PHARMACOKINETICS AND DRUG DISPOSITION

Intestinal expression of P-glycoprotein (*ABCB1*), multidrug resistance associated protein 2 (*ABCC2*), and uridine diphosphate–glucuronosyltransferase 1A1 predicts the disposition and modulates the effects of the cholesterol absorption inhibitor ezetimibe in humans

Background and Aims: **Ezetimibe is an inhibitor of the cholesterol uptake transporter Niemann-Pick C1-like protein (NPC1L1). Target concentrations can be influenced by intestinal uridine diphosphate–glucuronosyltransferases (UGTs) and the efflux transporters P-glycoprotein (P-gp) (*ABCB1*) and multidrug resistance associated protein 2 (MRP2) (*ABCC2*). This study evaluates the contribution of these factors to the disposition and cholesterol-lowering effect of ezetimibe before and after induction of UGT1A1, P-gp, and MRP2 with rifampin (INN, rifampicin).**

Methods: **Serum concentrations of ezetimibe, as well as its glucuronide, and the plant sterols campesterol and sitosterol (surrogate for cholesterol absorption) were studied in 12 healthy subjects before and after rifampin comedication. In parallel, duodenal expression of UGT1A1, P-gp, MRP2, and NPC1L1 was quantified by use of real-time reverse transcriptase–polymerase chain reaction and quantitative immunohistochemical evaluation. The affinity of ezetimibe and its glucuronide to P-gp and MRP2 was assessed in P-gp–**

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Stefan Oswald, PhD, Sierk Haenisch, PhD, Christiane Fricke, Thomas Sudhop, MD, Cornelia Remmler, PhD, Thomas Giessmann, MD, Gabriele Jedlitschky, PhD, Ulrike Adam, PhD, Eike Dazert, PhD, Rolf Warzok, MD, Wolfram Wacke, MD, Ingolf Cascorbi, PhD, MD, Heyo K. Kroemer, PhD, Werner Weitschies, PhD, Klaus von Bergmann, MD, and Werner Siegmund, MD *Greifswald, Kiel, and Bonn, Germany*

From the Departments of Clinical Pharmacology, Pharmacology, and Biopharmaceutics and Pharmaceutical Technology, Peter Holtz Research Center of Pharmacology and Experimental Therapeutics, and Departments of Internal Medicine and Pathology, University of Greifswald, Greifswald; Department of Pharmacology, University of Kiel, Kiel; and Department of Clinical Pharmacology, University of Bonn, Bonn.

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Reprint requests: Werner Siegmund, MD, Department of Clinical Pharmacology, Ernst Moritz Arndt University, Friedrich-Loeffler-Strasse 23d, D-17487 Greifswald, Germany.

E-mail: *siegmuw@uni-greifswald.de*

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overexpressing Madin-Darby canine kidney II cells and P-gp–containing or MRP2-containing inside-out vesicles.

Results: **Up-regulation of intestinal P-gp, MRP2, and UGT1A1 (but not of NPC1L1) by rifampin was associated with markedly decreased areas under the curve of ezetimibe and its glucuronide (116 ± 78.1 ng · h/mL versus 49.9 ± 31.0 ng · h/mL and 635 ± 302 ng · h/mL versus 225 ± 86.4 ng · h/mL, respectively; both $P = .002$) and increased intestinal clearances (2400 ± 1560 mL/min versus 5500 ± 4610 mL/min [$P = .003$] and 76.6 ± 113 mL/min versus 316 ± 457 mL/min [$P = .010$], respectively) and nearly abolished sterol-lowering effects. Intestinal expression of *UGT1A1*, *ABCB1*, and *ABCC2* was inversely correlated with the effects of ezetimibe on plant sterol serum concentrations. Parallel in vitro studies confirmed that ezetimibe glucuronide is a high-affinity substrate of MRP2 and has a low affinity to P-gp whereas ezetimibe interacts with P-gp and MRP2.**

Conclusions: **The disposition and sterol-lowering effects of ezetimibe are modified by metabolic degradation of the drug via intestinal UGT1A1 and either intestinal or hepatic secretion (or both) via P-gp and MRP2. (Clin Pharmacol Ther 2006;79:206-17.)**

Recent clinical trials have demonstrated that intense lipid-lowering medication with high doses of 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) provides a significant reduction in the cardiovascular mortality rate. This benefit, however, is associated with a greater incidence of side effects than at moderate doses.[1,2] Increased efficacy and safety are expected from the combination of low-dose statins with ezetimibe, the new cholesterol-lowering agent that modulates intestinal sterol absorption by interaction with the aminopeptidase N and inhibition of the recently identified sterol transporter Niemann-Pick C1-like protein (NPC1L1), which is most highly expressed in the duodenum, jejunum, and ileum—the known sites of cholesterol uptake.[3-7] Contrary to statins, ezetimibe is a substrate of uridine diphosphate–glucuronosyltransferase (UGT) 1A1 and UGT1A3 and has a lower affinity for UGT2B15, but it is not metabolized by cytochrome P450s.[8-10] The orally administered drug undergoes nearly complete glucuronidation by UGTs located in the small-intestinal wall, of which 1A1 is the major isoform.[11,12] The glucuronide has been suggested to contribute to the long-lasting cholesterol-lowering properties.[12] It is unclear, however, whether NPC1L1, which is located in the apical membrane of enterocytes, is inhibited by binding of ezetimibe or its glucuronide to luminal or cytoplasmatic receptor areas of the transporter.[4,6,12,13] There is evidence from drug interaction studies with cyclosporine (INN, ciclosporin) and fibrates that ezetimibe and its glucuronide are substrates of the intestinal efflux transporters P-glycoprotein (P-gp) (*ABCB1*) or multidrug resistance associated protein 2 (MRP2) (*ABCC2*) (or both).[14-17] Both proteins are located in the vicinity of the cholesterol uptake transporter on the crest of the small intestinal villi.[18] A combined action of glucuronidation and subsequent elimination by transporters could modulate the concentration of ezetimibe and its glucuronide on apical and intracellular sites of action. Accordingly, to define the factors influencing ezetimibe disposition and hence pharmacologic action, we determined concentration-time profiles and renal and intestinal excretion of ezetimibe and its glucuronide in healthy subjects treated with the pregnane X receptor (PXR) inducer rifampin (INN, rifampicin), which is known to regulate P-gp, MRP2, and UGT1A1.[19,20] To confirm the affinity of ezetimibe and its glucuronide to multidrug transporter proteins, competition assays with substrates of P-gp and MRP2 were performed in *ABCB1*- and *ABCC2*-transfected cells. To evaluate the pharmacologic effects of ezetimibe, serum concentrations of the plant sterols campesterol, sitosterol, and cholesterol were monitored. The changes in campesterol and sitosterol serum levels were used as surrogates for the changes in cholesterol absorption after ezetimibe administration because plant sterols, unlike cholesterol, are not endogenously formed by rifampin-inducible hepatic enzymes.[21,22]

The study findings provide evidence that UGT1A1, P-gp, and MRP2 expression in the intestine determines the disposition and sterol-lowering effect of ezetimibe. Up-regulation of UGT1A1 and intestinal transporters by rifampin leads to markedly lower serum concentrations of ezetimibe and its glucuronide, which are paralleled by increased intestinal elimination. Consequently, coadministration of rifampin significantly reduces the effects of ezetimibe.

METHODS

Clinical study protocol

Subjects. Twelve healthy subjects (1 woman and 11 men; age range, 21-31 years; body mass index range, 19.2-26.4 kg/m^2) were selected according to their *ABCC2* genotype.[23,24] Of the subjects, 6 were carriers of the TT allele and 6 were carriers of the CC allele of the *ABCC2* − 24C> T polymorphism. Additional screening for *ABCB1* polymorphisms identified 3 GG carriers, 7 GT carriers, 1 TT carrier, and 1 GA carrier of G2677T/A and 4 CC carriers, 7 CT carriers, and 1 TT carrier of C3435T.[25] The subjects were in good health as confirmed by medical histories, physical examinations, and routine clinical-chemical and hematologic screenings. The subjects abstained from alcohol and cigarette smoking and took no medication except for 1 woman who took hormonal contraceptives. A standardized diet was served during hospitalization. The study protocol was approved by the local ethical committee, and all subjects gave written informed consent.

Study protocol. After inclusion, the subjects underwent a gastroduodenoscopy and 4 tissue specimens (about 3 × 3 mm each) were taken with multibite forceps from the lower duodenal mucosa, of which 1 piece was immediately transferred into formaldehyde for semiquantitative immunohistochemical analysis and the other 3 pieces were transferred into liquid nitrogen for messenger ribonucleic acid (mRNA) analysis. One to 4 days later, the subjects were hospitalized in the evening before the pharmacokinetic study. After overnight fasting for at least 10 hours, 20 mg ezetimibe (10-mg fast-release tablets; MSD Sharp & Dome, Haar, Germany) was given orally with 200 mL tap water. A standard lunch and dinner were served 5 hours and 11 hours, respectively, after drug intake. Forearm venous blood (5 mL) was sampled before and at 0.33, 0.66, 1, 1.33, 1.66, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, and 48 hours after drug intake. Urine and feces were collected for 7 days. In the evenings of study days 8 to 15, rifampin (600-mg fast-release coated tablet; Grünenthal, Aachen, Germany) was given orally with 100 mL tap water. The last rifampin dose in the evening of the 15th study day was given exactly 12 hours before administration of ezetimibe the next morning. A second duodenal biopsy was performed on the last day of rifampin treatment. The disposition of ezetimibe on the 16th study day was measured again as described previously. Serum, aliquots of urine, and homogenized feces were stored at least at − 80°C until quantitative analysis.

Quantitative assay of ezetimibe

Ezetimibe in serum, urine, and feces was quantified by use of a liquid chromatography–tandem mass spectrometry system via the column XTerra MS (C_{18}; 2.1 × 100 mm; particle size, 3.5 μm) (Waters, Milford, Mass) and the PE Sciex API 2000 mass spectrometer (Applied Biosystems, Darmstadt, Germany). The mobile phase (flow rate, 200 μL/min) was acetonitrile/water (60:40 [vol/vol]). Unchanged ezetimibe and total ezetimibe after hydrolysis with β-glucuronidase (>100,000 Fishman units/mL) (Sigma-Aldrich, Taufkirchen, Germany) at 50°C for 60 minutes were extracted with methyl *tert*-butyl ether. The mass-to-charge transition was monitored in the negative mode at 408 to 271 for ezetimibe and 223 to 117 for the internal standard (4-hydroxychalcone). The limits of quantification for serum were 0.1 ng/mL for ezetimibe and 1.0 ng/mL for total ezetimibe. The limits of quantification for urine and feces were 0.025 and 0.1 μg/mL, respectively. The recovery of ezetimibe from serum, urine, and feces ranged from 91.3% to 113.7%, 87.1% to 102.1%, and 83.6% to 92.2% respectively. Within-day and between-day accuracy and precision of calibration curves and quality controls were within 13.5%. Ezetimibe was stable in human feces at room temperature for at least 24 hours, whereas the glucuronide was nearly completely hydrolyzed (93%), the major portion during the first hour (84%).

Analysis of sterols

The concentrations of total cholesterol, lathosterol, and cholic acid, as well as the plant sterols campesterol and sitosterol, were measured in serum samples taken before and 48 hours after ezetimibe administration (before and after rifampin comedication) by use of a gas chromatographic method with 5α-cholestane as the internal standard.[22] The method was specific for the respective sterols with a limit of quantification of 0.1 mg/dL for cholesterol, lathosterol, cholic acid, sitosterol, and campesterol. The variability of within-day and between-day accuracy and precision for all analytes was lower than 4% of the respective nominal and mean values, respectively.

Quantification of gene expression

ABCB1 mRNA and *ABCC2* mRNA expression was quantified by real-time reverse transcriptase–polymerase chain reaction according to *Taq*Man technology (Applied Biosystems).[23,24] Expression of *UGT1A1* mRNA was quantified by use of the primers UGT1A1_12F 5′-GGA ATC AAC TGC CTT CAC CAA AAT C-3′ and UGT1A1_12-12R 5′-ACA ATT

CCA TGT TCT CCA GAA GCA T-3′ and the *Taq*Man probe 5′-FAM-CAC TAT CCC AGG AAT TTG-TAMRA-3′. *NPC1L1* mRNA was quantified by use of an Assay-on-Demand assay (Hs00203602_m1) as recommended by the manufacturer (Applied Biosystems). The reference gene for all quantifications was *18S* mRNA by use of the delta CT (cycles of threshold) method.[26]

Duodenal P-gp and MRP2 content was quantified immunohistochemically with the monoclonal antibodies JSB-1 and M_2III-6 (Alexis, Grünberg, Germany), respectively, by use of the labeled streptavidin-biotin method.[24,27]

In vitro transport assays

Competition assays in P-gp–overexpressing cells. Competition assays in P-gp–overexpressing cells were performed on the basis of the calcein-AM transport.[28] Parental and *ABCB1*-transfected Madin-Darby canine kidney II (MDCKII) cells were cultured in Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Biochrom, Berlin, Germany, and Merck, Darmstadt, Germany). The cells were seeded on 96-well plates (BD Falcon, Bedford, Mass) (density, 70,000 cells per well). After culturing for 3 days, washing, and equilibration, the cells were preincubated for 30 minutes in buffer (142-mmol/L sodium chloride, 1-mmol/L potassium bishydrogen phosphate, 5-mmol/L glucose, 5-mmol/L potassium chloride, 1.5-mmol/L calcium chloride, 1.2-mmol/L magnesium sulfate, and 12.5-mmol/L HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)]. Subsequently, calcein AM (final concentration, 1 μmol/L) (A.G. Scientific, San Diego, Calif) was added, and the intracellular fluorescence signal of free calcein was measured at 485 nm (excitation) and 538 nm (emission) with the Fluoroskan II microplate reader (Labsystems, Helsinki, Finland) at defined times. The influence of ezetimibe and its glucuronide on calcein uptake was compared with the effect of the P-gp inhibitor PSC833 (valspodar, 0.01 mmol/L).

Transport studies with isolated membrane vesicles. Plasma membrane vesicles from *ABCB1*- and *ABCC2*-transfected LLC and 2008 cells, respectively (kindly provided by Dr A. H. Schinkel and Dr P. Borst, Netherlands Cancer Institute, Amsterdam, The Netherlands), were prepared, and adenosine triphosphate (ATP)–dependent transport of rhodamine 123 and [6,7-^{3}H]estradiol-17β–glucuronide into the inside-out membrane vesicles was measured by centrifugation of the vesicles through a gel matrix by use of nick-translation spin columns or by rapid filtration through nitrocellulose filters essentially as described.[29] Membrane vesicles (130 or 50 μg protein/100 μL) were incubated in the presence of 4-mmol/L ATP or 5′–adenosine monophosphate, 10-mmol/L magnesium chloride, 10-mmol/L creatine phosphate, 100 μg/mL creatine kinase, and 100 μmol/L of the P-gp substrate rhodamine 123 or 200 nmol/L of the MRP2 substrate [6,7-^{3}H]estradiol-17β–glucuronide (1.67 TBq/mmol) (Perkin-Elmer, Boston, Mass) in an incubation buffer containing 250-mmol/L sucrose and 10-mmol/L Tris–hydrochloric acid, pH 7.4 (final volume, 75 μL). Aliquots (20 μL) of the incubations were taken at the times indicated. In the case of rhodamine 123 transport, these aliquots were diluted in 80 μL ice-cold incubation buffer and immediately loaded onto Sephadex G50 (Amersham Biosciences, Uppsala, Sweden) nick-translation spin columns. The columns were rinsed with 100 μL of ice-cold incubation buffer and centrifuged for 3 minutes at 4°C and 400*g*. The effluents were collected into vials containing 20 μL of solubilization buffer (2% sodium dodecyl sulfate in 5-mmol/L Tris–hydrochloric acid, pH 7.4). The rhodamine 123 concentration was measured fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 485 nm. In the case of [^{3}H]estradiol-17β–glucuronide transport, aliquots were diluted in 1 mL of ice-cold incubation buffer and filtered immediately through nitrocellulose filters (pore size of 0.2 μm, presoaked in incubation buffer). Filters were rinsed with 5 mL of incubation buffer and dissolved in liquid scintillation fluid, and radioactivity was measured. Rates of ATP-dependent transport were calculated by subtracting the control values measured in the presence of 5′–adenosine monophosphate from those measured in the presence of ATP. The competition assays were done with 0.01-mmol/L and 0.1-mmol/L ezetimibe and its glucuronide, 0.1-mmol/L verapamil (in the case of P-gp), or the respective concentration of the solvent.

Pharmacokinetic and statistical evaluation

Values for maximum plasma concentration (C_{max}) and time to C_{max} were taken from the concentration-time curves. The area under the serum concentration-time curve (AUC) from 0 to 48 hours ($AUC_{0\text{-}48}$) was calculated via the trapezoidal rule. The elimination half-life was estimated from logarithmic values of the terminal data points. Oral clearance was assessed by dose/$AUC_{0\text{-}48}$, and renal clearance, metabolic clearance, and intestinal clearance were derived from the respective cumulative amounts of ezetimibe and the glucuronide excreted into the urine and feces over the

Table I. Expression of intestinal P-gp, MRP2, UGT1A1, and NPC1L1 in 12 healthy subjects before and after induction with rifampin

	P-glycoprotein		*MRP2*		*UGT1A1*	*NPC1L1*
	mRNA	*Protein*	*mRNA*	*Protein*	*mRNA*	*mRNA*
Before rifampin	1.01 ± 0.45	28.8 ± 37.8	0.88 ± 0.33	32.1 ± 12.3	0.71 ± 0.33	0.96 ± 0.35
After rifampin	4.37 ± 1.61	44.1 ± 39.9	2.21 ± 0.88	74.0 ± 18.6	1.93 ± 0.84	0.97 ± 0.44
P value (Wilcoxon test)	.002	.004	.005	.002	.003	NS

The mRNA values were normalized to *18S* mRNA. Protein content is given in arbitrary units. Data are given as mean ± SD.
P-gp, P-glycoprotein; MRP2, multidrug resistance associated protein 2; UGT1A1, uridine diphosphate–glucuronosyltransferase 1A1; NPC1L1, Niemann-Pick C1-like 1 protein; mRNA, messenger ribonucleic acid; NS, not significant.

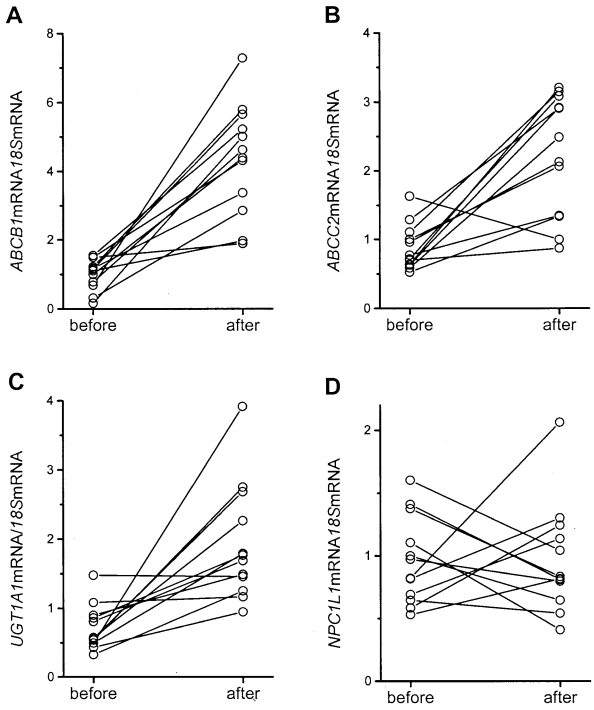

Fig 1. Individual messenger ribonucleic acid (mRNA) expression of duodenal *ABCB1* (**A**), *ABCC2* (**B**), uridine diphosphate–glucuronosyltransferase (UGT) 1A1 (**C**), and Niemann-Pick C1-like protein (*NPC1L1*) (**D**) relative to *18S* mRNA in 12 healthy subjects before and after induction with rifampin.

respective $AUC_{0\text{-}48}$ values. The biometric evaluation was done with appropriate methods of the sample statistics and Spearman rank test.

RESULTS

Pharmacokinetics

After 8 days of treatment with 600 mg rifampin, duodenal mRNA and protein content of P-gp, MRP2, and UGT1A1, but not NPC1L1, was significantly increased by rifampin (Table I). The basal expression in subjects without induction and the inducibility of P-gp, MRP2, and UGT1A1 by rifampin were highly variable as shown for mRNA expression in Fig 1. We observed significant correlation between *ABCC2* mRNA and MRP2 protein content ($r = 0.568$, $P < .004$) but not between *ABCB1* mRNA and P-gp protein content ($r = 0.324$, $P = .122$). A significant coregulation was measured between *ABCB1* mRNA and *ABCC2* mRNA ($r = 0.920$, $P < .001$), *UGT1A1* mRNA and *ABCB1* mRNA ($r = 0.894$, $P < .001$), and *UGT1A1* mRNA and *ABCC2* mRNA ($r = 0.855$, $P < .001$) expression.

The concentration-time curves of ezetimibe after oral administration showed an initial peak within 1 hour and at least 2 additional, even higher maximums just after lunch and dinner. The glucuronide was rapidly formed and exceeded the maximum serum concentrations of the parent compound in a significant manner (Fig 2 and Table II).

Induction of intestinal P-gp, MRP2, and UGT1A1 expression by rifampin resulted in more than a 50% decrease in the AUC of ezetimibe and its glucuronide. This was accompanied by a marked increase in the metabolic clearance of ezetimibe and the intestinal clearance of ezetimibe and of its glucuronide (Table II). The AUC of the glucuronide was correlated with intestinal *ABCB1* mRNA and *ABCC2* mRNA. In addition, there was a significant inverse correlation of the glucuronide with the intestinal *UGT1A1* mRNA content (Fig 3). The AUC also correlated significantly with the intestinal protein content of MRP2 ($r = -0.598$, $P = .002$) but not with P-gp ($r = -0.126$, $P = .557$). Nearly 60% of the dose was excreted via the feces as unchanged ezetimibe and about 10% as glucuronide. Rifampin comedication did not significantly change elimination rates and amounts of ezetimibe and its glucuronide excreted via feces (Table II).

The *ABCB1* and *ABCC2* genotype lacked any significant effects either on basal expression and inducibility of P-gp and MRP2 or on ezetimibe disposition.

Drug effects

Rifampin treatment was without a significant effect on cholesterol, plant sterol, and cholic acid serum levels. The initial values before and after induction were as follows: cholesterol, 192 ± 38.0 mg/dL versus 207 ± 34.4 mg/dL; sitosterol, 0.30 ± 0.08 mg/dL versus 0.28 ± 0.07 mg/dL; campesterol, 0.48 ± 0.14 mg/dL versus 0.44 ± 0.12 mg/dL; and cholic acid, 0.35 ± 0.06 mg/dL versus 0.35 ± 0.05 mg/dL. Lathosterol concentrations after rifampin were significantly increased (0.33 ± 0.18 mg/dL versus 0.50 ± 0.23 mg/dL, $P = .004$). A single oral dose of 20 mg ezetimibe reduced the serum concentrations of total cholesterol and the plant sterols campesterol and sitosterol in a significant manner but had no effect on lathosterol and cholic acid. After rifampin comedication, the effects of ezetimibe on plant sterol absorption were nearly abolished. Similar results were obtained by use of the ratios of plant sterols to cholesterol. The lathosterol-to-cholesterol ratio as a marker of endogenous cholesterol synthesis, however, was significantly increased after rifampin (Fig 4).

Induction of intestinal *UGT1A1* mRNA expression by rifampin resulted in a significantly reduced campesterol- and sitosterol-lowering effect of ezetimibe (Fig 5). Significant correlations were found between the ezetimibe effect on sterol serum levels and intestinal *ABCB1* mRNA ($r = -0.562$ and $P = .004$ for campesterol and $r = -0.500$ and $P = .013$ for sitosterol) and *ABCC2* mRNA ($r = -0.507$ and $P = .012$ for campesterol and $r = -0.445$ and $P = .029$ for sitosterol).

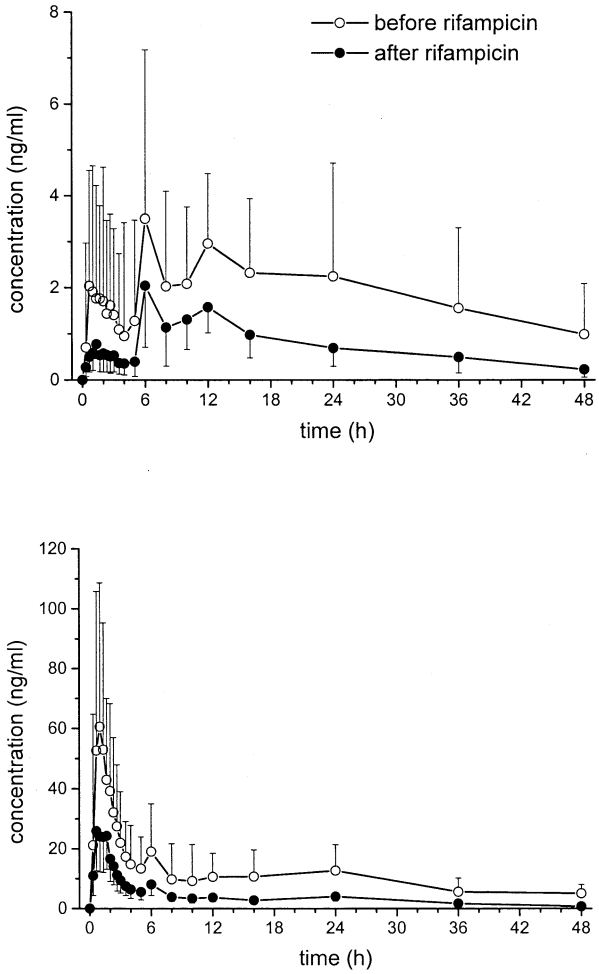

Fig 2. Serum concentration–time curves of ezetimibe (*top*) and its glucuronide (*bottom*) in 12 healthy subjects before pretreatment (*open circles*) and after pretreatment (*solid circles*) with 600 mg rifampin for 8 days. Geometric mean and geometric SD are given.

In vitro transport studies

In *ABCB1*-transfected MDCKII cells the intracellular fluorescence of the fluorophore calcein increased as a function of the ezetimibe concentration (0.06-0.6 mmol/L). The potency of ezetimibe as assessed by calculation of the concentration needed to double baseline fluorescence was approximately 0.2 mmol/L, whereas the P-gp inhibitor PSC833 (valspodar) showed a significantly higher inhibitory activity (Fig 6). Ezetimibe glucuronide had no inhibitory effect on P-gp in these cells (data not shown). In P-gp–containing inside-out vesicles, 0.1-mmol/L ezetimibe and its glucuronide inhibited rhodamine 123 transport by more than 60%. The ATP-dependent transport of estradiol-17β–glucuronide into isolated MRP2-containing inside-out vesicles was completely inhibited by the ezetimibe glucuronide at a low concentration (0.01 mmol/L). The parent ezetimibe was a weaker inhibitor (Fig 6).

DISCUSSION

The cholesterol-lowering drug ezetimibe modulates intestinal absorption of sterols by reducing intestinal sterol uptake.[4,6,7] After a single 20-mg oral dose, cholesterol, campesterol, and sitosterol serum levels and the plant sterol–to–cholesterol ratios were significantly reduced 48 hours after administration. The observed data indicate that intestinal glucuronidation by UGT1A1 and efflux via P-gp and MRP2 are major

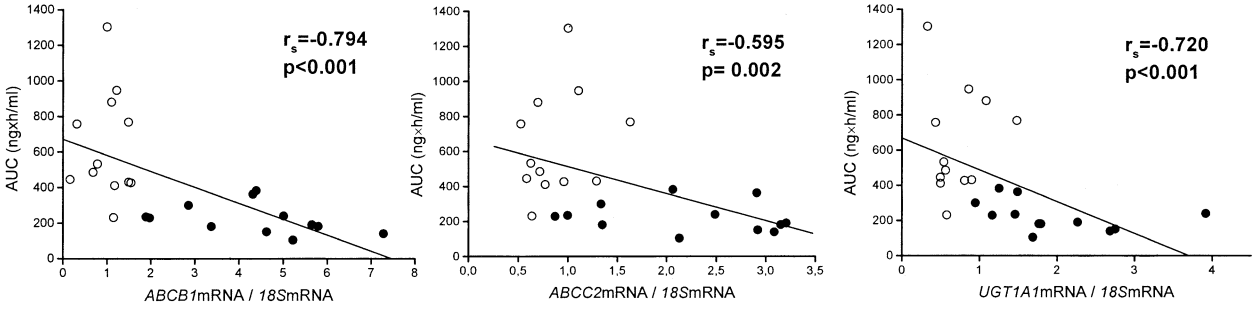

Fig 3. Correlations between area under concentration-time curve (AUC) of ezetimibe glucuronide and intestinal mRNA expression of *ABCB1* (*left*), *ABCC2* (*middle*), and *UGT1A1* (*right*). *Open circles* and *solid circles* indicate data before and after rifampin induction, respectively.

Table II. Pharmacokinetic characteristics of ezetimibe and its glucuronide before and after induction with rifampin in 12 healthy subjects

	Ezetimibe		*Ezetimibe glucuronide*	
	Before rifampin	*After rifampin*	*Before rifampin*	*After rifampin*
$AUC_{0\text{-}48}$ (ng · h/mL)	116 ± 78.1	49.9 ± 31.0 (P = .002)	635 ± 302	225 ± 86.4 (P = .002)
C_{max} (ng/mL)	5.09 ± 3.36	3.49 ± 2.17 (P = .041)	75.6 ± 43.1	40.5 ± 20.6 (P = .006)
t_{max} (h)	0.86 ± 0.22	0.94 ± 0.37	1.06 ± 0.51	1.22 ± 0.95
$t_{½}$ (h)	26.6 ± 20.3	27.1 ± 20.6	29.3 ± 32.5	25.3 ± 29.1
$CL_{intestinal}$ (mL/min)	2400 ± 1560	5500 ± 4610 (P = .003)	76.6 ± 113	316 ± 457 (P = .01)
CL_R (mL/min)	19.9 ± 22.1	Below LOQ	56.7 ± 30.6	57.2 ± 26.4
CL_M (mL/min)	903 ± 921	2080 ± 2380 (P = .007)	—	—
Ae_{feces} (mg)	11.5 ± 3.92	10.4 ± 4.56	2.15 ± 3.01	3.02 ± 3.16
Ae_{urine} (mg)	0.10 ± 0.12	Below LOQ	1.87 ± 0.74	0.72 ± 0.30 (P = .004)

Data are given as mean ± SD. P values indicate significance levels for comparisons to values before rifampin induction (Wilcoxon test).

$AUC_{0\text{-}48}$, Area under serum concentration–time curve from 0 to 48 hours; C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration; $t_{½}$, elimination half-life; $CL_{intestinal}$, apparent intestinal clearance; CL_R, renal clearance; CL_M, metabolic clearance; Ae_{feces}, cumulative amount excreted into feces; Ae_{urine}, cumulative amount excreted into urine; LOQ, limit of quantification.

determinants of the sterol-lowering effect. Up-regulation of glucuronidation and efflux transport by rifampin essentially abolished the effect of ezetimibe on campesterol and sitosterol serum concentrations. Serum plant sterol levels are suitable surrogate measures of the sterol-lowering effect of ezetimibe.[21,30] Rifampin has not been shown to regulate the sterol uptake transporter NPC1L1, and it is also not an inducer of the sterol efflux pumps ABCG5/G8 located on the apical membrane of enterocytes.[31] Therefore the reduced sterol-lowering effect of ezetimibe is not explainable by modulation of the specific sterol transporters. In accordance with published data the initial serum levels of campesterol and sitosterol remained unchanged in our study after treatment with rifampin.[22] By contrast, cholesterol disposition was influenced by rifampin-inducible endogenous processes as shown by the significant increase in the lathosterol-to-cholesterol ratio, an indirect marker of cholesterol synthesis.[22] Up-regulation of intestinal UGT1A1 by rifampin was associated, as expected, with lower serum concentrations of ezetimibe. The serum levels of the glucuronide (C_{max} and AUC), however, were reduced by about 60%; that is, induction of intestinal glucuronidation resulted in markedly lower rather than higher availability of the metabolite in the systemic circulation. Concurrently, a significant increase in the apparent intestinal clearance of the glucuronide was observed. Moreover, the intestinal clearance of unchanged ezetimibe was doubled after rifampin administration whereas renal clearance was reduced. On the basis of these data, it is concluded that the overall elimination of ezetimibe from the intestinal absorption compartment is caused by the interplay of P-gp, MRP2, and UGT1A1 as shown in Fig 7. The in vitro experiments with P-gp–overexpressing MDCKII cells and P-gp–containing or *MRP2*-

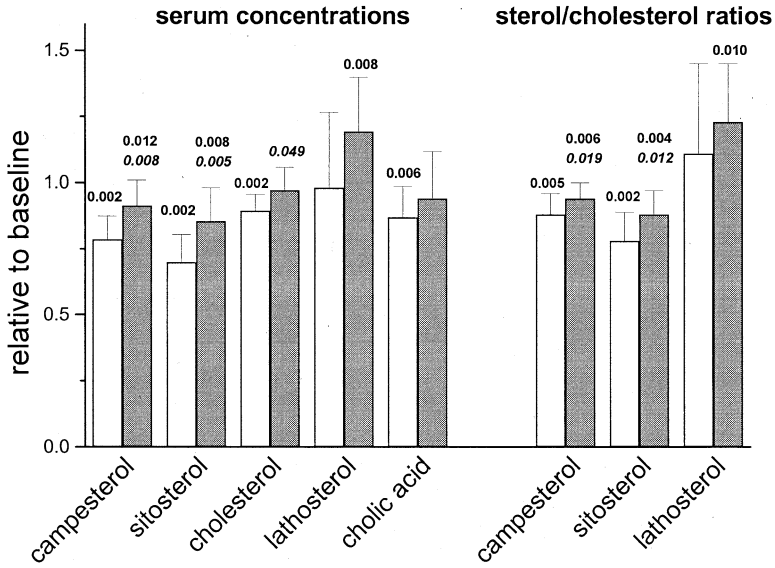

Fig 4. Sterol serum concentrations and sterol-to-cholesterol ratios 48 hours after single oral dose of 20 mg ezetimibe in 12 healthy subjects. The concentrations and ratios are given relative to the respective baseline values before (*white columns*) and after (*gray columns*) rifampin induction. *Columns* and *bars* indicate arithmetic means ± SD. *Nonitalic numbers* indicate significance levels for comparisons with the respective initial values. *Italic numbers* are for comparisons with values before rifampin induction (Wilcoxon test).

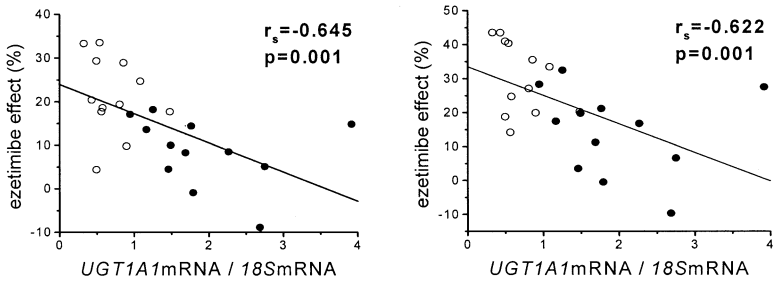

Fig 5. Campesterol-lowering effect (*left*) and sitosterol-lowering effect (*right*) of ezetimibe (in percent of baseline values) 48 hours after oral administration in correlation to intestinal contents of *UGT1A1* mRNA. *Open circles* and *solid circles* indicate data before and after rifampin induction, respectively.

containing vesicles confirmed that the glucuronide is a high-affinity substrate of MRP2 and has a moderate affinity to P-gp. The parent compound binds to MRP2 and P-gp with a low affinity. Therefore we hypothesize that the concentrations of the active ezetimibe on the NPC1L1 receptor side are dependent on expression of UGT1A1, P-gp, and MRP2 within the intestinal absorption compartment.

The appearance of the glucuronide in blood, however, seems to be determined by either intestinal or hepatic P-gp (or both) or MRP2-mediated secretion because of the inverse correlation between UGT1A1 expression and AUC of the glucuronide. An open question associated with ezetimibe treatment is whether the pharmacologic binding sites of NPC1L1 for ezetimibe are located on the apical (luminal) surface or the cytoplasmatic site of the apical enterocytic membrane and whether either the parent drug or its glucuronide, or both, is the active moiety in humans.[4,6,7,12,13] A recent report suggests that ezetimibe glucuronide seems to be the active principle. The parent ezetimibe, however, was not investigated in this study.[7]

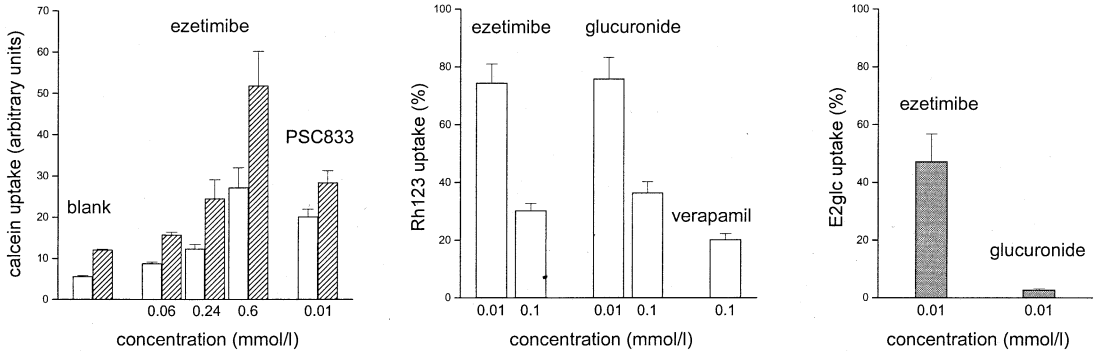

Fig 6. *Left*, Calcein transport into P-glycoprotein (P-gp)–overexpressing Madin-Darby canine kidney II (MDCKII) cells 60 minutes (*white columns*) and 120 minutes (*gray columns*) after incubation with blank (solvent), ezetimibe, and PSC833. *Middle*, Adenosine triphosphate (ATP)–dependent transport of rhodamine 123 (Rh123) into inside-out vesicles obtained from *ABCB1*-transfected LLC cells (in percent of control) after incubation for 10 minutes in the presence of ezetimibe, ezetimibe glucuronide, and verapamil. *Right*, ATP-dependent transport of [^{3}H]estradiol-glucuronide (E2glc, *gray columns*) into inside-out vesicles obtained from *ABCC2*-transfected 2008 cells after incubation with ezetimibe and its glucuronide for 10 minutes. Data are given as mean ± SD.

We concluded from our findings that the cholesterol-lowering effect of ezetimibe is attributed to the intracellular activity of the parent compound, for which intestinal glucuronidation and intestinal and hepatic P-gp–mediated and MRP2-mediated secretion are detoxifying processes. If the glucuronide would be the active principle, up-regulation of UGT1A1 should be associated with a stronger sterol-lowering effect. The pharmacologic receptor of ezetimibe is likely to be located within the enterocytes because an increase in the intestinal clearance of ezetimibe by rifampin-type induction decreased the pharmacodynamic effect. Alternatively, if the binding sites would be accessible from the luminal side, rifampin comedication should increase the sterol-lowering effect rather than decrease it. Our conclusions on the side of ezetimibe action are in agreement with very recent findings in HepG2 cells, which confirmed the intracellular localization of the NPC1L1 protein.[5]

Contrary to the discussion in the literature, our data indicate that the glucuronide represents the storage form for long-lasting recycling of the active ezetimibe to the intestinal absorption compartment via the systemic circulation.[7,12] From our point of view, ezetimibe is rapidly absorbed from gut lumen into enterocytes and subjected to extensive glucuronidation. The extent of absorption seems to be dependent on the function of intestinal P-gp or MRP2 (or both) on the absorption side. Only a minor part of the dose escapes first-pass metabolism and efflux transport and appears in blood in rather low concentrations. The major part of the glucuronide, however, is secreted by intestinal or hepatic P-gp or MRP2 into gut lumen. It cannot be predicted from our data to which extent intestinal and hepatic efflux contributes to the overall disposition and sterol-lowering effect of ezetimibe because rifampin regulates both elimination pathways. Furthermore, there is evidence that P-gp is not coordinately regulated in the liver and intestine.[32]

After hydrolysis of the glucuronide within the large intestine, which has been shown in humans and rats, the active moiety is reabsorbed and recycled to the place of intestinal sterol absorption.[9,12] Therefore ezetimibe is rather completely but extremely slowly excreted via the feces. The additional "(re)-absorption" peaks of ezetimibe were even higher than the initial maximum and were not accompanied by adequate glucuronide peaks, because colonic UGTs are of lower activity.[11,33] Interestingly, the additional absorption peaks of the ezetimibe moiety occur in association with the next meals[34] (at lunch and dinner times in our study). We postulate that the glucuronide accumulates during the fasting periods in the distal small intestine, preferentially in the ileum. Eating induces a so-called gastroileocecal reflex by which the ileal content is translocated as a bolus into the colon from which the meal-associated serum peaks of ezetimibe originate.[35,36] Food-triggered emptying of the gallbladder and associated release of ezetimibe and its glucuronide

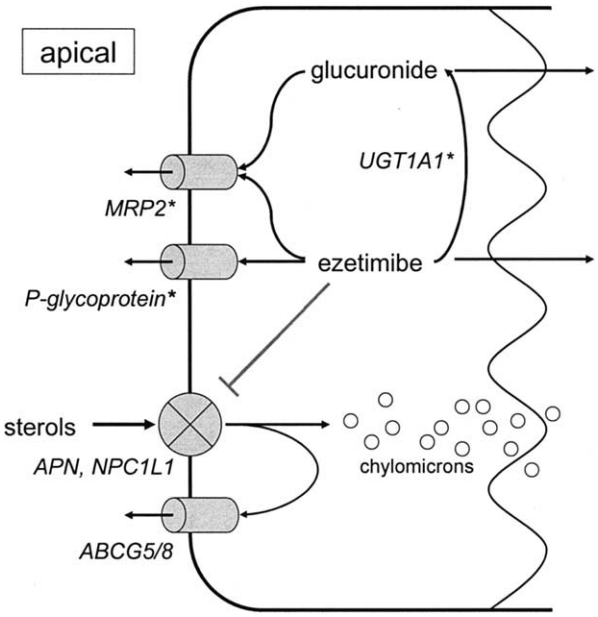

Fig 7. Disposition of ezetimibe in enterocytes and interaction of ezetimibe with intestinal sterol absorption. Pharmacologic targets of ezetimibe action are the aminopeptidase N (APN) and NPC1L1 at the initiation site of the sterol absorption pathway, which are located in the apical membrane of enterocytes. The cellular sterol traffic is limited by kickback via the efflux transporter proteins ABCG5 and ABCG8. The sterol-lowering effect of ezetimibe is dependent on the intestinal expression of UGT1A1, the efflux transporter protein P-gp, and the multidrug resistance associated protein 2 (MRP2). *Asterisks* indicate rifampin-inducible pathways.

from bile flow most likely reinforce this multiple-peak phenomenon.[34]

Rifampin regulates P-gp and MRP2 via the nuclear PXR pathway, which cross-activates the constitutive androstane receptor pathway for UGT1A1 induction.[19,20] Similar unfavorable effects on ezetimibe disposition and efficiency are hypothesized after comedication of the PXR ligands carbamazepine and St John's wort or after UGT1A1 induction with ligands of the constitutive androstane receptor (eg, phenobarbital, dexamethasone, and even dietary flavonoids).[19,20] By contrast, inhibition of intestinal glucuronidation and P-gp–mediated or MRP2-mediated secretion might result in higher ezetimibe concentrations within the sterol absorption compartment and therefore an increased ezetimibe effect. This is supported by evidence from drug interaction studies with P-gp–modulating and MRP2-modulating drugs (eg, cyclosporine, fenofibrate, and gemfibrozil).[37-39] Combination treatment with these drugs leads in part to several-fold elevation of the ezetimibe plasma concentrations.[14-17] Rifampin is also a modulator of P-gp and MRP2.[40-42] Therefore increased plasma levels of ezetimibe are also expected if a single dose of rifampin is given concomitantly with ezetimibe and not as in our study, with an interval of 12 hours between both drug administrations.

In conclusion, the disposition and plant sterol–lowering efficiency of ezetimibe in humans are determined by expression of intestinal UGT1A1, P-gp, and MRP2. Comedication of the PXR-type inducer rifampin leads to reduced effects of ezetimibe caused by faster elimination of ezetimibe via glucuronidation and subsequent intestinal or hepatic secretion via P-gp and MRP2.

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