Telithromycin, but not montelukast, increases the plasma concentrations and effects of the cytochrome P450 3A4 and 2C8 substrate repaglinide

Background and Objective: **The antidiabetic repaglinide is metabolized by cytochrome P450 (CYP) 2C8 and CYP3A4. Telithromycin, an antimicrobial agent, inhibits CYP3A4 in vitro and in vivo. Montelukast, an antiasthmatic drug, is a potent inhibitor of CYP2C8 in vitro. We studied the effects of telithromycin, montelukast, and the combination of telithromycin and montelukast on the pharmacokinetics and pharmacodynamics of repaglinide.**

Methods: **In a randomized 4-phase crossover study, 12 healthy volunteers received 800 mg telithromycin, 10 mg montelukast, both telithromycin and montelukast, or placebo once daily for 3 days. On day 3, they ingested a single 0.25-mg dose of repaglinide. Plasma and urine concentrations of repaglinide and its metabolites M1, M2, and M4, as well as blood glucose concentrations, were measured for 12 hours.**

Results: **Telithromycin alone raised the mean peak plasma repaglinide concentration to 138% (range, 91%-209%; $P = .006$) and the total area under the plasma concentration–time curve from 0 hours to infinity [AUC(0-∞)] of repaglinide to 177% (range, 125%-257%; $P < .001$) of control (placebo). Telithromycin reduced the AUC(0-∞) ratio of the metabolite M1 to repaglinide by 68% ($P < .001$) and the urinary excretion ratio of M1 to repaglinide by 77% ($P = .001$). In contrast to previous estimates based on in vitro CYP2C8 inhibition data, montelukast had no significant effect on the pharmacokinetics of repaglinide or its metabolites and did not significantly alter the effect of telithromycin on repaglinide pharmacokinetics. Telithromycin, unlike montelukast, lowered the maximum blood glucose concentration ($P = .002$) and mean blood glucose concentration from 0 to 3 hours ($P = .008$) after repaglinide intake, as compared with placebo.**

Conclusions: **Telithromycin increases the plasma concentrations and blood glucose–lowering effect of repaglinide by inhibiting its CYP3A4-catalyzed biotransformation and may increase the risk of hypoglycemia. Unexpectedly, montelukast has no significant effect on repaglinide pharmacokinetics, suggesting that it does not significantly inhibit CYP2C8 in vivo. The low free fraction of montelukast in plasma may explain the lack of effect on CYP2C8 in vivo, despite the low in vitro inhibition constant, highlighting the importance of incorporating plasma protein binding to interaction predictions. (Clin Pharmacol Ther 2006;79:231-42.)**

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Telithromycin, a novel antimicrobial drug of the ketolide class, is a potent inhibitor of cytochrome P450 (CYP) 3A4 in vitro.[1,2] Telithromycin can also considerably raise the plasma concentrations of CYP3A4 substrates in vivo.[1] For example, the area under the concentration-time curve (AUC) of simvastatin is raised by about 10-fold by telithromycin.[2] Montelukast is a leukotriene receptor antagonist used in the treatment of asthma and related diseases.[3,4] It is a potent and selective inhibitor of CYP2C8 in vitro, with inhibition constant (K_i) values ranging from 0.0092 to 0.15 μmol/L.[5,6] On the basis of in vitro inhibition data and the therapeutic plasma concentrations of montelukast, it has been estimated that montelukast could increase the AUC of a CYP2C8-cleared drug by 2-fold to over 100-fold.[5] However, the effect of montelukast on the pharmacokinetics of CYP2C8 substrate drugs has not been studied in vivo.

Repaglinide, a short-acting meglitinide analog antidiabetic drug, is used to reduce postprandial glucose levels in patients with type 2 diabetes.[7,8] Repaglinide has an oral bioavailability of about 60% as a result of considerable first-pass metabolism.[9] The metabolites of repaglinide are mainly inactive; they are excreted principally into feces and, to a lesser extent, into urine.[10] CYP2C8 and CYP3A4 are the main enzymes that participate in its oxidative biotransformation.[11,12] The plasma concentrations of repaglinide have been found to be raised by drugs that inhibit either CYP2C8 or CYP3A4 enzymes.[13-16] Thus trimethoprim (an inhibitor of CYP2C8) has increased the AUC of repaglinide by about 60%,[16] and clarithromycin[13] and itraconazole[14] (inhibitors of CYP3A4) have increased its AUC by about 40%. In addition, genetic polymorphism in CYP2C8 has been associated with altered pharmacokinetics of repaglinide.[17] In addition to CYP enzymes, membrane transporters seem to be important in the pharmacokinetics of repaglinide. Recently, the AUC of repaglinide was found to be greater in carriers than in noncarriers of the functionally significant single-nucleotide polymorphism (c.521T>C) in the *SLCO1B1* gene, encoding organic anion transporting polypeptide 1B1 (OATP1B1), suggesting that repaglinide is a substrate for this hepatic uptake transporter.[18]

Because telithromycin inhibits CYP3A4[1] and markedly increases the plasma concentrations of simvastatin[19] and because montelukast is a potent and selective inhibitor of CYP2C8 in vitro,[5] we hypothesized that telithromycin and montelukast might interact with repaglinide. Accordingly, our aim was to investigate the effects of telithromycin, montelukast, and the combination of telithromycin and montelukast on the pharmacokinetics, pharmacodynamics, and metabolic fate of repaglinide in vivo in healthy subjects.

METHODS

Subjects. Twelve healthy, self-reported nonsmoking volunteers (8 men and 4 women; age range, 18-24 years; weight range, 52-85 kg) participated in the study after giving written informed consent. 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.

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 4 phases and a washout period of 2 weeks was carried out. The subjects ingested 800 mg telithromycin (two 400-mg Ketek tablets; Aventis Pharma, Scoppito, Italy), 10 mg montelukast (one 10-mg Singulair tablet; Merck, Sharp & Dohme, Haarlem, Holland), both telithromycin and montelukast, or placebo once daily at 8 AM for 3 days. On day 3 at 9 AM, after an overnight fast and 1 hour after the last pretreatment dose, they ingested a single 0.25-mg dose of repaglinide (half of a 0.5-mg tablet of NovoNorm; Novo Nordisk, Bagsvaerd, Denmark) with 150 mL water. All 3 drugs used in this study have been approved by the Food and Drug Administration and European Medicines Agency. The small dose of repaglinide was chosen because of safety reasons. The volunteers remained seated for 3 hours after the administration of repaglinide. The timing of repaglinide administration was chosen to ensure adequate absorption of telithromycin and montelukast before repaglinide ingestion.

Food intake on day 3 was identical in all phases. The volunteers received a standardized light breakfast precisely 15 minutes after repaglinide administration, a standardized snack rich in carbohydrates precisely 1 hour and 2 hours after repaglinide administration, 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 consisted of 150 mL orange juice (Valio, Helsinki, Finland), 1 medium-sized banana, and 1 sandwich (2 pieces of dark bread, 2 slices of cheese, lettuce, tomato, and margarine) and contained approximately 1550 kJ energy, 70 g carbohydrates, 8 g protein, and 6 g fat. The snacks were identical, consisted of 100 mL orange juice (Valio) and 1 medium-sized banana, 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.

Sampling and determination of blood glucose concentrations. On the days of repaglinide administration, timed blood samples (10 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. Urine was collected cumulatively from 0 to 12 hours after the administration of repaglinide. Plasma and urine samples were stored at −70°C until analysis.

Determination of drug concentrations. Concentrations of repaglinide and its metabolites M1, M2, and M4 were measured in plasma and urine samples by use of an API 3000 liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada). Reversed-phase chromatographic separation was achieved on a Symmetry C_8 column (internal diameter of 150 × 2.1 mm and particle size of 3.5 μm) (Waters, Milford, Mass) by use of a mobile phase consisting of 10-mmol/L ammonium formate (pH 3.5, adjusted with 99% formic acid) and acetonitrile. A 15-μL aliquot was injected, and the mobile phase flow rate was 180 μL/min. The mobile phase gradient comprised 3 minutes at 40% acetonitrile, 3 minutes to 65% acetonitrile, 2 minutes at 65% acetonitrile, 4 minutes to 100% acetonitrile, 4 minutes at 100% acetonitrile, and 8 minutes at 40% acetonitrile, yielding a total chromatographic run time of 24 minutes. Clopidogrel served as the internal standard. The mass spectrometer was operated in positive TurboIonSpray (Sciex Division of MDS) 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 as follows: 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 0.01 ng/mL, and the day-to-day coefficients of variation (CVs) were 9.4% at 0.1 ng/mL and 4.9% at 2.0 ng/mL (n = 11). Because authentic metabolite standards were not available, 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. Telithromycin and montelukast did not interfere with the assay.

The concentration of telithromycin in plasma was measured from samples taken before and at 80 minutes and 2, 3, 5, and 13 hours after telithromycin administration by use of an API 2000 liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS).[20] The ion transition monitored was *m/z* 812 to *m/z* 655. The limit of quantification for telithromycin was 5.0 ng/mL, and the CVs were 5.9% at 7.0 ng/mL, 6.8% at 70 ng/mL, and 2.5% at 360 ng/mL (n = 7).

The concentration of montelukast in plasma was measured from samples taken before and at 80 minutes and 3, 5, and 13 hours after montelukast administration by HPLC by use of fluorescence detection.[21] The limit of quantification for montelukast was 5 ng/mL, and the CVs were 11.4% at 25 ng/mL, 11.7% at 250 ng/mL, and 14.1% at 700 ng/mL (n = 5).

Pharmacokinetics. The pharmacokinetics of repaglinide and its metabolites M1, M2, and M4 was characterized by the peak concentration in plasma (C_{max}), time to C_{max} (t_{max}), AUC from 0 hours to infinity [AUC(0-∞)], and elimination half-life ($t_{½}$). 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 via 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 concentration–time curve and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity by dividing the last measured concentration by k_e. The renal clearance (Cl_{renal}) of repaglinide was calculated by dividing the amount excreted into urine within 12 hours by repaglinide AUC from 0 to 12 hours [AUC(0-12)]. The pharmacokinetics of telithromycin was characterized by C_{max} 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 minimum and maximum blood glucose concentrations, as well as by mean blood glucose concentrations from 0 to 3 hours, from 0 to 7 hours, and from 0 to 12 hours. The mean concentrations were calculated by dividing the area under the

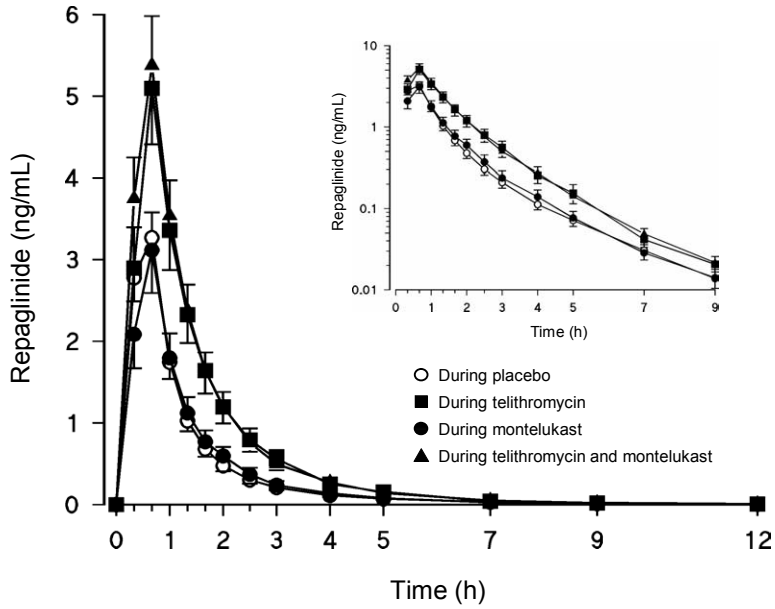

Fig 1. Mean ± SEM plasma concentrations of repaglinide in 12 healthy volunteers after single oral dose of 0.25 mg repaglinide after 3-day treatment with 800 mg telithromycin (*squares*), 10 mg montelukast (*solid circles*), both telithromycin and montelukast (*triangles*), or placebo (*open circles*) once daily. The *inset* depicts the same data on a semilogarithmic scale.

blood glucose concentration–time curve by the corresponding time interval.

Statistical analysis. Results are expressed as mean ± SD in the text and tables and, for clarity, as mean ± SEM in the figures. The pharmacokinetic and pharmacodynamic variables between the placebo, telithromycin, montelukast, and telithromycin and montelukast phases were compared by use of repeated-measures ANOVA, as well as a posteriori testing with the paired *t* test. The t_{max} values were compared via Friedman 2-way ANOVA followed by the Wilcoxon signed rank test. The Pearson correlation coefficient was used to investigate possible relationships between telithromycin pharmacokinetic variables and the extent of interaction between telithromycin and repaglinide. The analysis was performed with the statistical program Systat for Windows, version 6.0.1 (SPSS, Chicago, Ill). Differences were considered statistically significant at $P < .05$. With Bonferroni correction for multiple comparisons, the threshold for significance would be $P = .0083$. The sample size was chosen so that a possible clinically significant pharmacokinetic drug interaction could be verified without the use of an unnecessarily large group of healthy subjects. The number of subjects ($n = 12$) was estimated to be sufficient to detect a 30% change in the AUC(0-∞) of repaglinide with a power of 80% (α level, 5%).

RESULTS

Effect of telithromycin on repaglinide pharmacokinetics. Telithromycin alone considerably increased the plasma concentrations and urinary excretion of unchanged repaglinide (Fig 1 and Table I). Telithromycin did not affect the $t_{½}$ of repaglinide but raised its mean C_{max} and AUC(0-∞) to 138% ($P = .006$) and 177% ($P < .001$) of the control (placebo) values, respectively. Telithromycin decreased the C_{max} and AUC(0-∞) of the metabolite M1 and increased the C_{max} and AUC(0-∞) of the metabolites M2 and M4 (Fig 2 and Table II). The M1-to-repaglinide AUC(0-∞) ratio was lowered by 68% ($P < .001$), and the M2-to-repaglinide and M4-to-repaglinide AUC(0-∞) ratios were raised to 125% ($P = .012$) and 153% ($P < .001$) by telithromycin, respectively.

Telithromycin increased the urinary excretion of unchanged repaglinide (to 229%, $P < .001$) and its metabolites M2 (to 180%, $P < .001$) and M4 (to 198%, $P < .001$) by about 2-fold but reduced the excretion of M1 (by 41%, $P < .001$) compared with control (placebo) (Fig 3). The ratio of M2 or M4 to repaglinide in urine was not changed significantly, whereas that of M1 to repaglinide was reduced by telithromycin, as compared with the placebo or montelukast phase (Fig 3). The Cl_{renal} of repaglinide was increased by telithromycin (to 138%, $P = .010$).

Table I. Pharmacokinetic variables of repaglinide after single oral dose of 0.25 mg repaglinide in 12 healthy volunteers after 3-day treatment with 800 mg telithromycin, 10 mg montelukast, both telithromycin and montelukast, or placebo once daily

Variable	*Placebo phase (control)*	*Telithromycin phase*	*Montelukast phase*	*Telithromycin and montelukast phase*
C_{max} (ng/mL)	3.7 ± 1.7	5.1 ± 2.4*	3.4 ± 1.8†	5.4 ± 2.1*‡
% of control and range	100%	138% (91%-209%)	92% (61%-181%)	146% (97%-222%)
t_{max} (min)	40 (20-40)	40 (40)*	40 (20-40)	40 (20-40)
$t_{½}$ (h)	1.4 ± 0.3	1.2 ± 0.2	1.4 ± 0.2§	1.2 ± 0.2‖
% of control and range	100%	85% (39%-106%)	100% (75%-145%)	87% (54%-129%)
AUC(0-∞) (ng · h/mL)	3.9 ± 1.6	6.9 ± 3.1¶	3.9 ± 2.0†	7.4 ± 3.1¶‡
% of control and range	100%	177% (125%-257%)	99% (61%-147%)	188% (132%-265%)
Ae (ng)	32.0 ± 16.7	73.5 ± 32.3¶	32.7 ± 13.6†	75.3 ± 23.3¶‡
% of control and range	100%	229% (123%-632%)	102% (43%-222%)	235% (162%-541%)
Cl_{renal} (mL/h)	8.3 ± 3.1	11.4 ± 4.9*	9.2 ± 3.0	11.3 ± 4.2*
% of control and range	100%	138% (75%-265%)	111% (70%-211%)	137% (70%-237%)

Data are given as mean ± SD, except for t_{max} data, which are given as median and range.
C_{max}, Peak concentration in plasma; t_{max}, time to reach peak plasma concentration; $t_{1/2}$, elimination half-life; AUC(0-∞), area under plasma concentration–time curve from time 0 to infinity; Ae, amount excreted into urine; Cl_{renal}, renal clearance of repaglinide.
*$P < .05$, versus placebo phase.
†$P < .001$, versus telithromycin phase.
‡$P < .001$, versus montelukast phase.
§$P < .05$, versus telithromycin phase.
‖$P < .05$, versus montelukast phase.
¶$P < .001$, versus placebo phase.

Effect of montelukast on repaglinide pharmacokinetics. Montelukast alone had no statistically significant effect on any of the pharmacokinetic variables of the parent repaglinide or its metabolites M1, M2, or M4 (Figs 1, 2, and 3 and Tables I and II).

Effect of telithromycin-montelukast combination on repaglinide pharmacokinetics. The combination of telithromycin and montelukast raised the mean C_{max} and AUC(0-∞) of repaglinide to 146% ($P = .002$) and 188% ($P < .001$) of placebo values, respectively (Fig 1 and Table I). There were no significant differences in any of the pharmacokinetic variables of repaglinide between the combination phase and the telithromycin-alone phase. During the combination phase, the C_{max} and AUC(0-∞) of repaglinide were greater than during the montelukast phase (Table I).

The combination of telithromycin and montelukast decreased the C_{max} and AUC(0-∞) of the metabolite M1 but increased those of the metabolites M2 and M4 (Fig 2 and Table II), when compared with either the placebo or montelukast phase. The combination of telithromycin and montelukast lowered the M1-to-repaglinide AUC(0-∞) ratio and raised the M2-to-repaglinide and M4-to-repaglinide AUC(0-∞) ratios, when compared with the placebo phase (Table II).

The combination also increased the urinary excretion of unchanged repaglinide (to 235%, $P < .001$) and its metabolites M2 (to 186%, $P = .001$) and M4 (to 191%, $P < .001$) but reduced the excretion of M1 (by 44%, $P < .001$). The combination decreased the M1-to-repaglinide, M2-to-repaglinide, and M4-to-repaglinide ratios in urine when compared with placebo (Fig 3). The Cl_{renal} of repaglinide was increased by the combination (to 137%, $P = .002$).

Repaglinide pharmacodynamics. Compared with the placebo phase, telithromycin reduced the maximum blood glucose concentration (by 0.7 mmol/L, $P = .002$) and mean blood glucose concentration (0 to 3 hours) (by 0.5 mmol/L, $P = .008$) (Fig 4 and Table III). No significant differences were observed in the blood glucose concentrations between the placebo and montelukast phases. The maximum blood glucose concentration was reduced in the combination phase compared with the placebo phase (by 0.5 mmol/L, $P = .030$). None of the subjects had symptomatic hypoglycemia.

Telithromycin and montelukast. The C_{max} and AUC from 0 to 13 hours [AUC(0-13)] of telithromycin were 1180 ± 250 ng/mL and 5350 ± 1520 ng · h/mL, respectively, in the telithromycin phase (Fig 5). The plasma concentration of telithromycin was about 500 ng/mL at the time of repaglinide ingestion. The C_{max} and AUC(0-13) of montelukast were 540 ± 170 ng/mL and 3050 ± 1130 ng · h/mL, respectively, in the montelukast phase and 430 ± 200 ng/mL and 2440 ± 1060 ng · h/mL, respectively, in the telithromycin and montelukast phase. The plasma concentration of montelukast was around 100

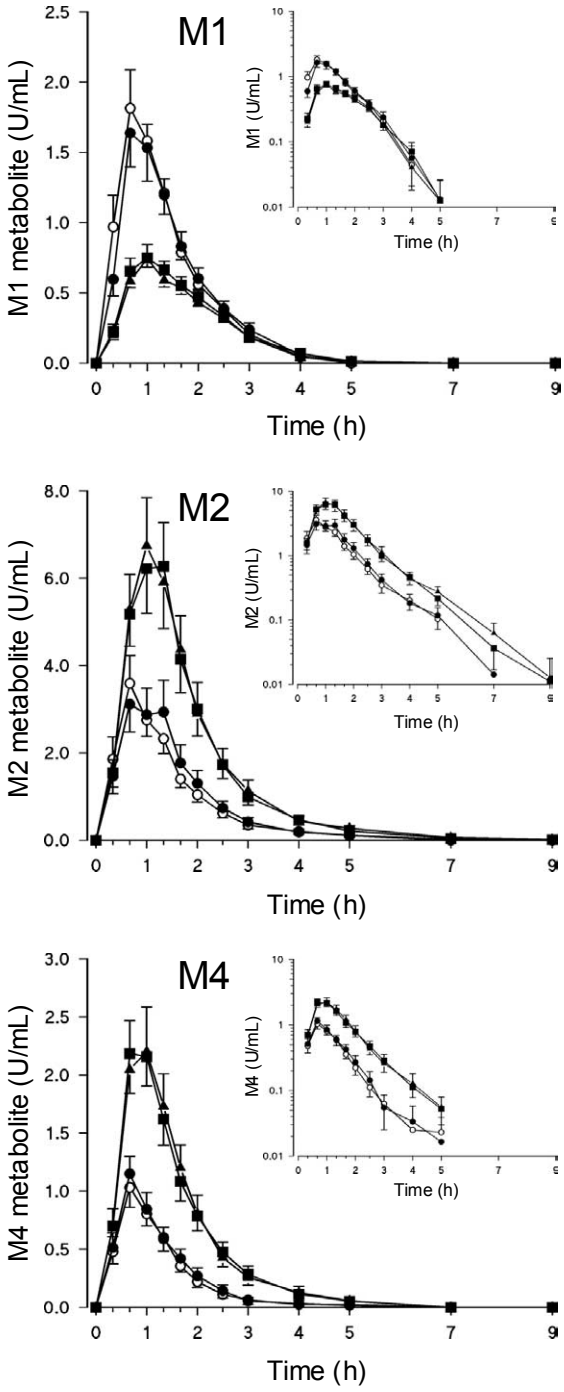

Fig 2. Mean ± SEM plasma concentrations of M1, M2, and M4 metabolites of repaglinide in 12 healthy volunteers after single oral dose of 0.25 mg repaglinide after 3-day treatment with 800 mg telithromycin (*squares*), 10 mg montelukast (*solid circles*), both telithromycin and montelukast (*triangles*), or placebo (*open circles*) once daily. The *insets* depict the same data on a semilogarithmic scale.

to 250 ng/mL at the time of repaglinide ingestion (Fig 5). Montelukast had no statistically significant effect on the pharmacokinetics of telithromycin, and telithromycin had no statistically significant effect on the pharmacokinetics of montelukast. There were no significant correlations between the pharmacokinetic variables of telithromycin and the extent of interaction between telithromycin and repaglinide.

DISCUSSION

This study shows that telithromycin significantly increases the plasma concentrations and effects of repaglinide. The ratio of the metabolite M1 to repaglinide in plasma and urine was decreased by telithromycin, indicating that telithromycin inhibited the formation of this metabolite, formed primarily by CYP3A4.[11,22] On the other hand, montelukast, a potent inhibitor of CYP2C8 in vitro,[5] unexpectedly had no effect on the pharmacokinetics of repaglinide or its metabolites, even when CYP3A4 was simultaneously inhibited by telithromycin.

In vitro, CYP2C8 and CYP3A4 contribute similarly to the metabolism of therapeutic repaglinide concentrations, with no significant biotransformation by other CYP enzymes.[11,12] In addition, genetic association data suggest that *SLCO1B1* (encoding OATP1B1) polymorphism is an important determinant of interindividual variation in repaglinide pharmacokinetics.[18] OATP1B1 is an uptake transporter expressed in the basolateral membrane of hepatocytes[23] and seems to have an important role in the elimination of repaglinide in vivo.[18]

In pharmacokinetic interaction studies in humans, inhibition of CYP3A4 (by itraconazole or clarithromycin)[13,14] and CYP2C8 (by trimethoprim)[16] has raised the AUC of repaglinide by about 40% and 60%, respectively, indicating that both enzymes also metabolize repaglinide in vivo. However, simultaneous inhibition of CYP-mediated biotransformation and OATP1B1-mediated hepatic uptake has caused the largest drug interactions with repaglinide.[14,22] Cyclosporine (INN, ciclosporin), an inhibitor of CYP3A4 and OATP1B1,[24] has raised the AUC of repaglinide by 2.4-fold.[22] In that study the effect of cyclosporine on repaglinide pharmacokinetics was smaller in subjects with the *SLCO1B1* (encoding OATP1B1) c.521TC genotype than in subjects with the reference genotype, supporting the conclusion that inhibition of OATP1B1 by cyclosporine contributes to the interaction. Gemfibrozil has raised the AUC of repaglinide by about 8-fold, and the combination of itraconazole and gemfibrozil has increased the AUC of repaglinide by about 20-fold.[14] Gemfibrozil and its glucuronide metabolite are inhibitors of CYP2C8 and OATP1B1.[25,26] Thus a combination of itraconazole

Table II. Pharmacokinetic variables of M1, M2, and M4 metabolites of repaglinide after single oral dose of 0.25 mg repaglinide in 12 healthy volunteers after 3-day treatment with 800 mg telithromycin, 10 mg montelukast, both telithromycin and montelukast, or placebo once daily

Variable	*Placebo phase (control)*	*Telithromycin phase*	*Montelukast phase*	*Telithromycin and montelukast phase*
M1 metabolite				
C_{max} (U/mL)	2.0 ± 0.8	0.8 ± 0.3*	1.7 ± 0.8†	0.8 ± 0.2*‡
% of control and range	100%	41% (25%-69%)	88% (54%-162%)	38% (25%-59%)
t_{max} (min)	60 (40-60)	60 (40-100)	60 (20-80)	60 (40-80)§
$t_{½}$ (h)	0.8 ± 0.2	1.2 ± 0.3*	0.8 ± 0.2†	1.2 ± 0.3*‖
% of control and range	100%	156% (106%-278%)	110% (85%-184%)	156% (100%-277%)
AUC(0-∞) (U · h/mL)	2.7 ± 0.9	1.5 ± 0.5*	2.6 ± 1.2¶	1.4 ± 0.4*‖
% of control and range	100%	56% (36%-81%)	96% (60%-126%)	52% (30%-96%)
M1-to-repaglinide AUC(0-∞) ratio (U/ng)	0.80 ± 0.41	0.25 ± 0.11*	0.77 ± 0.40†	0.22 ± 0.10*‡
% of control and range	100%	32% (20%-55%)	96% (56%-176%)	27% (17%-60%)
M2 metabolite				
C_{max} (U/mL)	3.7 ± 2.1	6.9 ± 3.5*	3.5 ± 2.6†	7.0 ± 3.9*‡
% of control and range	100%	188% (127%-288%)	95% (49%-146%)	190% (133%-314%)
t_{max} (min)	40 (40-80)	60 (40-80)§	40 (20-80)¶	60 (40-80) §‖
$t_{1/2}$ (h)	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.3
% of control and range	100%	107% (58%-155%)	109% (82%-202%)	114% (72%-171%)
AUC(0-∞) (U · h/mL)	5.3 ± 2.7	11.4 ± 5.7*	5.6 ± 4.0†	11.7 ± 6.3*‡
% of control and range	100%	216% (152%-304%)	106% (57%-172%)	223% (138%-318%)
M2-to-repaglinide AUC(0-∞) ratio (U/ng)	1.3 ± 0.4	1.7 ± 0.5§	1.4 ± 0.6	1.6 ± 0.5§
% of control and range	100%	125% (87%-218%)	104% (65%-156%)	118% (83%-187%)
M4 metabolite				
C_{max} (U/mL)	1.1 ± 0.5	2.4 ± 1.0*	1.2 ± 0.5†	2.4 ± 1.2‡§
% of control and range	100%	217% (118%-345%)	104% (62%-189%)	212% (114%-409%)
t_{max} (min)	40 (40-60)	40 (40-60)§	40 (20-80)	40 (40-60)§
$t_{½}$ (h)	0.7 ± 0.3	0.8 ± 0.3	0.7 ± 0.2	0.8 ± 0.2
% of control and range	100%	106% (57%-180%)	93% (49%-133%)	107% (66%-148%)
AUC(0-∞) (U · h/mL)	1.3 ± 0.7	3.5 ± 1.6*	1.4 ± 1.0†	3.5 ± 1.9*‡
% of control and range	100%	264% (172%-391%)	108% (55%-214%)	266% (154%-411%)
M4-to-repaglinide AUC(0-∞) ratio (U/ng)	0.35 ± 0.15	0.54 ± 0.21*	0.39 ± 0.16†	0.50 ± 0.19*‖
% of control and range	100%	153% (113%-281%)	109% (68%-192%)	141% (82%-201%)

Data are given as mean ± SD.
*$P < .001$, versus placebo phase.
†$P < .001$, versus telithromycin phase.
‡$P < .001$, versus montelukast phase.
§$P < .05$, versus placebo phase.
‖$P < .05$, versus montelukast phase.
¶$P < .05$, versus telithromycin phase.

and gemfibrozil results in simultaneous inhibition of CYP2C8, CYP3A4, and OATP1B1.

In this study telithromycin increased repaglinide AUC by almost 2-fold, which is even more than other potent inhibitors of CYP3A4 (itraconazole and clarithromycin)[13,14] have increased the AUC of repaglinide. Telithromycin also increased the excretion of unchanged repaglinide into urine and decreased the ratio of the M1 metabolite (formed primarily by CYP3A4)[11,22] to repaglinide in urine and plasma. These data indicate that telithromycin inhibited the CYP3A4-catalyzed biotransformation of repaglinide. In previous studies the CYP3A4 inhibitors cyclosporine[22] and itraconazole[14] have similarly reduced the ratio of M1 to repaglinide. In plasma the AUC ratios of M2 (formed by both CYP2C8 and CYP3A4 in vitro)[11] and M4 (formed primarily by CYP2C8 in vitro)[11,22] to repaglinide were increased by telithromycin, but there

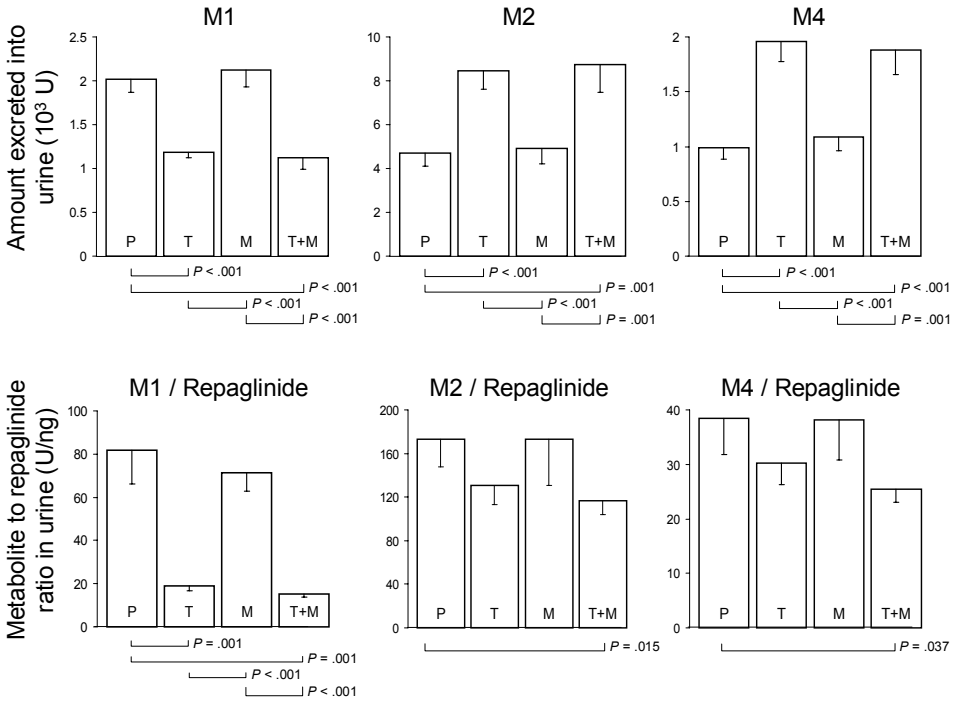

Fig 3. Mean − SEM excretion of M1, M2, and M4 metabolites of repaglinide into urine (*top*) and M1-, M2-, and M4-to-repaglinide ratios in urine (*bottom*). P, Placebo phase; T, telithromycin phase; M, montelukast phase; T+M, telithromycin and montelukast phase.

was no increase (and, in fact, there was a slight decrease) in the ratios of M2 and M4 to repaglinide in urine (Fig 3). These findings may be explained by the increased renal clearance of repaglinide in the telithromycin and telithromycin-montelukast phases and suggest that CYP3A4 plays only a minor role in the formation of M2 and M4 in vivo.

Both CYP2C8 and CYP3A4 are functionally expressed in the gut mucosa, as well as in the liver.[27] The CYP3A4 content of the intestinal mucosa exceeds its CYP2C8 content,[28,29] and at least CYP3A4 can markedly contribute to the first-pass metabolism of drugs. In our study the C_{max} and AUC of repaglinide were increased by telithromycin, whereas no effect on $t_{½}$ was seen. Moreover, as shown in Fig 2, the formation of the M1 metabolite (formed primarily by CYP3A4)[11,22] was already inhibited before the C_{max} of repaglinide was reached, indicating inhibition of the first-pass metabolism of repaglinide. These findings suggest that telithromycin raised the plasma concentrations of repaglinide mainly by inhibiting its presystemic metabolism, occurring presumably in the gut wall and the liver.

In the telithromycin phase the maximum and mean blood glucose concentrations (from 0 to 3 hours) were lower than in the placebo phase. The differences in blood glucose concentrations between the telithromycin-montelukast phase and the placebo phase were smaller. The relatively modest differences in the glucose-lowering effect of repaglinide between the study phases, despite a nearly doubled repaglinide AUC by telithromycin, can be explained by the small repaglinide dose used (for safety reasons), as well as the frequent carbohydrate intake after repaglinide ingestion to prevent hypoglycemia. However, because the blood glucose–lowering effect of repaglinide is dose- and concentration-dependent,[8] concomitant use of telithromycin with repaglinide may increase the risk of hypoglycemia, particularly if higher repaglinide doses are used.

Montelukast had no significant effect on the pharmacokinetics or pharmacodynamics of repaglinide when administered alone or with telithromycin. The ratio of M4 (formed almost exclusively by CYP2C8)[11,22] to repaglinide in plasma and in urine was unaffected by montelukast, even when the CYP3A4-mediated part of repaglinide metabolism was simultaneously inhibited by telithromycin. Moreover, montelukast had no effect on the M1 metabolite of repaglinide, formed by CYP3A4, or on the pharmacokinetics of telithromycin, a substrate of CYP3A4.[30]

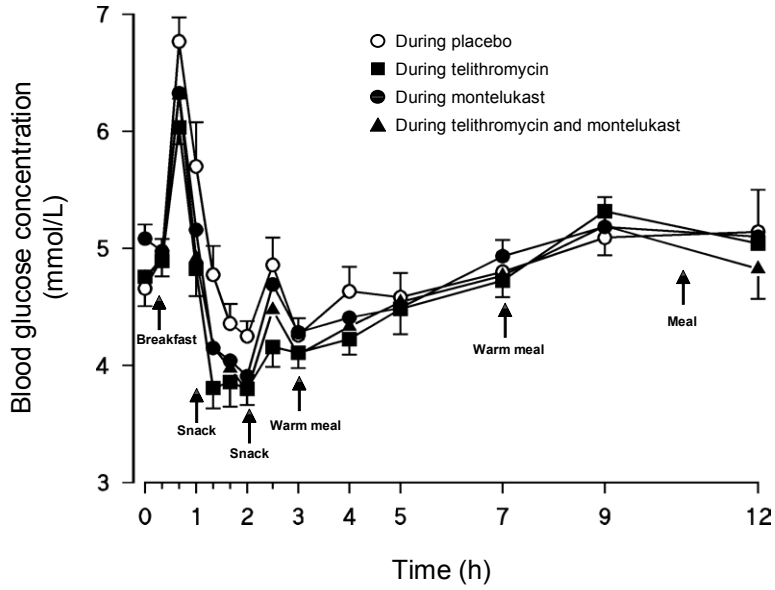

Fig 4. Mean ± SEM blood glucose concentrations in 12 healthy volunteers after single oral dose of 0.25 mg repaglinide after 3-day treatment with 800 mg telithromycin (*squares*), 10 mg montelukast (*solid circles*), both telithromycin and montelukast (*triangles*), or placebo (*open circles*) once daily. Representative *error bars* are shown, but for reasons of clarity, some have been omitted.

Table III. Blood glucose concentrations after single oral dose of 0.25 mg repaglinide in 12 healthy volunteers after 3-day treatment with 800 mg telithromycin, 10 mg montelukast, both telithromycin and montelukast, or placebo once daily

Variable	*Placebo phase (control)*	*Telithromycin phase*	*Montelukast phase*	*Telithromycin and montelukast phase*
Minimum concentration (mmol/L)	3.9 ± 0.5	3.5 ± 0.5	3.7 ± 0.4	3.5 ± 0.4
Maximum concentration (mmol/L)	6.9 ± 0.8	6.2 ± 0.5*	6.5 ± 0.7	6.4 ± 0.7*
Mean concentration from 0 to 3 h (mmol/L)	5.0 ± 0.6	4.4 ± 0.3*	4.7 ± 0.4†	4.6 ± 0.4
Mean concentration from 0 to 7 h (mmol/L)	4.8 ± 0.5	4.4 ± 0.3	4.6 ± 0.3†	4.5 ± 0.4
Mean concentration from 0 to 12 h (mmol/L)	4.9 ± 0.5	4.7 ± 0.3	4.8 ± 0.3	4.7 ± 0.3

Data are given as mean ± SD.
*$P < .05$, versus placebo phase.
†$P < .05$, versus telithromycin phase.

It has been estimated previously, based on in vitro CYP2C8 inhibition data and therapeutic concentrations of montelukast in plasma, that the plasma concentrations of a CYP2C8 substrate such as repaglinide could be increased considerably by montelukast in humans.[5] The estimated increase in the AUC of a CYP2C8-cleared drug ranged from 2-fold (based on unbound montelukast C_{max} and assuming a free fraction of 1%) to over 100-fold (based on total montelukast C_{max}).[5]

The available in vivo and in vitro evidence suggests that the contribution of CYP2C8 to the metabolic clearance of repaglinide is at least 50%. However, it is likely that, during the study phases that included inhibition of CYP3A4 by telithromycin, the contribution of CYP2C8 was closer to 100%. Accordingly, the lack of effect of montelukast on repaglinide pharmacokinetics and CYP2C8-mediated metabolism in our study suggests that the effect of montelukast on CYP2C8 in vivo is very weak. The apparent discrepancy between the in vitro estimates and our in vivo findings could be explained by the protein binding of montelukast being higher than that assumed in the estimation. The total plasma concentrations of montelukast observed in this study were similar to those previously reported.[31] The plasma protein binding of montelukast has been reported

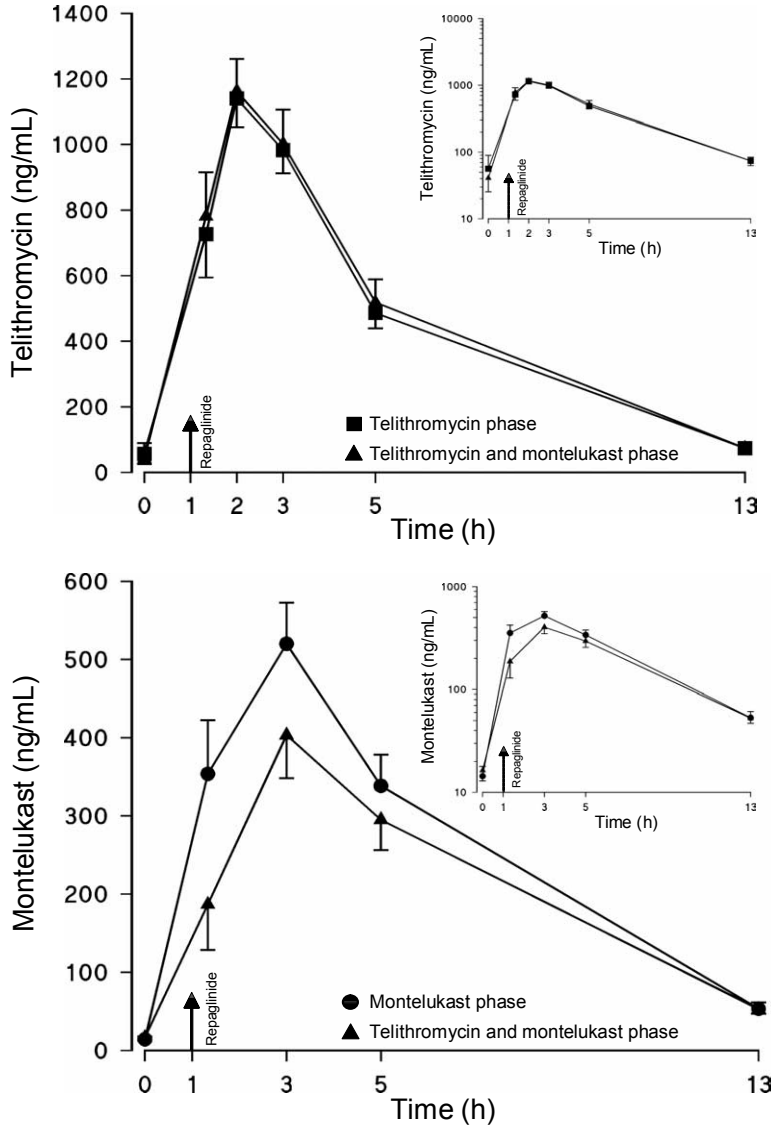

Fig 5. Mean ± SEM plasma concentrations of telithromycin (*top*) and montelukast (*bottom*) on day 3 in 12 healthy volunteers after last dose of 800 mg telithromycin or 10 mg montelukast (or both) in telithromycin (*squares*), montelukast (*circles*), or telithromycin and montelukast (*triangles*) phase. Time zero refers to administration of telithromycin or montelukast (or both) (ie, 1 hour before the administration of repaglinide). The *insets* depict the same data on a semilogarithmic scale.

to be more than 99%, but the exact free fraction is not known.[3,32] Assuming a free fraction of 1% for montelukast and that only 50% of the metabolism of repaglinide is mediated by CYP2C8, the equation and K_i (0.0092 μmol/L) applied by Walsky et al[5] would yield an estimate of a 31% to 32% increase in the AUC of repaglinide by montelukast (Table IV). However, when an assumed free fraction of 0.1% is used, montelukast would be estimated to cause only a 4% to 10% increase in the AUC of a drug cleared 50% to 100% by CYP2C8. The latter calculations agree well with our in vivo results and suggest that the high protein binding of montelukast limits its concentration available to CYP2C8 in vivo. The findings underline the importance of carrying out drug interaction studies in humans to confirm predictions based on in vitro data and highlight the significance of incorporating plasma protein binding to predictions of CYP inhibition–based interactions.

Table IV. Estimated effect of montelukast on the AUC of a drug metabolized by CYP2C8 in vivo, based on inhibition of CYP2C8 activity by montelukast in vitro

In vivo inhibitor concentration available to the enzyme	*Assumed unbound fraction (f_u)*	*Equation used to estimate the in vivo inhibitor concentration available to the enzyme ($[I]_{in\ vivo}$)*	*Magnitude of drug interaction ($AUC_{inhibited}/AUC_{control}$)*	
			Substrate metabolized 100% by CYP2C8 ($f_m = 1$)	*Substrate metabolized 50% by CYP2C8 ($f_m = 0.5$)*
Systemic total C_{max}	—	$[I]_{in\ vivo} = C_{max}$	91	1.98
Systemic free C_{max}	1%	$[I]_{in\ vivo} = f_u \cdot C_{max}$	1.89	1.31
Systemic free C_{max}	0.5%	$[I]_{in\ vivo} = f_u \cdot C_{max}$	1.45	1.18
Systemic free C_{max}	0.1%	$[I]_{in\ vivo} = f_u \cdot C_{max}$	1.09	1.04
Estimated total portal C_{max}	—	$[I]_{in\ vivo} = C_{max} + k_a \cdot F_a \cdot D/Q_h$	97	1.98
Estimated portal free C_{max}	1%	$[I]_{in\ vivo} = f_u \cdot (C_{max} + k_a \cdot F_a \cdot D/Q_h)$	1.96	1.32
Estimated portal free C_{max}	0.5%	$[I]_{in\ vivo} = f_u \cdot (C_{max} + k_a \cdot F_a \cdot D/Q_h)$	1.48	1.19
Estimated portal free C_{max}	0.1%	$[I]_{in\ vivo} = f_u \cdot (C_{max} + k_a \cdot F_a \cdot D/Q_h)$	1.10	1.05

Values used for montelukast include the following: mean peak concentration in plasma in the 2 phases of this study (C_{max}) (482 ng/mL), dose (D) (10 mg), bioavailability (F_a) (0.62),[5] unbound fraction (f_u) (0.1%-1%), absorption rate constant (k_a) (0.008 L/min),[5] and inhibition constant (K_i) (0.0092 μmol/L).[5] The equation used in the estimation is as follows:

$AUC_{inhibited}/AUC_{control} = 1/(f_m/[1 + [1]_{in\ vivo}/K_i] + [1 - f_m])$

AUC, Area under concentration-time curve; f_m, fraction metabolized by CYP2C8; Q_h, hepatic blood flow (1500 mL/min).[33]

In conclusion, telithromycin increased the plasma concentrations and blood glucose–lowering effect of repaglinide by inhibiting its CYP3A4-catalyzed biotransformation. The possibility of an increased risk of hypoglycemia should be considered when the 2 drugs are used concomitantly. Montelukast had no effect on the pharmacokinetics of repaglinide, suggesting that it does not significantly inhibit CYP2C8 in vivo. This lack of effect of montelukast on CYP2C8 in vivo is probably a result of its extensive binding to plasma proteins, limiting its concentration at the enzyme site in vivo.

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References

1. Clark JP, Langston E. Ketolides: a new class of antibacterial agents for treatment of community-acquired respiratory tract infections in a primary care setting. Mayo Clin Proc 2003;78:1113-24.
2. File TM. Telithromycin new product overview. J Allergy Clin Immunol 2005;115:S1-13.
3. Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast Clinical Research Study Group. Arch Intern Med 1998;158:1213-20.
4. Jarvis B, Markham A. Montelukast: a review of its therapeutic potential in persistent asthma. Drugs 2000;59: 891-928.
5. Walsky RL, Obach RS, Gaman EA, Gleeson JP, Proctor WR. Selective inhibition of human cytochrome P4502C8 by montelukast. Drug Metab Dispos 2005;33:413-8.
6. Walsky RL, Gaman EA, Obach RS. Examination of 209 drugs for inhibition of cytochrome P450 2C8. J Clin Pharmacol 2005;45:68-78.
7. 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.
8. Hatorp V. Clinical pharmacokinetics and pharmacodynamics of repaglinide. Clin Pharmacokinet 2002;41:471-83.
9. 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.
10. 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.
11. 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.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. 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.
19. Montay G, Chevalier P, Guimart C, Boudraa Y, Guillaume M, Shi J. A 12-hour dosing interval reduces the pharmacokinetic interaction between telithromycin and simvastatin [abstract]. In: Proceedings of the Forty-third Interscience Conference on Antimicrobial Agents and Chemotherapy; 2003 Sep 14-17; Chicago, Illinois. Chicago: The Conference; 2003.
20. Namour F, Sultan E, Pascual MH, Lenfant B. Penetration of telithromycin (HMR 3647), a new ketolide antimicrobial, into inflammatory blister fluid following oral administration. J Antimicrob Chemother 2002;49:1035-8.
21. Radhakrishna T, Narasaraju A, Ramakrishna M, Satyanarayana A. Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods. J Pharm Biomed Anal 2003;31:359-68.
22. Kajosaari LI, Niemi M, Neuvonen M, Laitila J, Neuvonen PJ, Backman JT. Cyclosporine markedly raises the plasma concentrations of repaglinide. Clin Pharmacol Ther 2005;78:388-99.
23. Kim RB. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) and genetic variability (single nucleotide polymorphisms) in a hepatic drug uptake transporter: what's it all about? Clin Pharmacol Ther 2004;75:381-5.
24. Åsberg A. Interactions between cyclosporin and lipid-lowering drugs: implications for organ transplant recipients. Drugs 2003;63:367-78.
25. 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.
26. 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.
27. Glaeser H, Drescher S, Eichelbaum M, Fromm MF. Influence of rifampicin on the expression and function of human intestinal cytochrome P450 enzymes. Br J Clin Pharmacol 2005;59:199-206.
28. Läpple F, von Richter O, Fromm MF, Richter T, Thon KP, Wisser H, et al. Differential expression and function of CYP2C isoforms in human intestine and liver. Pharmacogenetics 2003;13:565-75.
29. von Richter O, Burk O, Fromm MF, Thon KP, Eichelbaum M, Kivistö KT. Cytochrome P450 3A4 and P-glycoprotein expression in human small intestinal enterocytes and hepatocytes: a comparative analysis in paired tissue specimens. Clin Pharmacol Ther 2004;75:172-83.
30. Ketek [prescribing information]. Kansas City (MO): Aventis Pharmaceuticals; 2004.
31. Zhao JJ, Rogers JD, Holland SD, Larson P, Amin RD, Haesen R, et al. Pharmacokinetics and bioavailability of montelukast sodium (MK-0476) in healthy young and elderly volunteers. Biopharm Drug Dispos 1997;18:769-77.
32. Knorr B, Matz J, Bernstein JA, Nguyen H, Seidenberg BC, Reiss TF, et al. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. JAMA 1998;279:1181-6.
33. Lin JH, Lu AY. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev 1997;49:403-49.