variables of single oral dose of repaglinide (0.25 mg) in 12 healthy volunteers after oral administration of placebo or 100 mg cyclosporine at 13 hours and 1 hour before repaglinide administration

Variable	*Placebo phase (control)*	*Cyclosporine phase*	*Cyclosporine phase: Percentage of control and range*	*Geometric mean ratio and 95% CI*	*P value*
Repaglinide					
C_{max} (ng/mL)	3.9 ± 1.9	6.7 ± 2.1	175% (56%-365%)	1.82 (1.28-2.58)	.003
t_{max} (min)	40 (20-40)	40 (20-40)			.180
$t_{½}$ (h)	1.3 ± 0.3	1.3 ± 0.3	97% (69%-133%)	0.98 (0.86-1.13)	.806
AUC(0-∞) (ng · h/mL)	4.44 ± 1.68	10.82 ± 3.28	244% (119%-533%)	2.54 (1.91-3.35)	< .001
Cl_{renal} (mL/h)	12.8 ± 4.5	13.8 ± 5.6	108% (67%-189%)	1.08 (0.86-1.35)	.462
Amount excreted in urine					
Repaglinide (ng)	53.8 ± 18.3	142.6 ± 46.7	265% (155%-600%)	2.74 (2.11-3.54)	< .001
M1 metabolite (U)	379 ± 111	389 ± 85	103% (57%-142%)	1.05 (0.89-1.23)	.548
M2 metabolite (U)	955 ± 300	7142 ± 2574	748% (217%-1794%)	7.33 (5.14-10.45)	< .001
M4 metabolite (U)	126 ± 33	630 ± 165	499% (227%-1132%)	5.01 (3.70-6.78)	< .001
M1/repaglinide ratio (U/ng)	8.0 ± 3.6	3.0 ± 1.2	38% (17%-72%)	0.38 (0.29-0.50)	< .001
M2/repaglinide ratio (U/ng)	20.5 ± 10.3	52.9 ± 20.3	258% (129%-611%)	2.68 (1.94-3.72)	< .001
M4/repaglinide ratio (U/ng)	2.6 ± 1.0	4.7 ± 1.5	181% (106%-343%)	1.83 (1.51-2.22)	< .001

Data are given as mean ± SD, except for t_{max} data, which are given as median and range.
CI, Confidence interval; C_{max}, peak plasma concentration; t_{max}, time to peak plasma concentration; $t_{½}$, elimination half-life; AUC(0-∞), area under plasma concentration–time curve from time 0 to infinity; Cl_{renal}, renal clearance.

Table III. Pharmacodynamic variables of single oral dose of repaglinide (0.25 mg) in 12 healthy volunteers after administration of placebo or 100 mg cyclosporine at 13 hours and 1 hour before repaglinide administration

Variable	*Placebo phase (control)*	*Cyclosporine phase*	*Mean difference between phases and 95% CI*	*P value*
Mean change, 0-3 h (mmol/L)	−0.3 ± 0.7	−0.2 ± 0.5	0.1 (−0.4 to 0.7)	.644
Mean change, 0-12 h (mmol/L)	−0.2 ± 0.5	−0.2 ± 0.5	0.1 (−0.3 to 0.4)	.718
Maximum increase (mmol/L)	1.6 ± 0.9	1.6 ± 0.6	0.0 (−0.7 to 0.7)	.981
Maximum decrease (mmol/L)	1.5 ± 0.7	1.5 ± 0.6	0.0 (−0.6 to 0.6)	>.999

Data are given as mean ± SD.

Repaglinide pharmacodynamics and blood pressure. Although no statistically significant differences were observed in the mean blood glucose response between the placebo and cyclosporine phases (Table III), the blood glucose response of individual subjects correlated with the degree of pharmacokinetic interaction between cyclosporine and repaglinide. The ratio of repaglinide C_{max} values between the cyclosporine and placebo phases correlated with the difference in the mean blood glucose change from 0 to 3 hours ($r = -0.586$, $P = .045$) and with the difference in maximum blood glucose increase ($r = -0.623$, $P = .031$). The ratio of repaglinide AUC(0-∞) values between the phases correlated with the difference in the mean blood glucose change from 0 to 3 hours ($r = -0.602$, $P = .038$). The subject with the greatest (5-fold) increase in repaglinide AUC(0-∞) also had the greatest enhancement of the blood glucose–lowering effect of repaglinide (Fig 3). However, none of the subjects had symptomatic hypoglycemia in this low-dose repaglinide study with frequent carbohydrate intake. The mean blood pressure (systolic/diastolic) was 130 ± 10 mm Hg/74 ± 5 mm Hg and 133 ± 7 mm Hg/75 ± 5 mm Hg and the mean heart rate was 62 ± 11 beats/min and 62 ± 7 beats/min in the placebo and cyclosporine phases, respectively.

Excretion of repaglinide and its metabolites into urine. Compared with the corresponding control values, cyclosporine increased the urinary excretion of unchanged repaglinide (to 265%, $P < .001$) and its metabolites M2 (to 748%, $P < .001$) and M4 (to 499%, $P < .001$) but had no significant effect on the excretion of M1 (Table II). However, cyclosporine significantly reduced the ratio of M1 to repaglinide

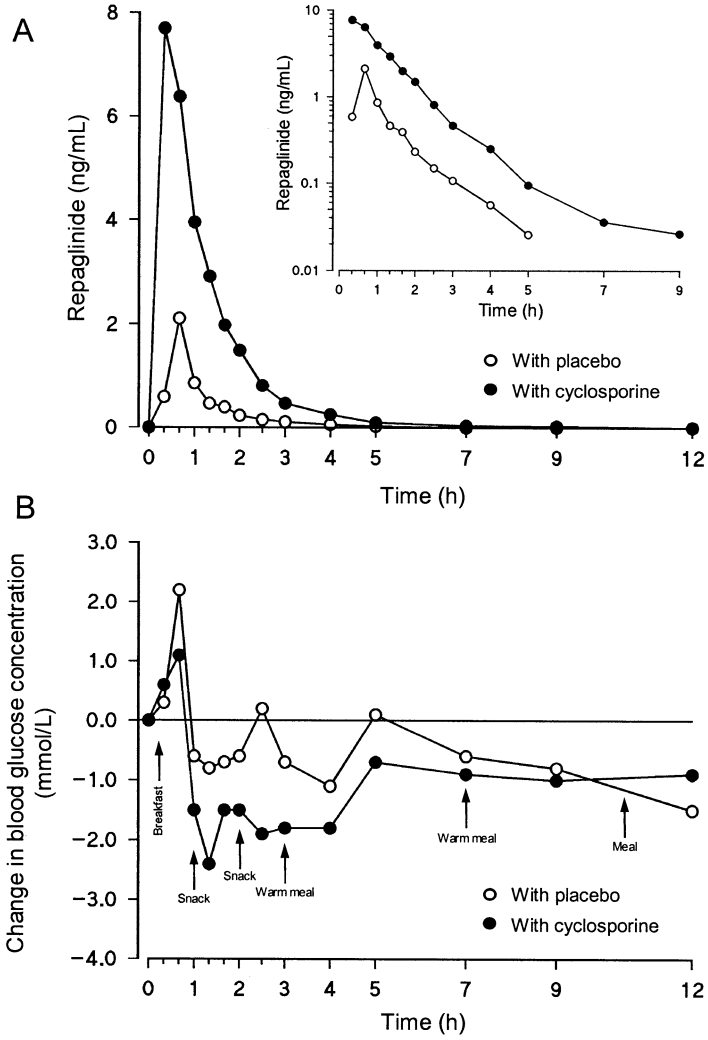

Fig 3. Plasma concentrations of repaglinide (**A**) and change in blood glucose concentrations (**B**) in subject 12, who had the greatest increase in repaglinide concentrations and the greatest increase in the blood glucose–lowering effect of repaglinide. *Inset* in **A** depicts the same data on a semilogarithmic scale. *Open circles*, Placebo phase; *solid circles*, cyclosporine phase.

(−62%, $P < .001$) and increased the ratio of M2 to repaglinide (+158%, $P < .001$) and M4 to repaglinide (+81%, $P < .001$) in urine. The renal clearance of repaglinide remained unchanged by cyclosporine ($P = .521$).

Cyclosporine pharmacokinetics. The mean C_{max} and AUC(0-∞) and median t_{max} values of cyclosporine were 664 ± 158 ng/mL, 1998 ± 636 ng/mL, and 80 minutes (range, 80-120 minutes), respectively (Fig 3 and Table I). There was a significant correlation between the AUC(0-∞) of cyclosporine and the increase in the AUC(0-∞) of repaglinide caused by cyclosporine (Pearson $r = 0.61$, $P = .034$) (Fig 4).

Genotypes and repaglinide pharmacokinetics. The *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* genotypes of the subjects are shown in Table I. In subjects with the *SLCO1B1* 521TC genotype, the increase in the AUC(0-∞) of repaglinide by cyclosporine (4.3 ± 3.8 ng · h/mL) was 42% smaller than in subjects with the 521TT (reference) genotype (7.4 ± 2.8 ng · h/mL) ($P = .047$) (Fig 5). No other statistically significant associations between the investigated SNPs or haplotypes and the extent of interaction or repaglinide baseline pharmacokinetics were found.

In vitro study. Cyclosporine inhibited the formation of the repaglinide metabolites M1 (IC_{50}, 0.2 μmol/L)

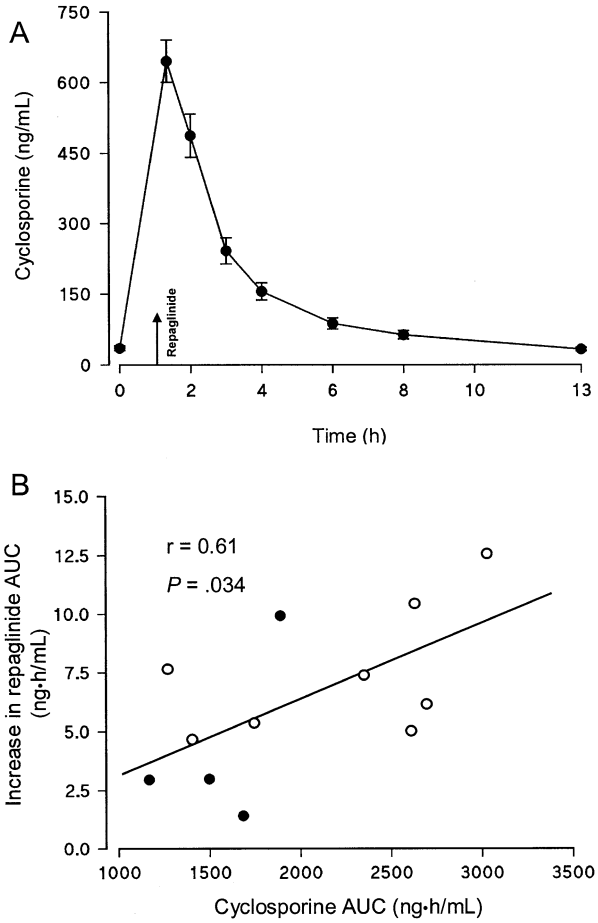

Fig 4. **A**, Mean (±SEM) plasma concentrations of cyclosporine after second (last) 100-mg dose. Time 0 refers to administration of cyclosporine, that is, 1 hour before the administration of repaglinide. **B**, Relationship between cyclosporine area under the concentration-time curve from time 0 to infinity [AUC(0-∞)] and increase in repaglinide AUC(0-∞) by cyclosporine. *Open circles*, Subjects with *SLCO1B1* 521TT genotype; *solid circles*, subjects with *SLCO1B1* 521TC genotype.

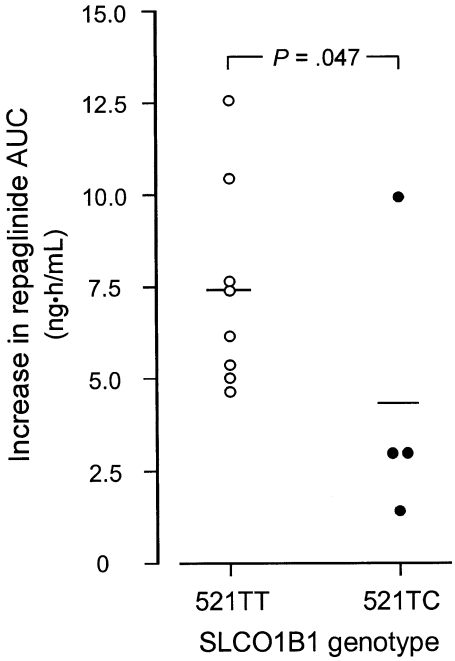

Fig 5. Increase in AUC(0-∞) of repaglinide caused by cyclosporine in relation to *SLCO1B1* 521T>C single-nucleotide polymorphism. *Bar*, Group mean; *open circles*, subjects with *SLCO1B1* 521TT genotype; *solid circles*, subjects with *SLCO1B1* 521TC genotype.

and M2 (IC_{50}, 4.3 μmol/L) in HLMs, with no effect on the formation of M4 (Fig 6, *A*). The maximum inhibition values of M1 and M2 formation were approximately 90% and 60%, respectively, and the IC_{50} values for the inhibitory processes were 0.13 μmol/L and 0.47 μmol/L, respectively.

All metabolites were formed in incubations with recombinant CYP2C8 and CYP3A4. M1 and M2 were predominantly formed by recombinant CYP3A4, whereas M4 was formed mainly by recombinant CYP2C8 (Fig 6, *B*).

DISCUSSION

This study shows that even short-term use of cyclosporine markedly increases the plasma concentrations of repaglinide. There was large variation in the extent of the interaction, with the increase in the AUC of repaglinide ranging from 1.2-fold to over 5-fold even in this homogeneous group of young healthy volunteers. This variation can be partly explained by genetic factors and variability in cyclosporine concentrations. However, cyclosporine had no significant effect on the blood glucose–lowering effect of repaglinide, which can be explained, at least partially, by the use of a subtherapeutic dose of repaglinide and the frequent food intake after repaglinide administration.

Both CYP2C8 and CYP3A4 participate in the metabolism of repaglinide.[4,5] There is evidence suggesting that the role of CYP2C8 might be more important than that of CYP3A4; inhibition of CYP3A4 by itraconazole or clarithromycin has resulted in an increase of about 40% in the AUC of repaglinide[9,11]; inhibition of CYP2C8 by trimethoprim has resulted in a 61% increase.[10] In addition, genetic variation in CYP2C8 is associated with altered repaglinide pharmacokinetics.[7,25] Gemfibrozil, an inhibitor of CYP2C8,[26] has

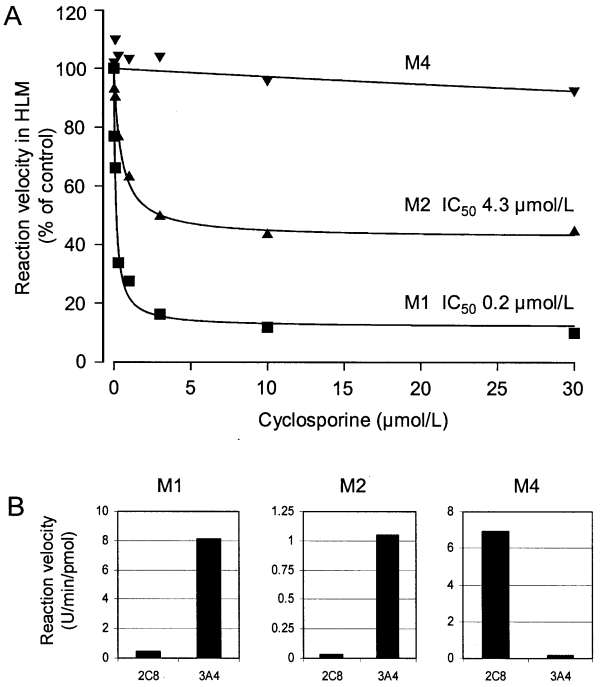

Fig 6. A, Effect of cyclosporine on formation of metabolites M1, M2, and M4 from repaglinide (2 µmol/L) by human liver microsomes (HLM). **B**, Formation of repaglinide metabolites M1, M2, and M4 by recombinant CYP2C8 and recombinant CYP3A4. IC_{50}, Concentration of inhibitor to cause 50% inhibition of original enzyme activity.

raised the AUC of repaglinide about 8-fold, greatly enhancing its blood glucose–lowering effect.[9] The glucuronide conjugate of gemfibrozil inhibits CYP2C8 more strongly than parent gemfibrozil does, without inhibiting CYP3A4,[14] and gemfibrozil and this conjugate metabolite also inhibit OATP1B1.[14] In a recent study the AUC of repaglinide was 3-fold higher in subjects homozygous for the functionally significant *SLCO1B1* 521T>C SNP compared with subjects with the reference genotype, consistent with a major role for OATP1B1 in repaglinide pharmacokinetics.[7] Thus combined inhibition of the hepatic uptake of repaglinide and its CYP2C8-mediated biotransformation could explain the observed in vivo interaction between gemfibrozil and repaglinide.

Inhibition of a single CYP enzyme by specific inhibitors, without an additional mechanism, has produced only moderate increases in the AUC of repaglinide. In the current study the mean AUC of repaglinide was raised about 2.5-fold by cyclosporine. Inhibition of CYP-mediated biotransformation of repaglinide by cyclosporine is unlikely to solely explain this cyclosporine-repaglinide interaction, because cyclosporine is weaker than itraconazole or clarithromycin as an inhibitor of CYP3A4 in vivo, and cyclosporine does not significantly inhibit CYP2C8.[27-30] Our in vitro results demonstrated that cyclosporine potently inhibited the CYP3A4-mediated repaglinide metabolism to M1, without an effect on M4 formed by CYP2C8. In this study the peak concentrations of cyclosporine (range, 423-956 ng/mL) were higher than the in vitro IC_{50} for repaglinide metabolism to M1 but not to M2.

In our study the ratio of M1 (formed primarily by CYP3A4)[5] to repaglinide in urine was significantly decreased by cyclosporine, suggesting that cyclosporine also inhibited the formation of M1 in vivo, similar to that which occurred in vitro. On the other hand, the ratios of M2 (formed only partially by CYP3A4)[5] to repaglinide and M4 (formed primarily by CYP2C8)[5] to repaglinide in urine were increased, suggesting that cyclosporine did not inhibit the formation of these major metabolites in vivo, although it caused a partial inhibition of M2 formation by HLMs in vitro. The increases in the ratios of M2 and M4 to repaglinide in urine by cyclosporine may be explained by inhibition of the hepatic (or biliary) elimination of M2 and M4. For example, cyclosporine inhibits the P-glycoprotein[31] and multidrug resistance–associated protein 2[32] efflux transporters, expressed in the canalicular membrane of the hepatocyte, and can thus affect the biliary elimination of drugs and their metabolites.

Although cyclosporine also inhibited the formation of M1 in vivo, this inhibition alone is unlikely to explain the effect of cyclosporine on the pharmacokinetics of parent repaglinide, because M1 is quantitatively only a minor metabolite of repaglinide.[6] Furthermore, itraconazole, which raised the AUC of repaglinide by only 40%, reduced the plasma AUC ratio of M1 to repaglinide by 80%,[9] whereas cyclosporine reduced the ratio of M1 to repaglinide in urine by only 62%. Given that the pharmacokinetics of repaglinide depends largely on the *SLCO1B1* (encoding OATP1B1) polymorphism[7] and that cyclosporine is a potent inhibitor of OATP1B1,[17] inhibition of the OATP1B1-mediated hepatic uptake of repaglinide by cyclosporine probably contributes to their interaction. This conclusion is further supported by the finding that the effect of cyclosporine on repaglinide pharmacokinetics was smaller in carriers of *SLCO1B1* 521T>C SNP than in noncarriers. Thus inhibition of both the CYP3A4-mediated biotransformation and the OATP1B1-mediated hepatic uptake by cyclosporine probably explains the interaction between cyclosporine and repaglinide.

Although cyclosporine increased the AUC of repaglinide by about 2.5-fold, it had no effect on the $t_{½}$ of repaglinide in this study. In a previous study in children who had received heart transplants, cyclosporine increased the AUC of pravastatin by 10-fold without affecting its $t_{½}$.[23] Moreover, *SLCO1B1* 521CC genotype did not affect repaglinide $t_{½}$, despite a 3-fold increase in AUC.[7] Increased bioavailability can only partly explain these increases in plasma drug concentrations, and further studies are needed to clarify the pharmacokinetic mechanisms involved.

In transplant recipients treated with cyclosporine, the exposure to all statins studied has been, on average, 3- to 20-fold higher than in control subjects.[16,20,21,24,33-35] Gemfibrozil has raised the concentrations of statins 2- to 5-fold.[12,26,36-38] OATP1B1 is involved in the hepatic uptake of pravastatin,[39,40] cerivastatin,[17] and rosuvastatin.[21] It is likely that atorvastatin, simvastatin, and lovastatin are also substrates of this hepatic uptake transporter.[7,21,39] Cyclosporine inhibits CYP3A4,[41] P-glycoprotein,[18,19] and OATP1B1,[17] whereas gemfibrozil (and its glucuronide conjugate) inhibits CYP2C8 and OATP1B1 but not CYP3A4 or P-glycoprotein.[12,13,42] The similarities in the observed drug interactions of gemfibrozil and cyclosporine suggest that they share a common pharmacokinetic interaction mechanism, that is, inhibition of OATP1B1.

There was no statistically significant difference in the mean glucose-lowering effect between the 2 phases in our study, despite a marked difference in the exposure to repaglinide between the phases. The obvious reason for this apparent discrepancy is that only a small dose of repaglinide was given, followed by frequent carbohydrate intake to prevent hypoglycemia. The blood glucose–lowering effect of repaglinide is dose- and concentration-dependent.[2] Accordingly, the greatest increases in the plasma repaglinide concentrations were associated with the greatest increases in the blood glucose–lowering effect of repaglinide. Thus concomitant use of cyclosporine may increase the blood glucose–lowering effect of repaglinide and the risk of hypoglycemia, particularly if higher cyclosporine and repaglinide doses are used. Of note, there was a linear relationship between cyclosporine AUC and the increase in repaglinide AUC by cyclosporine. It is, therefore, advisable to monitor blood glucose concentrations closely if cyclosporine is started in a patient taking repaglinide. In a previous study the combination of gemfibrozil and itraconazole synergistically increased the plasma concentrations (by nearly 20-fold) and effects of repaglinide.[9] Such synergism may occur if cyclosporine is combined with an inhibitor of CYP2C8, leading to simultaneous inhibition of OATP1B1-mediated hepatic uptake and CYP-mediated biotransformation of repaglinide.

In conclusion, cyclosporine considerably raised the plasma concentrations of repaglinide. This interaction is probably caused by inhibition of both the CYP3A4-catalyzed metabolism and the OATP1B1-mediated hepatic uptake of repaglinide by cyclosporine. The possibility of an increased risk of hypoglycemia should be considered when the 2 drugs are used concomitantly.

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References

1. Gromada J, Dissing S, Kofod H, Frøkjaer-Jensen J. Effects of the hypoglycaemic drugs repaglinide and glibenclamide on ATP-sensitive potassium-channels and cytosolic calcium levels in beta TC3 cells and rat pancreatic beta cells. Diabetologia 1995;38:1025-32.
2. Hatorp V. Clinical pharmacokinetics and pharmacodynamics of repaglinide. Clin Pharmacokinet 2002;41:471-83.
3. Hatorp V, Oliver S, Su CA. Bioavailability of repaglinide, a novel antidiabetic agent, administered orally in tablet or solution form or intravenously in healthy male volunteers. Int J Clin Pharmacol Ther 1998;36:636-41.
4. Kajosaari LI, Laitila J, Neuvonen PJ, Backman JT. Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. Basic Clin Pharmacol Toxicol 2005;97:249-56.
5. Bidstrup TB, Bjørnsdottir I, Sidelmann UG, Thomsen MS, Hansen KT. CYP2C8 and CYP3A4 are the principal enzymes involved in the human in vitro biotransformation of the insulin secretagogue repaglinide. Br J Clin Pharmacol 2003;56:305-14.
6. van Heiningen PN, Hatorp V, Kramer Nielsen K, Hansen KT, van Lier JJ, De Merbel NC, et al. Absorption, metabolism and excretion of a single oral dose of (14)C-repaglinide during repaglinide multiple dosing. Eur J Clin Pharmacol 1999;55:521-5.
7. Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, et al. Polymorphic organic anion transporting polypeptide 1B1 (OATP1B1) is a major determinant of repaglinide pharmacokinetics. Clin Pharmacol Ther 2005;77:468-78.
8. Hatorp V, Hansen KT, Thomsen MS. Influence of drugs interacting with CYP3A4 on the pharmacokinetics, pharmacodynamics, and safety of the prandial glucose regulator repaglinide. J Clin Pharmacol 2003;43:649-60.
9. Niemi M, Backman JT, Neuvonen M, Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. Diabetologia 2003;46:347-51.

10. Niemi M, Kajosaari LI, Neuvonen M, Backman JT, Neuvonen PJ. The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. Br J Clin Pharmacol 2004;57:441-7.
11. Niemi M, Neuvonen PJ, Kivistö KT. The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. Clin Pharmacol Ther 2001;70:58-65.
12. Backman JT, Kyrklund C, Kivistö KT, Wang JS, Neuvonen PJ. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. Clin Pharmacol Ther 2000;68:122-9.
13. Wang JS, Neuvonen M, Wen X, Backman JT, Neuvonen PJ. Gemfibrozil inhibits CYP2C8-mediated cerivastatin metabolism in human liver microsomes. Drug Metab Dispos 2002;30:1352-6.
14. Shitara Y, Hirano M, Sato H, Sugiyama Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. J Pharmacol Exp Ther 2004;311:228-36.
15. Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin: an updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (neoral)1 in organ transplantation. Drugs 2001;61:1957-2016.
16. Åsberg A. Interactions between cyclosporin and lipid-lowering drugs: implications for organ transplant recipients. Drugs 2003;63:367-78.
17. Shitara Y, Itoh T, Sato H, Li AP, Sugiyama Y. Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. J Pharmacol Exp Ther 2003;304:610-6.
18. Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Adv Drug Deliv Rev 1997;27: 201-14.
19. Stapf V, Thalhammer T, Huber-Huber R, Felberbauer F, Gajdzik L, Graf J. Inhibition of rhodamine 123 secretion by cyclosporin A as a model of P-glycoprotein mediated transport in liver. Anticancer Res 1994;14:581-5.
20. Renders L, Czock D, Schocklmann H, Kunzendorf U. Determination of the pharmacokinetics of cerivastatin when administered in combination with sirolimus and cyclosporin A in patients with kidney transplant, and review of the relevant literature. Int J Clin Pharmacol Ther 2003;41:499-503.
21. Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, et al. Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. Clin Pharmacol Ther 2004;76:167-77.
22. Park JW, Siekmeier R, Merz M, Krell B, Harder S, Marz W, et al. Pharmacokinetics of pravastatin in heart-transplant patients taking cyclosporin A. Int J Clin Pharmacol Ther 2002;40:439-50.
23. Hedman M, Neuvonen PJ, Neuvonen M, Holmberg C, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in pediatric and adolescent cardiac transplant recipients on a regimen of triple immunosuppression. Clin Pharmacol Ther 2004;75:101-9.
24. Regazzi MB, Iacona I, Campana C, Gavazzi A, Vigano M, Perani G. Clinical efficacy and pharmacokinetics of HMG-CoA reductase inhibitors in heart transplant patients treated with cyclosporin A. Transplant Proc 1994; 26:2644-5.
25. Niemi M, Leathart JB, Neuvonen M, Backman JT, Daly AK, Neuvonen PJ. Polymorphism in CYP2C8 is associated with reduced plasma concentrations of repaglinide. Clin Pharmacol Ther 2003;74:380-7.
26. Backman JT, Kyrklund C, Neuvonen M, Neuvonen PJ. Gemfibrozil greatly increases plasma concentrations of cerivastatin. Clin Pharmacol Ther 2002;72:685-91.
27. Ong CE, Coulter S, Birkett DJ, Bhasker CR, Miners JO. The xenobiotic inhibitor profile of cytochrome P4502C8. Br J Clin Pharmacol 2000;50:573-80.
28. Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. Clin Pharmacol Ther 1994;55:481-5.
29. Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr, Hall SD. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. Clin Pharmacol Ther 1998; 64:133-43.
30. Li G, Treiber G, Meinshausen J, Wolf J, Werringloer J, Klotz U. Is cyclosporin A an inhibitor of drug metabolism? Br J Clin Pharmacol 1990;30:71-7.
31. Tamai I, Safa AR. Competitive interaction of cyclosporins with the Vinca alkaloid-binding site of P-glycoprotein in multidrug-resistant cells. J Biol Chem 1990;265: 16509-13.
32. Chen ZS, Kawabe T, Ono M, Aoki S, Sumizawa T, Furukawa T, et al. Effect of multidrug resistance-reversing agents on transporting activity of human canalicular multispecific organic anion transporter. Mol Pharmacol 1999;56:1219-28.
33. Arnadottir M, Eriksson LO, Thysell H, Karkas JD. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron 1993;65:410-3.
34. Goldberg R, Roth D. Evaluation of fluvastatin in the treatment of hypercholesterolemia in renal transplant recipients taking cyclosporine. Transplantation 1996; 62:1559-64.
35. Gullestad L, Nordal KP, Berg KJ, Cheng H, Schwartz MS, Simonsen S. Interaction between lovastatin and cyclosporine A after heart and kidney transplantation. Transplant Proc 1999;31:2163-5.

36. Kyrklund C, Backman JT, Neuvonen M, Neuvonen PJ. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. Clin Pharmacol Ther 2003;73:538-44.
37. Kyrklund C, Backman JT, Kivistö KT, Neuvonen M, Laitila J, Neuvonen PJ. Plasma concentrations of active lovastatin acid are markedly increased by gemfibrozil but not by bezafibrate. Clin Pharmacol Ther 2001;69:340-5.
38. Schneck DW, Birmingham BK, Zalikowski JA, Mitchell PD, Wang Y, Martin PD, et al. The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. Clin Pharmacol Ther 2004;75:455-63.
39. Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem 1999;274: 37161-8.
40. Nakai D, Nakagomi R, Furuta Y, Tokui T, Abe T, Ikeda T, et al. Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. J Pharmacol Exp Ther 2001;297:861-7.
41. Martin J, Krum H. Cytochrome P450 drug interactions within the HMG-CoA reductase inhibitor class: are they clinically relevant? Drug Saf 2003;26:13-21.
42. Kivistö KT, Zukunft J, Hofmann U, Niemi M, Rekersbrink S, Schneider S, et al. Characterisation of cerivastatin as a P-glycoprotein substrate: studies in P-glycoprotein-expressing cell monolayers and mdr1a/b knock-out mice. Naunyn Schmiedebergs Arch Pharmacol 2004;370:124-30.