Enterohepatic Disposition of Rosuvastatin in Pigs and the Impact of Concomitant Dosing with Cyclosporine and Gemfibrozil

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ABSTRACT:
The hepatobiliary transport and local disposition of rosuvastatin in pig were investigated, along with the impact of concomitant dosing with two known multiple transport inhibitors; cyclosporine and gemfibrozil. Rosuvastatin (80 mg) was administered as an intrajejunal bolus dose in treatments I, II, and III (T, TII, and TIII, respectively; n = 6 per treatment). Cyclosporine (300 mg) and gemfibrozil (600 mg) were administered in addition to the rosuvastatin dose in TII and TIII, respectively. Cyclosporine was administered as a 2-h intravenous infusion and gemfibrozil as an intrajejunal bolus dose. In treatment IV (TIV, n = 4) 5.9 mg of rosuvastatin was administered as an intravenous bolus dose. The study was conducted using a pig model, which enabled plasma sampling from the portal (VP), hepatic (VH), and femoral veins and bile from the common hepatic duct. The biliary recoveries of the administered rosuvastatin dose were 9.0 ± 3.5 and 35.7 ± 15.6% in T and TIV, respectively. Rosuvastatin was highly transported into bile as shown by the median AUCbile/AUCVH ratio in T of 1770 (1640–11,300). Gemfibrozil did not have an effect on the plasma pharmacokinetics of rosuvastatin, most likely because the unbound inhibitor concentrations did not exceed the reported IC50 values. However, cyclosporine significantly reduced the hepatic extraction of rosuvastatin (T, 0.89 ± 0.06; TII, 0.46 ± 0.13) and increased the AUCVP and AUCVH by 1.6- and 9.1-fold, respectively. In addition, the biliary exposure and f0, bile were reduced by ~50%. The strong effect of cyclosporine was in accordance with inhibition of sinusoidal uptake transporters, such as members of the organic anion-transporting polypeptide family, rather than canalicular transporters.

Drug-drug interactions (DDIs) involving rosuvastatin that result in increased plasma exposure of rosuvastatin might, in rare cases, result in severe unwanted side effects such as myopathy and possibly rhabdomyolysis (Thompson et al., 2003). A 7.1- and 10.6-fold increase in the plasma exposure (AUC) and maximum plasma concentration (Cmax) of a single 10-mg oral rosuvastatin dose was observed in heart transplant patients receiving cyclosporine treatment (75–200 mg b.i.d.) (Simonson et al., 2004). In addition, gemfibrozil (600 mg b.i.d.) increased the AUC and Cmax of a single 80-mg rosuvastatin dose by 1.88- and 2.21-fold, respectively (Schneck et al., 2004).

These clinically relevant DDIs are considered to involve inhibition of hepatic transport proteins although the in vivo relevant mechanism(s) remains to be elucidated. High hepatic exposure is essential for rosuvastatin, which exhibits its pharmacological effects by inhibiting HMG-CoA reductase, localized to the endoplasmic reticulum of the hepatocyte. Rosuvastatin has an estimated hepatic extraction of 0.63 in humans and the hepatic clearance accounts for approximately 70% of the total plasma clearance (Martin et al., 2003a). OATP1B1 (SLCO1B1) is reported to be the primary transporter involved; however, additional sinusoidal transporters, OATP1B3 (SLCO1B3), OATP2B1 (SLCO2B1), OATP1A2 (SLCO1A2), and NTCP (SLC10A1), mediate the hepatic uptake of rosuvastatin (Ho et al., 2006; Kitamura et al., 2008). Rosuvastatin is also a substrate of canalicular transporters, BCRP (ABCG2) and MRP2 (ABCC2), and approximately 10% of an orally administered dose was recovered in bile during the absorption phase in healthy volunteers (Bergman et al., 2006; Huang et al., 2006; Kitamura et al., 2008).

Rosuvastatin is a potent inhibitor of OATP1B1-, OATP2B1-, OATP1B3-, and NTCP-mediated transport of rosuvastatin (IC50 values 0.31–2.2, 0.07, 0.06, and 0.37 μM) (Simonson et al., 2004; Ho et al., 2006). In addition, cyclosporine inhibits BCRP- and MRP2-mediated transport (Chen et al., 1999; Gupta et al., 2006; Xia et al., 2007). The OATP1B1-mediated transport of rosuvastatin is also inhibited by gemfibrozil (IC50 values of 4.0 ± 1.3 and 25 μM) (Schneck et al., 2004; Ho et al., 2006). Furthermore, gemfibrozil inhibits the OATP2B1 and NTCP-mediated transport of...
CYP2C9, CYP2C19, and UDP glucuronosyltransferase 1A1 and 1A3 are considered to primarily mediate the metabolism of rosuvastatin; however, only marginal effects on its pharmacokinetics in vivo were observed when it was coadministered with the potent CYP2C9 inhibitor, fluconazole (Cooper et al., 2002; Martin et al., 2003b). In humans, the majority of an orally administered dose (~80%) was excreted unchanged in feces (fe = 0.90), and three metabolites have been identified: rosuvastatin-55-lactone, N-desmethyl rosuvastatin, and a β-1-O-acyl glucuronide conjugate (Prueksaritanont et al., 2002; Martin et al., 2003b; Fujino et al., 2004). Approximately 50% of an oral dose is absorbed, and the absolute oral bioavailability is estimated to be 20.1% in humans (Martin et al., 2003a). Many of the transport proteins involved in the hepatobiliary disposition of rosuvastatin are also expressed in the small intestine in humans and could possibly affect the intestinal absorption of rosuvastatin and, thus, be a potential site for DDIs (Glaeser et al., 2007; Zair et al., 2008).

The effect of cyclosporine and gemfibrozil on the disposition of rosuvastatin was investigated in this study using an advanced method in pigs, which enables in vivo pharmacokinetics to be assessed at multiple plasma sites simultaneously, along with direct monitoring of the excretion of rosuvastatin into bile and the upper part of the small intestine (Petri et al., 2006; Persson et al., 2007, 2008; Sjödin et al., 2008). The pig is an appropriate animal for the investigation of drug disposition, as it is known to display high similarities with humans with regard to its gastrointestinal physiology and important transport and enzymatic proteins have been identified in pigs such as CYP3A4, CYP2E1, CYP2C, OATP1B1, OATP1A2, and MRP2 (Karari, 1995; Lampen et al., 1995; Anzenbacher et al., 1998; Goh et al., 2002; Tang et al., 2004; Baranová et al., 2005; Upton, 2008). In addition, a homolog to BCRP mRNA has been detected in pig liver and intestine tissue, which shares a 86% amino acid identity with human BCRP (Eisenblätter and Gall, 2002).

Because the hepatic transport of rosuvastatin is important from a pharmacokinetic and a pharmacodynamic perspective, the main objective of this study was to investigate the effect on the hepatic uptake and biliary excretion of a single intrajejunal bolus dose of rosuvastatin when coadministered with cyclosporine (as an intravenous infusion) and gemfibrozil (as an intrajejunal bolus dose). The hepatic extraction was determined in vivo in pigs by simultaneous sampling in the portal and hepatic vein compartments and offers a direct measurement of the capacity of the liver to extract rosuvastatin, which is of special interest for drugs that exhibit their pharmacological effect in the liver.

Materials and Methods

Animals. The study was approved by the local ethics committee for animal experiments, and the handling of animals followed national regulations. The study included 23 pigs (3 female and 20 castrated male) of mixed breed (Yorkshire and Swedish Landrace) of which one experienced complications during surgery and did not complete the study. The animals were 10 to 12 weeks old and weighed 28.0 ± 2.2 kg (23–32.5 kg). No food intake was allowed the night before the experiment, although they had unlimited access to water.

Study Design and Investigational Drugs. The study was of parallel design and included four different treatment groups: treatments I to IV (T–TIV), as summarized in Table 1. Six animals were included in T to TIII and four in TIV. In T to TIII the dose was 80 mg of rosuvastatin (Crestor, mol. wt. 480.53; AstraZeneca, Södertälje, Sweden) administered as an intrajejunal bolus dose by use of the Loc-I-Gut catheter (Snyectics Medical, Stockholm, Sweden), which has been described in detail previously (Petri et al., 2006; Sjödin et al., 2008). Cyclosporine (Sandimmune, mol. wt. 1202.61; Novartis, Täby, Sweden) and gemfibrozil (Lopid, mol. wt. 250.35; Pfizer, Täby, Sweden) were coadministered with rosuvastatin in TII and TIII, respectively. In TIV, rosuvastatin was administered alone as an intravenous bolus dose into a vein in the right ear.

The drug solution of rosuvastatin administered intrajejunally in T to TIII was prepared by dissolving two 40-mg Crestor tablets in 50 ml of 37°C sodium chloride (9 mg/ml) (Fresenius Kabi, Halden, Norway). The concentration of rosuvastatin in the final drug solution in T to TIII was 1.7 ± 0.15 mg/ml. In T and TII, phenol red was added to the solution to provide a final concentration of 0.2 mg/ml. The pH of the final drug dispersion was corrected, if necessary, to pH 6 to 7. After drug administration, the dispersion vial and catheter were rinsed with 50 ml of 37°C sodium chloride (9 mg/ml). In TIV, rosuvastatin was administered as an intravenous dose, which was prepared by dissolving six 40-mg tablets in 100 ml of sodium chloride (9 mg/ml) followed by filtration (0.1 μm Minisart; Sartorius AG, Goettingen, Germany). The final concentration of the intravenous solution was 1.97 ± 0.07 mg/ml, and doses were stored as prefilled syringes at −20°C. Subsequently 3 ml of the intravenous drug solution (corresponding to 5.9 mg of rosuvastatin) was administered into the right ear vein. The cyclosporine solution for infusion (2.5 mg/ml) in TII was composed of cyclosporine concentrate for infusion and sodium chloride (9 mg/ml). Cyclosporine was infused into the central venous catheter, and the infusion was started 1 h before the rosuvastatin dose with an infusion rate as follows: 0 to 10 min, 180 ml/min; 10 min and onward, 50 ml/min (calibrated infusion pump, Asena TIVA, MKIII; Alaris Medical Systems, Stockholm, Sweden). The infusion was terminated 1 h after the administration of rosuvastatin and a total volume of 120 ml was infused over 2 h, corresponding to a total cyclosporine dose of 300 mg. In TIV, one 600-mg tablet of gemfibrozil was dispersed in 50 ml of 37°C sodium chloride (9 mg/ml) and administered intrajejunally 20 min before the rosuvastatin dose by use of the Loc-I-Gut catheter. The gemfibrozil dose was administered as a drug suspension with a final concentration of 0.67 ± 0.38 mg/ml. After the administration of the gemfibrozil dose, the dispersion vial and catheter were rinsed with 50 ml of 37°C sodium chloride (9 mg/ml).

Experimental Procedures. In vivo study. This animal model has been developed, validated, and described in detail in previous reports (Petri et al., 2006; Sjödin et al., 2008). In brief, an initial sedative dosage of 50 mg of xylazine (Rompun Vet, 20 mg/ml; Bayer AG, Leverkusen, Germany) was administered intramuscularly, followed by a mixture of 2.2 mg/kg xylazine and 0.04 mg/kg atropine (Atropin NM Pharma, 0.5 mg/ml; Merck AB, Stockholm, Sweden) and a mixture of 3 mg/kg tiletamine and 3 mg/kg zolazepam (Zoletil; Virbac S.A., Carros, France) administered intramuscularly. A continuous intravenous infusion of 20 mg/kg/h ketamine (Ketaminol Vet, 100 mg/ml; Intervet, Stockholm, Sweden), 0.5 mg/kg/h morphine (Morfin Meda, 10 mg/ml; Meda AB, Solna, Sweden), and 0.25 mg/kg/h pancuronium bromide (Pavulon, 2 mg/ml; Organon AB, Gothenburg, Sweden) was continuously infused intravenously at 10 ml/kg/h throughout the experiment. To compensate for blood loss, 250 ml of Macrodex with sodium chloride (60 mg/ml; Meda AB) was administered during surgery. Ringer’s acetate (Fresenius Kabi AB, Uppsala, Sweden) was continuously infused intravenously at 8 ml/kg/h; however, during the infusion of cyclosporine, the flow was adjusted to maintain an equal volume of fluid infused between TII and TIII. A tracheotomy was performed, and the pigs were ventilated using a 30% oxygen-air mix (Servo 900C ventilator; Siemens-
for 60 min at 4°C, after which the pellet was redissolved in cold sodium chloride, 35 mM mannitol, 10 mM D-glucose, and 1.0 g/l polyethylene pump (model 355; Sage Instruments, Orion Research Inc., Cambridge, MA) was perfused during 100 min at 2 ml/min using a calibrated syringe balloon, which created a 10-cm-long isolated jejunal segment. The isolated balloons of the Loc-I-Gut tube were inflated with 20 to 25 ml of air and jejunum. Once the Loc-I-Gut catheter was in position, the jejunum was rinsed of the investigational drugs, a catheter was placed in the right femoral vein (VF). The surgical procedures lasted approximately 90 min and were performed by experienced personnel at a validated laboratory. After the surgical procedures, the animals were left to stabilize for 30 min. Throughout the experiment body temperature, blood gases, electrocardiograms, heart rate, hemoglobin, and arterial and central venous pressures were monitored to ensure normal physiological values. As the animal model was carefully monitored the physiological conditions were comparable with those of previous studies (Petri et al., 2006; Persson et al., 2007, 2008; Sjödin et al., 2008).

An isolated jejunal segment was perfused in TI and TIII from 0 to 100 min, and the perfuse samples were quantitatively collected on ice every 10 min from the start of the experiment up to 100 min after the rosuvastatin dose was given. In TI to TIV, bile samples were quantitatively collected before the rosuvastatin dose and at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, and 300 min. In TI, TIII, and TIV a sample was also withdrawn 360 min after the rosuvastatin dose was given. Blood samples were withdrawn in TI to TIV from VP, VH, and VF simultaneously with the bile samples. Blood samples were collected in tubes spray coated with lithium heparin (BD Vacutainer, Plymouth, UK) and were immediately centrifuged at 1000 g for 10 min at 4°C. Cycleom was monitored in VP and VH using whole blood (KEDTA-containing tubes; BD Vacutainer) and stored at 2 to 8°C, awaiting analysis. Bile, plasma, and perfuse samples were buffered with an equal volume of 0.1 M sodium acetate buffer at pH 4 before being stored at −20°C. The blood/plasma ratio of rosuvastatin was determined in triplicate; 3 ml of whole blood was spiked with rosuvastatin (0.2 and 2 μM), and incubated in 37°C for 30 min. The plasma fraction was then obtained by centrifugation, and the blood fraction was obtained by addition of water (1:3 v/v of whole blood-water). The samples were mixed with an equal volume of 0.1 M sodium acetate buffer (pH 4) before storage at −20°C.

In vitro study. Incubations were performed with pooled human liver microsomes (HLMs) (BD Gentest, Woburn, MA) and pooled pig liver microsomes (PLMs). The PLMs were prepared by homogenization of liver biopsy samples from four pigs with 10 mM NaKPO4 in a buffer of pH 7.4, followed by centrifugation at 10,000 g for 20 min 4°C. The supernatant was ultracentrifuged at 105,000 g for 60 min at 4°C, after which the pellet was redissolved in cold phosphate buffer followed by an additional ultracentrifugation at 105,000 g for 60 min at 4°C. The final pellet was redissolved in 50 mM NaKPO4 buffer, pH 7.4, and the final protein concentration was determined by using the method of Lowry et al. (1951). The PLMs were immediately frozen in liquid nitrogen and stored at −70°C. Incubations were performed at a final protein concentration of 0.5 mg/ml in 0.1 mM NaKPO4 buffer, pH 7.4, at 37°C with constant shaking. The final rosuvastatin concentrations were 0.5, 5, and 28 μM, and samples were withdrawn at 7, 15, 30, and 60 min. The incubations were started by addition of 1 mM NADPH (Sigma-Aldrich, St. Louis, MO) and stopped by addition of 600 μl of acetonitrile to the 200-μl reaction mixture, followed by centrifugation at 10,000 g for 10 min. The supernatant was mixed with an equal volume of 0.1 M sodium acetate buffer (pH 4) and stored at −70°C, awaiting analysis. All incubations were performed in triplicate, and there was no binding or instability of rosuvastatin in the incubation mixture (HLMs and PLMs excluded) at 0.5 and 28 μM.

Rosuvastatin and gemfibrozil analysis. The calcium salt of rosuvastatin was purchased from APIN Chemicals Ltd. (Abingdon, Oxfordshire, UK). Gemfibrozil was obtained from Sigma-Aldrich. Hexadeuterated gemfibrozil (gemfibrozil-d6) was bought from CDN Isotopes (Pointe-Clair, QC, Canada). Tri-deuterated rosuvastatin (rosuvastatin-d3 sodium salt was purchased from SynFine Research (Richmond Hill, ON, Canada). All of the other chemicals were of analytical grade or better and were used without further purification. Water was purified using a Milli-Q water purification system (Millipore Corporation, Billerica, MA). Stock solutions of rosuvastatin, rosuvastatin-d3 (internal standard), gemfibrozil, and gemfibrozil-d6 (internal standard) were prepared in methanol at approximately 1 mg/ml and subsequently diluted and used for spiking blank matrices for construction of calibration samples. For plasma, the calibration interval for rosuvastatin was 0.15 to 5006 ng/ml prepared by adding 100 μl of a working standard solution to 1000 μl of blank matrix (1:1 v/v of 0.1 M plasma-sodium acetate buffer, pH 4.0) and for gemfibrozil was 2.5 to 45,800 ng/ml prepared by adding 100 μl of a working standard solution to the same matrix. For bile, the standard curve interval for rosuvastatin was 0.82 to 57,434 ng/ml prepared by adding 100 μl of the standard solution to 1000 μl of blank matrix (1:1 v/v of 0.1 M bile-sodium acetate buffer, pH 4.0), and for gemfibrozil 7.1 to 17,166 ng/ml was prepared by addition of 100 μl of a working standard solution to the same matrix. The quality control samples were prepared by adding 100 μl of separate stock solutions of rosuvastatin and gemfibrozil to blank matrix.

To a 1.0-ml plasma sample (diluted 1:1 with 0.1 M sodium acetate buffer, pH 4.0), 100 μl of the internal standard solutions was added (0.13 μg/ml rosuvastatin-d3 and 0.73 μg/ml gemfibrozil-d6). Extraction was performed with 5.0 ml of ethyl acetate/dichloromethane (1:1) for 20 min. After centrifugation for 10 min at 3500 rpm, the aqueous phase was removed, and the organic phase was centrifuged for an additional 5 min, after which it was transferred to a new tube and evaporated under nitrogen at 50°C. The residue was reconstituted in 75 μl of 0.002% acetic acid (aqueous)-methanol (75:25 v/v) before LC-MS/MS analysis. The bile samples (diluted 1:1 with 0.1 M sodium acetate buffer, pH 4.0) were prepared according to the same procedure, except that the sample volume of diluted bile was 350 μl. High-performance liquid chromatography (HPLC) was performed with an HP series 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) with a binary pump, degasser, and autosampler. The guard column was an ODS-octadecyl C18 (length 4 mm, 2.5 μm, 1 μl) and the chromatographic column was a Phenomenex Luna 5 μC18 (length 50 mm, i.d. 2.00 mm, particle size 5 μm) (both supplied from Scandinavian GeneTech, Västra Frölunda, Sweden). The mobile phase consisted of (A) 0.2% formic acid in water and (B) methanol and was adapted as a gradient of 59% B for 1 min, 59 to 90% B for 1 min and then constantly at 90% B for 6 min, 90 to 59% B for 0.1 min, and constantly at 59% B for 3.9 min. The total run time was 12 min, the flow rate was 200 μl/min, and the injection volume was 20 μl.

The HPLC column outlet was connected to a Quattro LC quadrupole-hexapole-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization interface (ESI). The instruments were controlled using MassLynx software (version 3.3). The mass spectrometry parameters were manually optimized for sensitivity during direct infusion of rosuvastatin and gemfibrozil solutions. Rosuvastatin was monitored in a positive mode and gemfibrozil in a negative mode. The two analytes were analyzed in the same chromatographic run with the positive ESI parameters for rosuvastatin (0–5.0 min), capillary voltage 3.7 kV, cone 48 V, extractor 2 V, and RF lens 0.60 V, and the negative ESI parameters (5.0–12.0 min) for gemfibrozil, capillary voltage −4.5 kV, cone −28 V, extractor −2 V, and RF lens −0.30 V. The desolvation and source block temperatures were 350 and 120°C, and the nebulizer and desolvation gas flows were 100 and 500 l/h, respectively. Quantifications were performed in the selected reaction monitor-
The apparent intestinal clearance (CL intestine) for the 10-cm isolated jejunal segment of bile were calculated for TI to TIII according to eqs. 5 and 6:

\[ V/F = \frac{\text{Dose}_i}{\text{AUC}_{i,j}} \times 0.693 \]  

\[ CL/F = \frac{\text{Dose}_i}{\text{AUC}_{i,j}} \]  

The hepatic plasma clearance (CLH), total plasma clearance (CL), and volume of distribution (V/F) were calculated for TI using eqs. 7 to 9. The ratio of blood/plasma for rosvastatin in pigs was determined to be 0.77 ± 0.08. The hepatic blood flow (QH) in pigs under the same anesthetic regimen and of the same breed and age was determined previously to be 52 ml/min/kg (Nordgren et al., 2002). The fraction absorbed, fAbs, was calculated according to eq. 10. Gut extraction, Egi, was assumed to equal zero to estimate fAbs (Martin et al., 2003a):

\[ CL_H = c_{\text{plasma}} \times \left( \frac{C_{\text{bile}}}{C_{\text{plasma}}} \right) \]  

\[ CL = \frac{\text{Dose}_i}{\text{AUC}_{i,j}} \]  

\[ V_{\text{H}} = \frac{\text{AUMC}_{i,j}}{\text{AUC}_{i,j}} \times \frac{\text{AUC}_{i,j}}{\text{AUC}_{i,j}} \]  

\[ F = f_{\text{Abs}} \times (1 - E_{\text{gi}}) \times (1 - E_{\text{H}}) \]  

### Data Analysis

The plasma and bile concentration-time data were analyzed using noncompartmental methods (WinNonlin version 4.0; Pharsight, Mountain View, CA), and the following pharmacokinetic variables were determined: maximum plasma/bile concentration (Cmax) and time to reach Cmax (tmax), the terminal rate constant (λt), the dwell time at 50% (t1/2), and the area under the curve (AUC). The areas under the concentration-time profile were analyzed with the linear trapezoidal rule for the ascending areas and the logarithmic trapezoidal rule for the descending ones. By monitoring the portal and hepatic vein compartments simultaneously, we were able to calculate the apparent hepatic extraction, Egi, using eq. 1, which gives a more precise measurement of the in vivo capacity of the liver to extract rosvastatin, as opposed to EI = CLH/QH, where the enterohepatic circulation is included in CLH (Petri et al., 2006; Sjödin et al., 2008):

\[ E_{\text{gi}} = \frac{\text{AUC}_{i,j} \times \text{AUC}_{i,j}}{\text{AUC}_{i,j}} \]  

Bile samples were quantitatively collected at specified time points, and the amount of drug excreted in the bile was calculated as the concentration in the collected sample during the collection interval multiplied by the collected volume of bile. The apparent biliary clearance (CLbile) and fraction of the dose excreted in the bile (fcbile) were calculated according to eqs. 2 and 3, respectively (Petri et al., 2006; Sjödin et al., 2008):

\[ CL_{\text{bile}} = \frac{\sum c_{\text{bile}} \times t_{\text{bile}}}{\text{Dose}} \]  

\[ f_{\text{cbile}} = \frac{\sum c_{\text{bile}}}{\text{Dose}} \]  

The apparent intestinal clearance (CLintestinal) for the 10-cm isolated jejunal segment was calculated according to eq. 4:

\[ CL_{\text{intestinal}} = \frac{\sum c_{\text{intestinal}} \times t_{\text{intestinal}}}{\text{AUC}_{i,j}} \]  

### Results

#### Effect of Intravenously Infused Cyclosporine on the Absorption and Disposition of Rosuvastatin (TI and TIIIA). A 2-h intravenous infusion with cyclosporine (300 mg) significantly increased the plasma exposure in all three compartments (VP, VH, and VF) of an intrajejunally administered rosvastatin dose (TIIIA) compared with rosvastatin administered alone (TI) (Fig. 1, A–C; Table 2). In the portal vein, the AUC0–τ of rosvastatin was increased 1.6-fold (95% CI: 1.0–2.6; P < 0.05), and the Cmax was increased 2.0-fold (95% CI: 1.2–3.3; P < 0.05) (Fig. 1A; Table 2). In the hepatic vein, the AUC was increased 9.1-fold (95% CI: 3.2–25.9; P < 0.001) and the Cmax was increased 16.0-fold (95% CI: 5.4–47.2; P < 0.001) (Fig. 1B; Table 2). In the femoral vein, the AUC was increased 6.2-fold (95% CI: 3.6–10.5; P < 0.001) and the Cmax was increased 9.2-fold (95% CI: 5.2–16.1; P < 0.001) (Fig. 1C; Table 2). Thus, the hepatic extraction was significantly reduced (P < 0.001); however, the terminal half-life was unaffected by cyclosporine in TIIIA (Table 2). In bile, the AUC and Cmax of rosvastatin were significantly decreased 2.1-fold (95% CI: 1.6–2.8; P < 0.001) and 1.8-fold (95% CI: 1.6–2.8; P < 0.001) for rosvastatin and gemfibrozil, respectively.
1.1–2.8, \( P < 0.05 \), respectively, in TII compared with TI, which was in accordance with the reduction of the biliary clearance from 115 to 53.1 \( \text{ml/min} \) \( (P < 0.05) \) (Fig. 2; Table 3). The fraction of the rosuvastatin dose excreted into bile during 5 h was significantly reduced by cyclosporine \( (P < 0.05) \) (Table 3). In total were 139 \( \pm 51 \) and 197 \( \pm 45 \) \( \text{ml of bile collected in} \) TI and TII, respectively, and the biliary flow was unaffected by cyclosporine (Table 3). Rosuvastatin was highly accumulated in bile \( (\text{AUC}_{\text{bile}} / \text{AUC}_{\text{VH}}) \), as presented in Table 3. The AUC bile/AUC VP was significantly \( (P < 0.01) \) reduced by cyclosporine as it ranged from 59 to 132 in TII and ranged from 185 to 342 in TI (Table 3).

The pharmacokinetic variables of cyclosporine in whole blood in VP and VH are presented in Table 2. There is a risk of cross-reactivity between metabolites of cyclosporine and the cyclosporine antibody. Thus, the reported concentrations using immunoassay actually represent cyclosporine and to some extent its metabolites. Mean whole-blood concentrations of cyclosporine in VP and VH are displayed in Fig. 3 along with mean plasma concentrations of rosuvastatin in TI and TII in VP and VH. It seems as if the most pronounced increase in the exposure of rosuvastatin in VP by cyclosporine occurred at inhibitor concentrations in the VP above 3 \( \mu \text{M} \) and the time profile suggests that the observed interaction was immediate. Cyclosporine is highly bound to plasma proteins \( (f_b = 0.9) \) and the average maximum unbound concentration, \( C_{\text{max, u}} \), of cyclosporine in TII was, therefore, approximately 0.38 \( \mu \text{M} \).

**Effects of Intrajejunally Administrated Gemfibrozil on the Absorption and Disposition of Rosuvastatin (TI and TIII).** A single bolus dose of gemfibrozil (600 mg) was administered using the Loc-I-Gut tube into the jejunum 20 min before the rosuvastatin dose. Despite the high intestinal luminal concentration of gemfibrozil, the intestinal absorption of rosuvastatin was unaffected by gemfibrozil as indicated by unchanged \( t_{\text{max}} \), \( C_{\text{max}} \), and AUC in the
Pharmacokinetic properties determined in plasma for rosuvastatin and gemfibrozil (TI, TII, and TIII) and in whole blood for cyclosporine (TII)

All parameters are given as arithmetic means and the variability is expressed as the S.D. unless otherwise stated. In TI, TII, and TIII, 80 mg of rosuvastatin was administered as an oral solution in the jejunum. Cyclosporine (300 mg) and gemfibrozil (600 mg) were administered concomitantly with rosuvastatin in TI and TIII respectively. Rosuvastatin and gemfibrozil were analyzed using LC-MS/MS, and cyclosporine was analyzed by immunoassay.

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<thead>
<tr>
<th>Parameter</th>
<th>TI</th>
<th>TII</th>
<th>TIII</th>
<th>TIII</th>
<th>TIV</th>
</tr>
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<tbody>
<tr>
<td>Time to max (min)*</td>
<td>40 (20–80)</td>
<td>40 (20–60)**</td>
<td>60 (40–100)</td>
<td>20 (20–60)</td>
<td>40 (20–60)</td>
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<tr>
<td>Cmax (μM)</td>
<td>1.60 ± 0.72</td>
<td>3.17 ± 1.28*</td>
<td>1.69 ± 1.27</td>
<td>1.69 ± 1.27</td>
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<tr>
<td>Terminal half-life (h)*</td>
<td>2.22 ± 0.67</td>
<td>3.67 ± 1.45*</td>
<td>2.30 ± 1.35</td>
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<tr>
<td>CL/F (ml/min/kg)</td>
<td>444 ± 303</td>
<td>75.4 ± 26.8*</td>
<td>288 ± 278</td>
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<tr>
<td>CL/F (l/kg)</td>
<td>91.5 ± 82.2*</td>
<td>12.8 ± 7.27*</td>
<td>25.2 ± 10.6*</td>
<td>25.2 ± 10.6*</td>
<td>25.2 ± 10.6*</td>
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</table>

Pharmacokinetic variables determined in bile for TI to TIV

All parameters are presented as arithmetic means and the variability is expressed as the S.D. unless otherwise stated. In TI, TII, and TIII, 80 mg of rosuvastatin was administered as an oral solution into the jejunum. One hour before the administration of the rosuvastatin dose in TII, a cyclosporine infusion was started and continued for 2 h into the central vein catheter (total dose 300 mg). In TIII, 600 mg of gemfibrozil was administered as a bolus dose into the jejunum 20 min before rosuvastatin was given. In TIV, a single bolus dose of 5 mg of rosuvastatin was administered into the right ear vein. The biliary concentrations of rosuvastatin and gemfibrozil were determined using LC-MS/MS analysis. The biliary concentration of cyclosporine was not determined.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TI</th>
<th>TII</th>
<th>TIII</th>
<th>TIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to max (min)*</td>
<td>60 (40–100)</td>
<td>40 (20–60)</td>
<td>40 (40–80)</td>
<td>20</td>
</tr>
<tr>
<td>Cmax (μM)</td>
<td>385 ± 144</td>
<td>209 ± 57.7</td>
<td>252 ± 163</td>
<td>347 ± 188</td>
</tr>
<tr>
<td>AUC0–5 h (h·μM)</td>
<td>580 ± 179</td>
<td>271 ± 42.5***</td>
<td>357 ± 118*</td>
<td>118 ± 65.5</td>
</tr>
<tr>
<td>AUC0–5 h/AUCvP ratio*</td>
<td>280 (185–342)</td>
<td>73.0 (58.5–132)***</td>
<td>157 (109–396)</td>
<td>2260 (805–3270)</td>
</tr>
<tr>
<td>CL/F (ml/min/kg)</td>
<td>4.21 ± 1.64</td>
<td>1.85 ± 0.82*</td>
<td>5.02 ± 3.24</td>
<td>46.5 ± 21.8</td>
</tr>
<tr>
<td>f0 (μl/min)</td>
<td>9.0 ± 3.5</td>
<td>5.4 ± 1.2*</td>
<td>9.3 ± 3.3</td>
<td>34.7 ± 15.6</td>
</tr>
<tr>
<td>Biliary flow (ml/min)</td>
<td>0.46 ± 0.17</td>
<td>0.64 ± 0.15</td>
<td>0.78 ± 0.36</td>
<td>0.48 ± 0.11</td>
</tr>
</tbody>
</table>

N.D., not determined.
* P < 0.05.
** P < 0.01.
*** P < 0.001.
Expressed as the median value and range.

The biliary exposure of rosuvastatin was significantly reduced in TIII compared with that in TI (P < 0.05) (Fig. 2; Table 3). However, there were no differences between the amount of rosuvastatin excreted into bile between TI and TIII. This result could be explained by a tendency toward an increase in the biliary flow in TIII compared with that in TI (P = 0.086) (Table 3).

The biliary concentration and the total and unbound plasma concentration of gemfibrozil in the portal vein are illustrated in Fig. 4. The plasma concentrations of gemfibrozil in VF were comparable with clinical concentrations after repeated oral dosing with 600 mg of gemfibrozil twice daily (Dollery, 1999; Schneck et al., 2004). Gemfibrozil is highly bound to plasma proteins (f0 = 0.9935) (Shitara et al., 2004). The maximum unbound plasma concentration of gemfibrozil in VP was approximately 0.55 μM (f0 = 0.9935), which was well below the reported IC50 values for the OATP1B1-mediated uptake of rosuvastatin (Schneck et al., 2004; Ho et al., 2006).

During the initial 100 min after the dosing of rosuvastatin in TI and TIII, a 10-cm-long jejunal segment was perfused with phosphate buffer to investigate the direct intestinal excretion of rosuvastatin and gemfibrozil. There was no intestinal excretion of gemfibrozil; however, rosuvastatin was excreted into the jejunal segment with a lag phase of 30 min, after which rosuvastatin was detected in most of the intestinal perfusate samples (Fig. 5). There was a significantly higher amount of rosuvastatin excreted in TIII compared with that in TI (186 ± 92.5 and 55.4 ± 54.4 mg, respectively; P < 0.05) (Fig. 5). The intestinal clearance of rosuvastatin in the isolated jejunal segment (10 cm) was 0.0021 ± 0.0019 ml/min/kg in TI and 0.0038 ± 0.0036 ml/min/kg in TIII. Subsequently, the intestinal clearance of the small
The intestinal was estimated to be 0.11 ± 0.10 and 0.10 ± 0.18 ml/min/kg in TI and TIII, respectively. The length of the small intestine in pig was estimated to be approximately 5 m and was based on the assumption that the pig small intestine length (11.8 m, n = 1) contracts to a degree similar to that in man (~40% in vivo compared with postmortem) (Kararli, 1995). One animal in TI differed from the others because there was no intestinal secretion of rosuvastatin during the entire intestinal perfusion.

**Intravenous Administration of Rosuvastatin (TIV).** The pharmacokinetic parameters derived from the intravenous rosuvastatin dose are presented in Table 3. The maximum plasma concentration, AUC0–6 h, and terminal half-life of rosuvastatin in the femoral vein were 0.14 ± 0.02 μM, 0.07 ± 0.01 h • μM, and 45 ± 19 min, respectively. The hepatic extraction was 0.59 ± 0.17 when rosuvastatin was administered intravenously. The bioavailability was 0.38 and the fraction absorbed was estimated to be 0.92. The distribution
into bile was rapid, and the maximum biliary concentration was 347 ± 188 µM and occurred 20 min after dosing. The \( \frac{AUC_{\text{bile}}}{AUC_{\text{VP}}} \) differed significantly between intravenous and intrajejunal dosing \( (P < 0.01) \); however, there was no difference between the \( \frac{AUC_{\text{bile}}}{AUC_{\text{VP}}} \) \( \geq 0.31 \) and \( 2.2 M \). The hepatic plasma clearance was calculated to be 23.5 ± 6.61 ml/min/kg using eq. 7.

The in vitro metabolism of rosuvastatin in human and pig liver microsomes was investigated using the multiple depletion curves method (Fig. 6) (Sjögren et al., 2009). As expected, the metabolism was minor, because 98 and 99% of the rosuvastatin remained unchanged in the PLMs and HLMs after 1 h of incubation.

Discussion

This in vivo pharmacokinetic study investigated the absorption and disposition of rosuvastatin after intrajejunal administration and simultaneous dosing with a transport inhibitor, i.e., cyclosporine (TII) or gemfibrozil (TII). The present in vivo model enables sampling at multiple plasma sites (VP, VH, and VF), direct collection of primary bile, and monitoring of drug excretion into the proximal small intestine. This validated model is currently used in our laboratory to assess mechanistic pharmacokinetics for drugs such as ximelagatran, fexofenadine, and verapamil, with a special focus on intestinal and liver pharmacokinetics of parent drug and metabolites (Petri et al., 2006; Sjödin et al., 2008).

The hepatic extraction of rosuvastatin was high \( (\approx 0.9) \), after intrajejunal administration in TII and was significantly reduced in TII when cyclosporine was given concomitantly as an intravenous infusion (Table 2). The effect of cyclosporine on the plasma exposure of rosuvastatin was immediate (Fig. 3). In the portal vein compartment, the main increase in AUC occurred during the initial 40 min after the rosuvastatin dose and coincided with total cyclosporine concentrations in VP of 3 to 3.5 µM. However, the effects on hepatic vein exposure of rosuvastatin seemed to persist longer, up to 2 h, after dosing and at total cyclosporine concentrations in VP of 1.5 µM. Thus, we conclude that the interaction was immediate in its action on hepatic uptake transporter(s) and, possibly, on intestinal efflux transporter(s) (Simonsen et al., 2004; Ho et al., 2006; Kitamura et al., 2008). The 9.1-fold increase in \( AUC_{\text{VP}} \) (postliver) in relation to the 1.6-fold increase in \( AUC_{\text{VP}} \) (preliver) shows that this clinical DDI was primarily allocated to the liver rather than the intestine (Fig. 1, A and B; Table 2). The reduction in the apparent volume of distribution and oral clearance of rosuvastatin by approximately 80% were also in accordance with an inhibition of various liver transporters that determine its pharmacokinetics. It is noteworthy that this hypothesis assumes that cyclosporine did not affect the plasma protein binding of rosuvastatin. In accordance with previous studies, this study showed that rosuvastatin was subjected to little metabolism in vitro, and the DDI was likely to be mechanistically located on hepatic transport proteins affecting the disposition and bioavailability (Fig. 6) (Prueksaritanont et al., 2002; Fujino et al., 2004). The consequence of inhibiting sinusoidal transport versus canalicular transport on the pharmacokinetics of pravastatin was recently reported by applying a computational physiologically based pharmacokinetic model (Watanabe et al., 2009). Inhibition of sinusoidal transport resulted in marked effects on plasma exposure but minor effects on the intraduodenal exposure. This theoretical finding supports our experimentally based thesis that the 9.1-fold increase in the hepatic plasma exposure of rosuvastatin in relation to the 2.1-fold reduction in biliary exposure was attributable primarily to inhibition of sinusoidal transport rather than to canalicular transport (Watanabe et al., 2009). Given that cyclosporine is an inhibitor of MRP2 and BCRP located on the canalicular membrane, it is possible that dual inhibition of the hepatobiliary transport occurred in this study. However, the observed reduction in the biliary clearance and the \( AUC_{\text{bile}} \) by \( \approx 50% \), along with the 60% reduction in \( f_{\text{e, bile}} \) were more likely attributable to the reduction in \( f_{\text{H}} \) by \( \approx 50% \) than to canalicular inhibition (Tables 2 and 3). To the best of our knowledge, rosuvastatin-cyclosporine \( K_a \) values for canalicular located transporters have not been reported. However, \( K_a \) values of 6.7, 7.8, and 4.7 µM have been reported for the inhibition by cyclosporine of BCRP-mediated transport of estrone-3-sulfate and methotrexate and MRP2-mediated transport of leukotriene C4, respectively (Chen et al., 1999; Gupta et al., 2006; Xia et al., 2007). OATP1B1 is considered to be the key transporter for the disposition of rosuvastatin (Kitamura et al., 2008). Two \( IC_{50} \) values have been reported for cyclosporine inhibition of the OATP1B1-mediated transport of rosuvastatin, 0.31 and 2.2 µM, using OATP1B1-transfected HeLa cells and Xenopus oocytes, respectively. The maximum unbound plasma concentration \( (C_{\text{max, u}} \) of cyclosporine in TII was 0.38 µM, and a potent DDI was observed, which is in accordance with the \( IC_{50} \) value of 0.31 µM but in disagreement with the \( IC_{50} \) value of 2.2 µM (Simonson et al., 2004; Ho et al., 2006). In a clinical study, Simonson et al. (2004) reported a 7.1-fold increase in plasma exposure of rosuvastatin at a \( C_{\text{max, u}} \) value of cyclosporine well below 2.2 µM.

Because of the involvement of multiple hepatic uptake transporters in the hepatic extraction of rosuvastatin, it is possible that multiple uptake transporters were inhibited in TII. The \( C_{\text{max, u}} \) of cyclosporine in TII was greater than the reported \( IC_{50} \) values for OATP2B1, OATP1B3, and NTCP inhibition as well (Ho et al., 2006). The CEDIA-PLUS assay is reported to overestimate the cyclosporine concentration by approximately 40% compared with HPLC analysis, but even if this fact is taken into consideration, the reported \( IC_{50} \) values were exceeded in TII (Loor et al., 2004; Cattaneo et al., 2005; Fujino et al., 2004). The consequence of inhibiting sinusoidal transport versus canalicular transport on the pharmacokinetics of pravastatin was recently reported by applying a computational physiologically based pharmacokinetic model (Watanabe et al., 2009). Inhibition of sinusoidal transport resulted in marked effects on plasma exposure but minor effects on the intraduodenal exposure. This theoretical finding supports our experimentally based thesis that the 9.1-fold increase in the hepatic plasma exposure of rosuvastatin in relation to the 2.1-fold reduction in biliary exposure was attributable primarily to inhibition of sinusoidal transport rather than to canalicular transport (Watanabe et al., 2009). Given that cyclosporine is an inhibitor of MRP2 and BCRP located on the canalicular membrane, it is possible that dual inhibition of the hepatobiliary transport occurred in this study. However, the observed reduction in the biliary clearance and the \( AUC_{\text{bile}} \) by \( \approx 50% \), along with the 60% reduction in \( f_{\text{e, bile}} \) were more likely attributable to the reduction in \( f_{\text{H}} \) by \( \approx 50% \) than to canalicular inhibition (Tables 2 and 3). To the best of our knowledge, rosuvastatin-cyclosporine \( K_a \) values for canalicular located transporters have not been reported. However, \( K_a \) values of 6.7, 7.8, and 4.7 µM have been reported for the inhibition by cyclosporine of BCRP-mediated transport of estrone-3-sulfate and methotrexate and MRP2-mediated transport of leukotriene C4, respectively (Chen et al., 1999; Gupta et al., 2006; Xia et al., 2007). OATP1B1 is considered to be the key transporter for the disposition of rosuvastatin (Kitamura et al., 2008). Two \( IC_{50} \) values have been reported for cyclosporine inhibition of the OATP1B1-mediated transport of rosuvastatin, 0.31 and 2.2 µM, using OATP1B1-transfected HeLa cells and Xenopus oocytes, respectively. The maximum unbound plasma concentration \( (C_{\text{max, u}} \) of cyclosporine in TII was 0.38 µM, and a potent DDI was observed, which is in accordance with the \( IC_{50} \) value of 0.31 µM but in disagreement with the \( IC_{50} \) value of 2.2 µM (Simonson et al., 2004; Ho et al., 2006). In a clinical study, Simonson et al. (2004) reported a 7.1-fold increase in plasma exposure of rosuvastatin at a \( C_{\text{max, u}} \) value of cyclosporine well below 2.2 µM.

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It is interesting to note that no effects were observed on biliary flow in TII compared with TII, which was surprising because cyclosporine is a known cholestatic substance. The biliary flow in rats was reduced by approximately 40 to 50% after single and repeated dosing of
cyclosporine at a variety of doses (5–30 mg/kg) (Böhne et al., 1994; Deters et al., 2001, 2004). The 2.0-fold increase in \( C_{\text{max}} \), along with the reduction in \( T_{\text{max}} \), in the portal vein compartment may indicate an increased intestinal absorption rate of rosuvastatin by cyclosporine, which possibly could be explained by inhibition of intestinal efflux transporters such as BCRP and/or MRP2 (Table 2). However, the increase may be a consequence of the pronounced increases in \( \text{VH} \). The scaled-up intestinal clearance suggests a minor role of intestinal efflux, as it was less than 1% of total plasma clearance. The efficient biliary clearance of rosuvastatin is mediated by transporters that also are expressed in the enterocyte (Glaser et al., 2007; Zair et al., 2008). A plausible explanation for these tissue differences may be the absence of an efficient basolateral uptake transporter in the intestine. Intestinal OATP may act as an uptake transporter although the absorption was unaffected in TIII, despite a high intrajejunal concentration of gemfibrozil (the concentration in the oral suspension was 2680 ± 1510 \( \mu \text{M} \), and the \( IC_{50} \) value was 4.0 ± 1.3 \( \mu \text{M} \) for OATP1B1), which suggests that intestinal OATP contributes only marginally to the oral absorption of rosuvastatin (Schneck et al., 2004; Hagenbuch and Gui, 2008).

The absence of a direct effect on the disposition of rosuvastatin by gemfibrozil is in accordance with the \( C_{\text{max}} \) 0.55 \( \mu \text{M} \) in VP being well below the reported \( IC_{50} \) value of 4.0 ± 1.3 \( \mu \text{M} \) for the inhibition of OATP1B1-mediated transport of rosuvastatin (Table 2; Fig. 4) (Schneck et al., 2004; Ho et al., 2006). Furthermore, a single 600 mg i.j. dose of gemfibrozil (\( C_{\text{max}} \), 0.40 \( \mu \text{M} \) did not have an effect on the pharmacokinetics of a single 20 mg i.j. dose of rosuvastatin in healthy volunteers (E. Bergman, E. Matsson, M. Hedeland, U. Bondesson, L. Knutson, and H. Lennernäs, manuscript submitted for publication). However, repeated dosing with gemfibrozil (\( C_{\text{max}} \), 0.66 \( \mu \text{M} \) resulted in a 1.88-fold increase in the plasma exposure of rosuvastatin in healthy volunteers (Schneck et al., 2004). We can only speculate on the observed differences between single and repeated dosing. Gemfibrozil is extensively metabolized, and a potential explanation is accumulation of metabolites able to affect OATP1B1. Gemfibrozil-1-O-glucuronide is a more potent OATP1B1 inhibitor than gemfibrozil and has a higher unbound fraction (\( f_u = 0.115 \)) and hepatic extraction (\( E_H = 0.09 \) for parent and \( E_H = 0.65 \) for glucuronide in rats) (Sallustio et al., 1996; Shitara et al., 2004). However, significant accumulation of the acyl glucuronide is less likely because it is chemically reactive and is expected to exhibit a formation rate-limited elimination as indicated by its reported half-life of 8.7 min in rat isolated perfused liver compared to parent compound (half-life of 76.9 min) (Sallustio et al., 1996).

The average \( E_H \) values were 0.89 and 0.59, respectively, after intrajejunal and intravenous administration. At present, we have no explanation as to why \( E_H \) differs between jejunal and intravenous dosing. The \( E_H \) after intrajejunal dosing in this study was higher than the previously reported value of 0.63 in humans, which was based on elimination clearance (\( E_H = \text{CL}_{\text{H}}/Q_{\text{H}} \)) (Martin et al., 2003a). For drugs that undergo enterohepatic circulation the \( E_H \) based on elimination clearances will underestimate the true \( E_H \), and hence an \( E_H \) value of approximately 0.9 better demonstrates the capacity of the liver to extract rosuvastatin. From a pharmacodynamic perspective, this finding is important because it shows efficient uptake into the drug target site and a prolongation of the pharmacological effect by repeated local liver exposure. The physiologically based pharmacokinetic model of Watanabe et al. (2009) and the experimental findings in this study demonstrate the vulnerability for significant alterations in the plasma pharmacokinetics of rosuvastatin as a consequence of DDIs or polymorphism (Watanabe et al., 2009).

In conclusion, intravenous infusion of cyclosporine markedly decreased the hepatic extraction of a single intrajejunal rosuvastatin dose and a 9.1-fold increase in \( \text{AUC}_{\text{VH}} \) was observed along with a 2.1-fold decrease in \( \text{AUC}_{\text{H}} \). We believe that the observed effects in TIII were attributed to the inhibition of the sinusoidal uptake of rosuvastatin, which primarily is considered to be mediated by OATP1B1 (Kitamura et al., 2008). No marked effects on the pharmacokinetics of rosuvastatin were observed after concomitant dosing with gemfibrozil in accordance with the unbound portal plasma concentrations being below the reported \( IC_{50} \) value for the OATP1B1-mediated transport. It is noteworthy that the intestinal absorption of rosuvastatin was not affected by the high local concentration of gemfibrozil. The results obtained in this study indicate that there is hepatic uptake and biliary efflux of rosuvastatin in pigs and that, in combination, these have a large impact on its liver kinetics. This study has provided further insight into the in vivo mechanisms underlying the clinical effect of rosuvastatin and also its reported DDIs with cyclosporine and gemfibrozil.

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Sjögren E, Lennernäs H, Andersson TB, Gräsö J, and Bredberg U (2009) The multiple depletion curves method provides accurate estimates of intrinsic clearance (CLint), maximum velocity of the metabolic reaction (Vmax), and Michaelis constant (Km): accuracy and robustness evaluated through experimental data and Monte Carlo simulations. *Drug Metab Dispos* **37**:47–58.


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