Different Effects of SLCO1B1 Polymorphism on the Pharmacokinetics of Atorvastatin and Rosuvastatin

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Thirty-two healthy volunteers with different SLCO1B1 genotypes ingested a 20 mg dose of atorvastatin and 10 mg dose of rosuvastatin with a washout period of 1 week. Subjects with the SLCO1B1 c.521CC genotype (n = 4) had a 144% (P < 0.001) or 61% (P = 0.049) greater mean area under the plasma atorvastatin concentration–time curve from 0 to 48 h (AUC0–48 h) than those with the c.521TT (n = 16) or c.521TC (n = 12) genotype, respectively. The AUC0–48h of 2-hydroxyatorvastatin was 100% greater in subjects with the c.521CC genotype than in those with the c.521TT genotype (P = 0.018). Rosuvastatin AUC0–48h and peak plasma concentration (Cmax) were 65% (P = 0.002) and 79% (P = 0.003) higher in subjects with the c.521CC genotype than in those with the c.521TT genotype. These results indicate that, unexpectedly, SLCO1B1 polymorphism has a larger effect on the AUC of atorvastatin than on the more hydrophilic rosuvastatin.

Atorvastatin and rosuvastatin are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, or statins, widely used in the treatment of hypercholesterolemia. Statins reduce cardiovascular morbidity and mortality in high risk patients and their efficacy and safety for the primary and secondary prevention of cardiovascular events have been demonstrated in various clinical trials. Large variability exists in the individual response to statins. Moreover, statins can cause myopathy, even rhabdomyolysis, as a rare adverse effect. Such toxicity is often associated with increased plasma concentrations of statins caused by drug interactions or hereditary differences in statin pharmacokinetics.

Transporters have been implicated as important determinants of the intestinal absorption and hepatobiliary clearance of hydrophilic statins, such as pravastatin and rosuvastatin. Multiple organic anion transporting polypeptide (OATP) family members are capable of statin transport, and some of them are abundant in the liver, where they may be involved in the hepatic uptake of statins from the sinusoidal blood. OATP1B1 is expressed on the sinusoidal membrane of human hepatocytes and facilitates the hepatic uptake of many endogenous and foreign compounds, such as estrogen conjugates, bile acids, and statins, including rosuvastatin and atorvastatin. Several single-nucleotide polymorphisms (SNPs) in the gene encoding OATP1B1, SLCO1B1, have been discovered lately. Some of them, in particular c.521T>C (Val174Ala), are associated with reduced activity of OATP1B1 in vitro and markedly increased plasma concentrations of pravastatin, rosuvastatin, pitavastatin, and simvastatin, but not of fluvastatin, in vivo in humans.

Atorvastatin is administered as the calcium salt of the active hydroxy acid form. It is well absorbed but undergoes marked first-pass metabolism resulting in an oral bioavailability of about 14%. Atorvastatin acid is biotransformed to a more lipophilic atorvastatin lactone, either by a coenzyme A-dependent or an acyl glucuronide intermediate pathway. Both atorvastatin and its lactone form are metabolized primarily by cytochrome P450 (CYP) 3A4, and also by CYP2C8 at a low rate. The lactone forms of atorvastatin and its metabolites can be hydrolyzed to the respective acid forms nonenzymatically or by esterases and paraoxonases. The major hydroxy acid metabolites, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin, are pharmacologically active contributing to 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibition during atorvastatin treatment. In addition to OATP1B1, atorvastatin acid is a substrate for some other transporters, including OATP2B1 and the efflux transporters P-glycoprotein and breast cancer...
resistance protein. Atorvastatin plasma concentrations have been elevated when administered concomitantly with CYP3A4 inhibitors, for example itraconazole or grapefruit juice, or with gemfibrozil, an inhibitor of CYP2C8 and OATP1B1. Cyclosporine inhibits CYP3A4, P-glycoprotein, OATP1B1, and some other hepatic uptake transporters and markedly elevates the plasma concentrations of atorvastatin. A 5-day treatment with oral rifampin (INN, rifampicin), an inducer of, for example, CYP3A4, OATP1B1, and P-glycoprotein, decreased the area under the plasma atorvastatin concentration-time curve (AUC) by about 80%. On the other hand, a single 600 mg intravenous dose of rifampin raised the mean AUC of atorvastatin by more than 600%, probably by inhibiting OATP-mediated hepatic uptake of atorvastatin.

The oral bioavailability of rosuvastatin is about 20%. Fecal excretion accounts for approximately 90% of oral rosuvastatin and its metabolites, and 10% is recovered in the urine. Metabolism is not a major mechanism for rosuvastatin elimination, in which CYP2C9-mediated N-demethylation and uridine diphosphate-glucuronosyltransferase 1A1- and uridine diphosphate-glucuronosyltransferase 1A3-mediated acyl-glucuronidation, followed by spontaneous lactonization, are minor pathways. Cyclosporine and gemfibrozil increase the AUC of rosuvastatin, about sevenfold and twofold, respectively, but itraconazole has had only a modest effect on its plasma concentrations. Rosuvastatin is a substrate of the organic anion transporting polypeptides 1B1, 2B1, 1B3, and 1A2. Human hepatic bile acid transporter sodium-taurocholate cotransporting polypeptide and breast cancer resistance protein, but not P-glycoprotein, also appear to facilitate the transmembrane passage of rosuvastatin.

Our aim was to compare the effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin in a prospective genotype panel study to investigate the role of OATP1B1-mediated hepatic uptake in the pharmacokinetics of these two statins in humans.

RESULTS

Effect of SLCO1B1 polymorphism on atorvastatin and its metabolites

Atorvastatin. The SLCO1B1 genotype was significantly associated with the pharmacokinetics of atorvastatin (Figure 1a; Table 1). In subjects with the SLCO1B1 c.521T genotype (n = 4), the mean AUC of atorvastatin (acid) was 144% larger than in the subjects with the c.521T (reference) genotype (n = 16) (P < 0.001) and 61% larger than in subjects with the c.521T genotype (n = 12) (P = 0.049). The mean AUC of atorvastatin in subjects with the c.521T genotype was 52% greater than in subjects with c.521T genotype (P = 0.040).

The effects of the SLCO1B1 genotype were not statistically significant on the peak plasma concentration (Cmax) (P = 0.118), tmax, or t1/2 of atorvastatin (Table 1). Moreover, no statistically significant differences were seen in the pharmacokinetics of atorvastatin lactone and its metabolites between subjects with different SLCO1B1 genotypes (Table 1).

Figure 1 Mean (± SEM) plasma concentrations of (a) atorvastatin, (b) atorvastatin lactone, (c) 2-hydroxyatorvastatin, and (d) 2-hydroxyatorvastatin lactone after a single 20 mg oral dose of atorvastatin in 32 healthy Caucasian subjects in relation to the SLCO1B1 c.521T>C SNP. Open circles indicate subjects with the c.521T > C SNP. Open circles indicate subjects with the c.521T genotype (n = 16); solid circles indicate subjects with the c.521T genotype (n = 12); solid triangles indicate subjects with the c.521T genotype (n = 4). Insets depict the same data on a semilogarithmic scale. For clarity, some of the error bars have been omitted.
The pharmacokinetic variables of atorvastatin between c.521TC heterozygous participants with different \textit{SLCO1B1} haplotypes. The mean ± SD \textit{AUC}_{0-48 h} of atorvastatin was 27.5 ± 9.6 ng/h/ml in c.521TC heterozygotes with the *15 haplotype, 28.2 ± 7.2 ng/h/ml in those with the *16 haplotype, and 49.7 ± 30.3 ng/h/ml in those with the *17 haplotype (\textit{P} = 0.171). The \textit{C}_{\text{max}} and \textit{AUC}_{0-48 h} of atorvastatin varied 17.2- and 7.1-fold between individual subjects (\textit{Figure 2}). The smallest \textit{AUC}_{0-48 h} was found in a male subject with the c.521TT genotype, and the largest \textit{AUC}_{0-48 h} in a female subject with the c.521TC genotype (*1A/*17 diploëtype).

\textbf{Atorvastatin lactone.} The mean plasma concentrations of atorvastatin lactone were lower in subjects with the \textit{SLCO1B1} c.521TT genotype than in those with the \textit{SLCO1B1} c.521TC or c.521CC genotype (\textit{Figure 1b}). However, the associations between the \textit{SLCO1B1} genotype and the pharmacokinetics of atorvastatin lactone were not statistically significant (\textit{Table 1}), although there was a tendency towards greater \textit{AUC}_{0-48 h} values in subjects with the c.521CC genotype than in those with the reference (c.521TT) genotype (\textit{P} = 0.094). The \textit{C}_{\text{max}} and \textit{AUC}_{0-48 h} of atorvastatin lactone varied 16.6- and 16.2-fold between individual subjects.

\textbf{2-Hydroxyatorvastatin.} The mean \textit{AUC}_{0-48 h} of 2-hydroxyatorvastatin was 100% greater in subjects with the \textit{SLCO1B1} c.521CC genotype than in those with the c.521TT (reference) genotype (\textit{P} = 0.018) (\textit{Figure 1c; Table 1}). The \textit{SLCO1B1} genotype had no statistically significant effect on the \textit{C}_{\text{max}} (\textit{P} = 0.156), \textit{t}_{\text{max}} or \textit{t}_{\text{1/2}} of 2-hydroxyatorvastatin. The \textit{C}_{\text{max}} and \textit{AUC}_{0-48 h} of 2-hydroxyatorvastatin varied 9.7- and 7.9-fold between individual subjects. No statistically significant differences were seen in the pharmacokinetic variables of 2-hydroxyatorvastatin between c.521TC heterozygous participants with different \textit{SLCO1B1} haplotypes.

\textbf{2-Hydroxyatorvastatin lactone.} The mean plasma concentrations of 2-hydroxyatorvastatin lactone were lower in subjects with the \textit{SLCO1B1} c.521TT (reference) genotype than in those with the \textit{SLCO1B1} c.521TC or c.521CC genotype (\textit{Figure 1d}). However, the effects of the \textit{SLCO1B1} genotype on the pharmacokinetic variables of 2-hydroxyatorvastatin lactone were not statistically significant (\textit{Table 1}). The \textit{C}_{\text{max}} and \textit{AUC}_{0-48 h} of 2-hydroxyatorvastatin lactone varied 15.2- and 9.8-fold between individual subjects.

\textbf{Effect of \textit{SLCO1B1} polymorphism on rosuvastatin.} The \textit{SLCO1B1} genotype had a significant effect on the pharmacokinetics of rosuvastatin (\textit{Figure 3; Table 2}). In subjects with the \textit{SLCO1B1} c.521CC genotype, the mean \textit{AUC}_{0-48 h} and \textit{C}_{\text{max}} of rosuvastatin were 65% (\textit{P} = 0.002) and 79% (\textit{P} = 0.003) higher, respectively, than in subjects with the

\begin{table}[h]
\centering
\caption{Pharmacokinetic variables of a single 20 mg oral dose of atorvastatin in relation to \textit{SLCO1B1} polymorphism in 32 healthy white Caucasian subjects.}
\begin{tabular}{|l|l|l|l|l|l|}
\hline
\textbf{\textit{SLCO1B1} genotype} & \textbf{\textit{C}_{\text{max}}} & \textbf{\textit{t}_{\text{max}}} & \textbf{\textit{t}_{\text{1/2}}} & \textbf{\textit{AUC}_{0-48 h}} & \textbf{\textit{AUC}_{0-\infty}} \\
\hline
\textbf{Atorvastatin} & & & & & \\
\text{c.521TT (n=16)} & 5.70 ± 2.57 ng/ml & 0.5 (0.5-5.0) h & 10.0 ± 2.6 h & 23.2 ± 8.3 ng h/ml & 24.2 ± 8.6 ng h/ml \\
c.521TC (n=12) & 8.03 ± 3.72 ng/ml & 0.75 (0.5-3.0) h & 9.2 ± 3.6 h & 35.2 ± 20.1 ng h/ml & 36.2 ± 20.3 ng h/ml \\
c.521CC (n=4) & 10.06 ± 9.02 ng/ml & 1.5 (0.5-2.0) h & 10.4 ± 2.9 h & 56.7 ± 17.4 ng h/ml & 59.3 ± 17.4 ng h/ml \\
\hline
\textbf{Atorvastatin lactone} & & & & & \\
c.521TT (n=16) & 0.28 ± 0.15 U/ml & 2.0 (0.5-5.0) h & 14.7 ± 4.6 h & 3.9 ± 2.0 U h/ml & 4.3 ± 2.1 U h/ml \\
c.521TC (n=12) & 0.38 ± 0.26 U/ml & 3.0 (1.0-5.0) h & 11.8 ± 3.3 h & 5.1 ± 2.6 U h/ml & 5.4 ± 2.7 U h/ml \\
c.521CC (n=4) & 0.58 ± 0.42 U/ml & 2.0 (1.0-7.0) h & 14.4 ± 2.7 h & 8.1 ± 4.9 U h/ml & 8.8 ± 5.0 U h/ml \\
\hline
\textbf{2-Hydroxyatorvastatin} & & & & & \\
c.521TT (n=16) & 0.11 ± 0.06 U/ml & 1.0 (0.5-7.0) h & 11.4 ± 4.9 h* & 1.0 ± 0.4 U h/ml & 1.0 ± 0.4 U h/ml* \\
c.521TC (n=12) & 0.15 ± 0.07 U/ml & 1.0 (0.5-5.0) h & 9.8 ± 3.7 h* & 1.3 ± 0.6 U h/ml & 1.4 ± 0.6 U h/ml* \\
c.521CC (n=4) & 0.18 ± 0.08 U/ml & 1.5 (1.0-5.0) h & 9.4 ± 1.7 h* & 2.0 ± 0.8 U h/ml** & 2.0 ± 0.9 U h/ml** \\
\hline
\textbf{2-Hydroxyatorvastatin lactone} & & & & & \\
c.521TT (n=16) & 0.25 ± 0.13 U/ml & 4.0 (1.0-9.0) h & 10.1 ± 3.4 h & 3.4 ± 1.4 U h/ml & 3.6 ± 1.6 U h/ml \\
c.521TC (n=12) & 0.38 ± 0.33 U/ml & 4.0 (1.0-12.0) h & 11.0 ± 2.9 h & 4.6 ± 2.9 U h/ml & 4.8 ± 3.0 U h/ml \\
c.521CC (n=4) & 0.39 ± 0.29 U/ml & 4.0 (2.0-7.0) h & 10.4 ± 2.5 h & 5.3 ± 3.2 U h/ml & 5.8 ± 3.5 U h/ml \\
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\textit{AUC}_{0-\infty}, area under the plasma concentration-time curve from 0 h to infinity; \textit{AUC}_{0-48 h}, area under the plasma concentration-time curve from 0 to 48 h; \textit{C}_{\text{max}}, peak plasma concentration; \textit{t}_{\text{1/2}}, elimination half-life; \textit{t}_{\text{max}}, time to \textit{C}_{\text{max}}. Data are given as mean ± SD. \textit{t}_{\text{max}} data are median (range). \textit{P}=0.040 vs subjects with the c.521TT genotype. ** \textit{P}=0.012 vs subjects with the c.521TT genotype. * \textit{P}=0.046 vs subjects with the c.521TT genotype. \textit{P}<0.001 vs subjects with the c.521TT genotype and \textit{P}=0.049 vs subjects with the c.521TC genotype. \textit{P}<0.001 vs subjects with the c.521TT genotype and \textit{P}=0.041 vs subjects with the c.521TT genotype. * Data collected from 30 subjects only (the \textit{k}_{\text{c}} could not be determined in two subjects). ** \textit{P}=0.018 vs subjects with the c.521TT genotype.}
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\end{table}
c.521TT (reference) genotype. There was a tendency toward higher AUC0–48h of rosuvastatin in subjects with the c.521TC genotype than in subjects with the c.521TT genotype (P = 0.053 and 0.062, respectively). In subjects with the c.521TT genotype, the AUC0–48h of atorvastatin was 41 ± 15% and the AUC0–48h of rosuvastatin 61 ± 32% (P = 0.027, paired t-test) of the corresponding mean AUC0–48h values of atorvastatin and rosuvastatin in subjects with the c.521CC genotype.

The Cmax and AUC0–48h of rosuvastatin varied 20.1- and 11.7-fold between individual subjects; the lowest values were in the same male subject with the c.521TT genotype who also had the lowest AUC0–48h of atorvastatin (Figure 2). The greatest rosuvastatin Cmax and AUC0–48h values were in the female subject with the *1A/*17 diplotype. No statistically significant differences were seen in the pharmacokinetic variables of rosuvastatin between c.521TC heterozygous participants with different SLCO1B1 haplotypes. The mean ± SD AUC0–48h of rosuvastatin was 43.6 ± 18.1 ng h/ml in c.521TC heterozygotes with the *15 haplotype, 58.3 ± 19.6 ng h/ml in those with the *16 haplotype, and 57.5 ± 30.5 ng h/ml in those with the *17 haplotype (P = 0.298). The SLCO1B1 genotype had no significant effect on the tmax or elimination t1/2 of rosuvastatin.

**DISCUSSION**

This study shows that SLCO1B1 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin.
The effect on the AUC of atorvastatin was greater than that of rosuvastatin, and SLCO1B1 polymorphism also significantly affected the plasma concentrations of one of the major metabolites of atorvastatin, 2-hydroxyatorvastatin.

These findings support the idea that atorvastatin acid and rosuvastatin need active transport to penetrate the hepatocyte plasma membrane. The main molecular properties affecting the transmembrane passage of a drug are size, lipophilicity, and charge. The octanol/water partition coefficient of atorvastatin (acid) at pH 7.0 (logD7.0) is 1.53 and it is considerably more hydrophilic than atorvastatin lactone (logD7.0 4.2).39 Unexpectedly, although rosuvastatin is a far more hydrophilic compound (logD7.0 −2.29; calculated value obtained from the SciFinder database) than atorvastatin, the effect of SLCO1B1 polymorphism was smaller on the AUC of rosuvastatin than on that of atorvastatin acid. Thus, the hydro/lipophilicity characteristics of statins do not alone explain the variable effects of SLCO1B1 polymorphism on their pharmacokinetics. In addition to OATP1B1, rosuvastatin is a substrate of a number of other uptake transporters including OATP1B3, OATP2B1, and OATP1A2 as well as sodium-taurocholate cotransporting polypeptide.8 They are all expressed in the liver and uptake via these transporters might compensate for the reduced activity of OATP1B1 and thus explain the relatively modest effect of SLCO1B1 polymorphism on rosuvastatin disposition.

Impaired OATP1B1 function should theoretically reduce the clearances of atorvastatin and rosuvastatin, because it decreases the entry into the liver, the main site of their metabolism and elimination. The lack of effect of SLCO1B1 polymorphism on the elimination $t_2$ of atorvastatin and rosuvastatin could be explained by corresponding decreases in the distribution volume ($V_d$) and clearance (CLtot) when access to the hepatocyte is limited, as the $t_2 = \ln 2 \cdot V_d/Cl_{tot}$. Similar increases in AUC and $C_{max}$ with unchanged $t_2$ have been seen in the effects of SLCO1B1 polymorphism on the pharmacokinetics of pravastatin, simvastatin acid, and repaglinide.16,17,40

Cyclosporine has increased the AUC of rosuvastatin and atorvastatin about sevenfold.28,36 These interactions probably are, at least partly, due to OATP1B1-inhibition,11 although atorvastatin is also a substrate of CYP3A4 and P-glycoprotein, and cyclosporine inhibits both of them.32,43 Cyclosporine, like SLCO1B1 polymorphism, has increased the AUC of statins without affecting their elimination $t_2$.3,28,36 Gemfibrozil has increased the $C_{max}$ and AUC of rosuvastatin about twofold, and this effect has been suggested to be due to OATP1B1 inhibition.37 Gemfibrozil also moderately raises the plasma concentrations of atorvastatin and its metabolites.27 It is possible that SLCO1B1 polymorphism could affect the extent of pharmacokinetic interactions of statins with OATP1B1 inhibitors, such as cyclosporine and gemfibrozil. In a recent study, cyclosporine raised the AUC of repaglinide 3.5-fold, and the effect was 42% lower in subjects with the SLCO1B1 c.521TC genotype than in those with the c.521TT (reference) genotype.43

In the same volunteer subjects as in this study, the mean AUC of simvastatin acid has been 221% higher in subjects with the c.521CC genotype than in subjects with the c.521TT genotype.17 The respective difference in the mean AUC of atorvastatin was 144% (this study), in that of pravastatin 91%,16 and in that of rosuvastatin it was 65% (this study). The mean AUC of fluvastatin was 19% larger in subjects with the c.521CC genotype than in those with the c.521TT genotype, but this difference was not statistically significant.16 Moreover, there have been sex-related differences in the pharmacokinetics of pravastatin,16 but not with other statins. In addition to OATP1B1, fluvastatin is a substrate of OATP1B3 and OATP2B1,7 and rosuvastatin is a substrate of OATP1B3, OATP2B1, OATP1A2, and sodium-taurocholate cotransporting polypeptide uptake transporters expressed in the liver as well.8 The function of these transporters might explain the smaller effect of SLCO1B1 polymorphism on the pharmacokinetics of fluvastatin and rosuvastatin. Pravastatin and atorvastatin are also substrates of OATP2B1.24,44

Interestingly, among all subjects (all genotype groups), the AUC of atorvastatin acid correlated significantly with that of simvastatin acid (Pearson correlation coefficient, $r = 0.86$, $P<0.001$), rosuvastatin ($r = 0.64$, $P<0.001$), fluvastatin ($r = 0.56$, $P<0.001$), and pravastatin ($r = 0.55$, $P = 0.001$). The correlations of the AUC of rosuvastatin with those of simvastatin acid ($r = 0.57$, $P<0.001$), fluvastatin ($r = 0.49$, $P = 0.005$), and pravastatin ($r = 0.49$, $P = 0.005$) were also significant. The AUC of simvastatin acid also correlated significantly with that of pravastatin ($r = 0.59$, $P<0.001$) and fluvastatin ($r = 0.47$, $P = 0.007$), but the AUC of pravastatin did not correlate with the AUC of fluvastatin ($r = 0.05$, $P = 0.794$).

The highest plasma concentrations of rosuvastatin and atorvastatin were seen in one subject with the SLCO1B1*1A/*17 diplotype. Two other subjects with the same diplotype had considerably lower atorvastatin and rosuvastatin $C_{max}$ and AUC values. This same subject also had the

### Table 2 Pharmacokinetic variables of a single 10 mg oral dose of rosuvastatin in relation to SLCO1B1 polymorphism in 32 healthy white Caucasian subjects

<table>
<thead>
<tr>
<th>SLCO1B1 genotype</th>
<th>$C_{max}$ (ng/ml)</th>
<th>$t_{max}$ (h)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC$_{0-48h}$ (ng h/ml)</th>
<th>AUC$_{0-\infty}$ (ng h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.521TT (n=16)</td>
<td>4.21 ± 2.41</td>
<td>5.0 (1.0-5.0)</td>
<td>13.7 ± 8.5</td>
<td>33.7 ± 17.5</td>
<td>35.0 ± 18.1</td>
</tr>
<tr>
<td>c.521TC (n=12)</td>
<td>6.38 ± 3.20</td>
<td>4.0 (2.0-5.0)</td>
<td>11.4 ± 2.5</td>
<td>53.1 ± 22.3</td>
<td>55.0 ± 22.7</td>
</tr>
<tr>
<td>c.521CC (n=4)</td>
<td>7.53 ± 1.20*</td>
<td>5.0 (3.0-5.0)</td>
<td>10.9 ± 2.2</td>
<td>55.6 ± 5.4</td>
<td>56.7 ± 5.1</td>
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AUC$_{0-\infty}$ area under the plasma concentration-time curve from 0 h to infinity; AUC$_{0-48h}$ area under the plasma concentration-time curve from 0 to 48 h; $C_{max}$ peak plasma concentration; $t_{max}$ elimination half-life; $t_{max}$ time to $C_{max}$. Data are given as mean ± SD. *$P=0.003$ vs subjects with the c.521TT genotype. 7$P=0.002$ vs subjects with the c.521TT genotype.
highest $C_{\text{max}}$ and AUC of simvastatin acid in our earlier study.\textsuperscript{17} Simvastatin and atorvastatin, unlike rosuvastatin, are largely metabolized, mainly by CYP3A enzymes.\textsuperscript{3} However, all subjects in these studies were nonexpressors of CYP3A5. CYP2C8 can also be significant in the metabolism of simvastatin acid,\textsuperscript{45} and uridine diphosphate-glucuronosyl-transferase enzymes metabolize it to a minor extent.\textsuperscript{20} Rosuvastatin undergoes minor metabolism by CYP2C9,\textsuperscript{34,35} but none of the subjects carried the CYP2C9*3 allele. Because the AUC of rosuvastatin was also particularly high in this subject, genetic variability in drug-metabolizing enzymes is unlikely to explain the high statin plasma concentrations observed. It is possible that this subject carries a rare variant of the SLCO1B1 gene, or of some other drug transporter gene affecting statin pharmacokinetics.

Statins are among the most prescribed drugs worldwide, as the need for lipid-lowering therapy grows together with increase in cardiovascular morbidity, metabolic syndrome, and obesity. Function of OATP1B1 seems to be an important step in the clearance of many, but not all, statins. The liver is both the site of therapeutic action and a major site of elimination of statins. Thus, reduced OATP1B1-mediated uptake of statins into hepatocytes, caused by genetic polymorphism or drug interactions, can result in both a reduced cholesterol-lowering efficacy and an increased risk of systemic adverse effects such as myopathy.\textsuperscript{3,46} In a study with Japanese patients using pravastatin, atorvastatin, or simvas- tatin, the SLCO1B1 c.521TC genotype was associated with impaired cholesterol-lowering efficacy compared with the c.521TT genotype.\textsuperscript{47} In addition, the short-term effect of pravastatin on cholesterol synthesis was reduced in carriers of the SLCO1B1*17 haplotype in a study with healthy Caucasian subjects.\textsuperscript{48} On the other hand, in one multiple-dose trial with pravastatin, no significant difference was found in the lipid-lowering efficacy of pravastatin between carriers of the SLCO1B1*15 or *17 haplotypes ($n = 8$) and the control group ($n = 8$).\textsuperscript{49} In a Japanese study, the SLCO1B1*15 haplotype was associated with pravastatin- or atorvastatin-induced myopathy.\textsuperscript{50} In a recent study, atorvastatin-related myopathy was associated with several fold increased systemic exposures of atorvastatin metabolites atorvastatin lactone and 4-hydroxy-atorvastatin, but not with altered pharmacokinetics of the parent atorvastatin.\textsuperscript{51} Further clinical studies are needed to characterize the impact of SLCO1B1 polymorphism on the lipid-lowering efficacy and tolerability of different statins.

In conclusion, SLCO1B1 polymorphism has a greater effect on the AUC of atorvastatin than the more hydrophilic rosuvastatin. Large differences exist in the effect of SLCO1B1 polymorphism on different statins in vivo in humans and SLCO1B1 polymorphism can partly explain why individual patients respond differently to various statins.

METHODS

**Subjects.** A total of 32 young healthy white volunteers participated in the study after giving their written informed consent. They had been genotyped for SLCO1B1 SNPs by TaqMan\textsuperscript{18} allelic discrimination with an Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) as described previously.\textsuperscript{53} In addition, the participants were genotyped for the CYP3A5*3 (g.6966A>G) allele as described previously.\textsuperscript{33} As the CYP3A5*3 genotype has been associated with the efficacy of atorvastatin,\textsuperscript{33} only subjects with the CYP3A5 nonexpressor genotype (CYP3A5*3/*3) were recruited. The participants were selected on the basis of the SLCO1B1 C.521T>C SNP as well as the g.-11187G>A, g.-10499A>C, and c.388A>G SNPs, by which the four major Caucasian haplotypes containing the c.521C allele (*5, *15, *16, and *17) can be distinguished.\textsuperscript{13,52} The haplotypes were assigned as described previously.\textsuperscript{2} The subjects were allocated into one of three groups according to the genotype. The control group included 16 subjects (eight women, eight men) with the homozygous reference genotype at each position (c.521TT group). Their mean $\pm $ SD age was $23 \pm 2$ years, height $174 \pm 9$ cm, and weight $68 \pm 10$ kg. The second group consisted of 12 subjects (five women, seven men) heterozygous for the c.521T>C SNP (c.521TC group), including four subjects heterozygous for the SLCO1B1*15 haplotype (g.-11187G, g.-10499A, c.388G, c.521C), four subjects heterozygous for the *16 haplotype (GGGC), and four subjects heterozygous for the *17 haplotype (AAGG). Their mean $\pm $ SD age was $24 \pm 4$ years, height $174 \pm 9$ cm, and weight $69 \pm 8$ kg. The third group comprised four subjects (one woman, three men) with the homozygous c.521CC genotype (c.521CC group). Their mean $\pm $ SD age was $23 \pm 2$ years, height $180 \pm 8$ cm, and weight $84 \pm 8$ kg. The subjects were ascertained to be healthy by medical history, physical examination, and routine laboratory tests before they were entered in the study. One subject with the c.521TT genotype was a tobacco smoker and none used any continuous medication. The effects of variant SLCO1B1 genotypes on the pharmacokinetics of simvastatin, pravastatin, and fluvastatin have been investigated previously in prospective genotype panel studies with the same subjects.\textsuperscript{16,37}

**Study design.** The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District and by the National Agency for Medicines. In phase 1, following an overnight fast, the subjects ingested a single 10 mg dose of rosuvastatin (Crestor, AstraZeneca AB, Södertälje, Sweden) with 150 ml water at 0800. In phase 2, after a washout period of at least 1 week and following an overnight fast, the subjects ingested a single 20 mg dose of atorvastatin (Lipitor, Pfizer, Freiburg, Germany) with 150 ml water at 0800. In both phases, a standard warm meal was served 4 h after statin ingestion and a standard light meal after 7 and 10 h. Timed blood samples (5–10 ml each) were drawn before and 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 34, and 48 h after statin ingestion into tubes that contained ethylenediaminetetraacetic acid and placed on ice immediately after sampling. Plasma was separated within 30 min after blood sampling and stored at $–70^\circ$C until analysis. Use of other drugs was prohibited for 1 week and use of grapefruit products was prohibited for 3 days before administration of rosuvastatin or atorvastatin.

**Determination of plasma drug concentrations.** The concentrations of atorvastatin and its metabolites atorvastatin lactone, 2-hydroxy-atorvastatin acid, and 2-hydroxyatorvastatin lactone, as well as rosuvastatin, were measured by use of the liquid chromatography-tandem mass spectrometer SCIEX Q Trap LC/MS/MS (Sciex Division of MDS Inc., Toronto, Ontario, Canada) operating in positive turbo ion spray mode as described previously,\textsuperscript{4,55} with some modifications. Chromatography was performed on a Symmetry C8 column (50 x 2.1 mm, internal diameter; 3.5 µm) protected by a Symmetry C8 guard column (10 x 2.1 mm, internal diameter; 3.5 µm) (Waters, Milford, MA) by use of a gradient of 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 34, and 48 h after statin ingestion into tubes that contained ethylenediaminetetraacetic acid and placed on ice immediately after sampling. Plasma was separated within 30 min after blood sampling and stored at $–70^\circ$C until analysis. Use of other drugs was prohibited for 1 week and use of grapefruit products was prohibited for 3 days before administration of rosuvastatin or atorvastatin.

**RESULTS**
250 for atorvastatin, m/z 541 to m/z 250 for atorvastatin lactone, m/z 575 to m/z 250 for 2-hydroxyatorvastatin, m/z 557 to m/z 250 for 2-hydroxyatorvastatin lactone, and m/z 482 to m/z 250 for rosuvastatin. The limit of quantification for atorvastatin was 0.1 ng/ml, and the between-day coefficient of variation (CV) was 7.5% at 0.25 ng/ml, 4.3% at 1.0 ng/ml, 9.7% at 5.0 ng/ml, and 10.4% at 25.0 ng/ml (n = 7). Atorvastatin metabolite concentrations are given in arbitrary units relative to the ratio of the peak height of each metabolite to that of the internal standard in the chromatogram. The limit of quantification of plasma rosuvastatin was 0.25 ng/ml, and the between-day CV was 14.5% at 0.25 ng/ml, 5.8% at 1.0 ng/ml, 5.4% at 5.0 ng/ml, and 4.6% at 25.0 ng/ml (n = 11).

Pharmacokinetics. \(C_{\text{max}}\) time to \(C_{\text{max}}\) (\(t_{\text{max}}\)), elimination half-life (\(t_2\)), and \(AUC_{\text{0-48h}}\) and \(AUC_{\infty}\) were calculated for atorvastatin, its metabolites, and rosuvastatin. The terminal log-linear part of each concentration-time curve was identified visually, and the elimination rate constant (\(k_e\)) was determined from In-transformed data with linear regression analysis. The \(t_2\) was calculated by the equation \(t_2 = \ln 2/k_e\). The \(AUC\) values were calculated by a combination of the linear and log-linear trapezoidal rules, with extrapolation to infinity, when appropriate, by division of the last measured concentration by \(k_e\).

Statistical analysis. Results are expressed as mean \pm SD in the text and tables and, for clarity, as mean \pm SEM in the figures. The data were analyzed with the statistical program SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). The pharmacokinetic variables of atorvastatin, atorvastatin metabolites, and rosuvastatin between the SLCO1B1 c.521TT, c.521TC, and c.521CC genotype groups were compared using analysis of variance. Homogeneity of variance was tested using Levene’s Test of Equality. Post hoc testing was done with the Tukey test (equal variances) or with the Games-Howell test (unequal variances). Compatibility of the residuals with normal distribution was assessed by the Shapiro-Wilk test. When appropriate, the data were log transformed before analysis or analyzed by the Kruskal-Wallis test with a posteriori testing with the Mann-Whitney U-test with the Bonferroni correction. \(t_{\text{max}}\) data were analyzed by the Kruskal-Wallis test and a posteriori testing with the Mann-Whitney U-test with the Bonferroni correction. Difference in the effect of SLCO1B1 polymorphism on the \(AUC_{\text{0-48h}}\) of atorvastatin and rosuvastatin was tested with the paired t-test. On the basis of previous data on the pharmacokinetics of rosuvastatin and atorvastatin,28,29 the number of subjects in each genotype group was estimated to be sufficient to detect a 50% larger AUC0–48 h (\(P\) = 0.05). Differences were considered statistically significant when \(P\) was below 0.05.

ACKNOWLEDGMENTS

We thank Ms Eija Mäkinen-Pulli, Ms Lisbet Partanen, Ms Kerttu Mäntensson, and Mr Jouko Lahtila for skilful technical assistance. This study was supported by grants from the Helsinki University Central Hospital Research Fund (Helsinki, Finland) and the Sigrid Juselius Foundation (Helsinki).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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