SHORT COMMUNICATION

Atorvastatin Treatment Induces Uptake and Efflux Transporters in Human Liver

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ABSTRACT

The metabolism and disposition of statins are highly dependent on different cytochrome P450 enzymes, such as CYP3A4 and CYP2C9, as well as membrane transporters SLCO1B1, SLCO2B1, ABCB1, and ABCG2. Interindividual gene expression differences among these enzymes may explain part of the variability in tolerance and effect for statin treatment. The aim of the present study was to investigate the effect of statin treatment on these genes in human liver tissue. Levels of CYP3A4, CYP2C9, SLCO1B1, SLCO2B1, ABCB1, and ABCG2 mRNA in liver tissue from a previously performed clinical trial in 29 patients randomized to treatment with placebo, 80 mg/day of atorvastatin, or 20 mg/day of fluvastatin for 4 weeks were measured using quantitative polymerase chain reaction. Treatment with atorvastatin (n = 10), but not with fluvastatin (n = 10), resulted in 3-fold higher expression of SLCO2B1 compared with placebo-treated patients (n = 9) (P < 0.05). Atorvastatin increased the expression of both ABCB1 and ABCG2 by more than 2-fold (P < 0.05). No difference was found in CYP2C9, CYP3A4, or SLCO1B1 mRNA expression in patients administered statins or those administered placebo. Premenopausal women (n = 8) had higher expression of CYP3A4 (P < 0.05) and lower expression of CYP2C9 (P < 0.05) compared with postmenopausal women (n = 10) and men (n = 11), respectively. Here we show for the first time that atorvastatin treatment leads to increased expression of the membrane transporters SLCO2B1, ABCB1, and ABCG2 in human liver tissue, which potentially may counteract the efficacy of the treatment, and our findings may cast light on the mechanisms of clinical problems with adverse reactions and drug interactions in statin treatment.

INTRODUCTION

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are used for treatment of hypercholesterolemia. Large outcome trials have convincingly demonstrated a preventive effect of statins on serious cardiovascular events (Baigent et al., 2005). Today statins are some of the most prescribed drugs in the Western world. Several reports have demonstrated that statin-induced muscular side effects, such as myalgia, are more common than first predicted in clinical trials. In fact, 10–15% of patients prescribed statins may experience muscle symptoms (Bruckert et al., 2005; Joy and Hegele, 2009; our own unpublished results). The statin-induced myotoxicity appears to be dose-dependent (Silva et al., 2007) but independent of the cholesterol-lowering effect (Abd and Jacobson, 2011). Patients experiencing myopathy were found to have higher plasma concentrations of statins and different statin metabolites (Hermann et al., 2006).

Many statins are metabolized predominantly by hepatic cytochrome P450 enzymes. CYP3A4 is the main enzyme involved in the phase I metabolism of lovastatin, simvastatin, atorvastatin, and cerivastatin, whereas CYP2C9 is involved in the metabolism of fluvastatin (Toda et al., 2009). Given their important role in statin pharmacokinetics, it would be expected that genetic variations in cytochromes would influence the efficacy and safety of these agents.

Disposition of a drug is also strongly influenced by different transport processes. Statins are transported into hepatocytes by the organic anion transporting polypeptide (OATP) encoded by the solute carrier organic anion transporter (SLCO) genes. SLCO1B1 and SLCO2B1 have been shown to be involved in the influx transportation of statins (Kameyama et al., 2005; Grube et al., 2006; Varna et al., 2011), and genetic variation in SLCOs has been recognized in statin-induced myotoxicity (Link et al., 2008; Puccetti et al., 2010). ATP-binding cassette B1 (ABCB1, also known as permeability glycoprotein) and G2 (ABCG2, also known as breast cancer resistance protein) are efflux transporters mediating the cellular efflux of a wide variety of xenobiotics, including statins (Highman et al., 2004; Huang et al., 2006; Frishman and Horn, 2008; Keskitulo et al., 2009). Genetic variation in ABCB1 has been shown to affect the pharmacokinetics of active forms of simvastatin and atorvastatin (Keskitulo et al., 2008).

Recent in vitro studies indicate that statins affect the gene expression of SLCOs and ABCs genes (Rodrigues et al., 2006, 2009; Hoffart et al., 2012). However, the statins’ impact on the expression of cytochromes, SLCOs, and ABCs in human liver tissues has not been studied. Our study was designed to determine the hepatic gene expression of CYP2C9, CYP3A4, SLCO1B1, SLCO2B1, ABCB1, and ABCG2 in patients administered 80 mg of atorvastatin or 20 mg of fluvastatin for 4 weeks.

ABBREVIATIONS: ABCB1, ATP-binding cassette subfamily B1; ABCG2, ATP-binding cassette subfamily G2; LDL, low-density lipoprotein; P450, cytochrome P450; SLCO1B1, solute carrier organic transporter family member 1B1; SLCO2B1, solute carrier organic transporter family member 2B1.
Materials and Methods

Study Population. In this study, we used hepatic tissue material from a previously performed clinical study (Parini et al., 2008). Premenopausal and postmenopausal women and male patients selected for elective cholecystectomy because of uncomplicated gallstone disease had been enrolled. Each of the three patient groups was randomized to three treatment arms: 1) placebo, 2) 20 mg/day of fluvastatin for 4 weeks before surgery, or 3) 80 mg/day of atorvastatin for 4 weeks before surgery. Twenty-nine RNA samples were available from the original study: placebo (n = 9), atorvastatin (n = 10), and fluvastatin (n = 10). The demographic data of these subjects are shown in Table 1, including the cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein values at baseline and change during the study period in the different treatment arms. Unfortunately, we had no data on whether the premenopausal women were taking contraceptive drugs or whether postmenopausal women were taking hormonal replacement therapy, which would have been of interest for this study. A biopsy was obtained from the left lobe of the liver at surgery, and the specimen was immediately frozen in liquid nitrogen. Inclusion criteria and surgical procedure are described in details elsewhere (Parini et al., 2008). Written informed consent was obtained from all patients before inclusion into the study, which was approved by the Human Ethics Committee of Karolinska Institutet and by the Swedish Medical Products Agency.

Quantitative Polymerase Chain Reaction. Stored RNA prepared from the liver biopsies (Parini et al., 2008) was used. cDNA was synthesized using 1 μg of RNA and first-strand synthesis kit with a random hexamer primer (GE Healthcare, Uppsala, Sweden). The mRNA levels of CYP2C9, CYP3A4, SLCO2B1, SLCO1B1, ABCB1, and ABCG2 were determined by real-time polymerase chain reaction (PCR) using Taqman Gene Expression Assays from Applied Biosystems Assay ID CYP2C9: Hs01682803_mH, CYP3A4: Hs00604506_m1, SLCO1B1: Hs00272374_m1, SLCO2B1: Hs00200670_m1, ABCB1: Hs00184500_m1 and ABCG2: Hs01053790_m1, 18S rRNA was chosen as an endogenous control gene (catalog number #4310893E; Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed using 7500 Fast Applied Biosystems. Reaction mixture contained Taqman reaction mix (Applied Biosystems), and 1 μl of cDNA template (corresponding to 500 ng/μl) in a total volume of 15 μl. Thermal cycling conditions included activation at 95°C (10 minutes) followed by 40 cycles each of denaturation at 95°C (15 seconds) and annealing/elongation at 60°C (1 minute). Each reaction was performed in triplicate, and no-template controls were included in each experiment. A control sample (placebo) was used as a calibrator, and the delta-delta-Ct (ddCt) formula was used as described by Livak and Schmittgen (2001).

Statistical Analysis. Statistical analysis of the mRNA expression was performed using GraphPad Prism software version 5 (GraphPad Software, San Diego, CA). Differences in means were analyzed by one-way analysis of variance, followed by Bonferroni post hoc test comparing each statin treatment with placebo. Results are presented as mean ± standard deviation in the text, and the figures show mean, 95% confidence interval, and minimum and maximum values in box whisker plots. Correlation between gene expression and LDL reduction were calculated using Spearman rank correlation.

Results

mRNA Expression of Cytochrome, SLCO, and ABC Patients Treated with Statins or Placebo. No difference was found in CYP2C9 and CYP3A4 mRNA expression between patients administered statins or those who received placebo (Fig. 1, A and B). The interindividual variation in cytochrome expression was large. A 225- and 101-fold variation in the relative mRNA expression of CYP2C9 and CYP3A4, respectively, was observed.

No difference was observed in SLCO1B1 mRNA expression between patients treated with statins and those treated with placebo (Fig. 1C). Atorvastatin, but not fluvastatin, significantly increased the expression of SLCO2B1 3-fold (P < 0.05) (Fig. 1D). The mean expression for SLCO2B1 was 1.13 (±0.43), 3.29 (±2.27), and 0.84 (±0.72) for placebo, atorvastatin, and fluvastatin, respectively.

Atorvastatin significantly increased the expression of both ABCB1 and ABCG2 by 2.4- and 2.2-fold, respectively (P < 0.05) (Fig. 1E). The interindividual variations in the gene expression of the transporters were between 15–40-fold.

Table 1
Demographic data of the study cohort

<table>
<thead>
<tr>
<th>All</th>
<th>Placebo (n = 9)</th>
<th>Atorvastatin (n = 10)</th>
<th>Fluvastatin (n = 10)</th>
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<td>Age (yr)</td>
<td>55 (28–76)</td>
<td>55 (28–68)</td>
<td>57 (35–76)</td>
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<td>BMI (kg/m²)</td>
<td>26.6 (20.9–39.4)</td>
<td>26.6 (20.9–39.4)</td>
<td>25.6 (22.2–36.0)</td>
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<td>Cholesterol baseline (mM)</td>
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<td>5.1 (3.3–8.8)</td>
<td>5.5 (5.0–7.8)</td>
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<td>Cholesterol change during study (min, max)</td>
<td>−5% (+12, −16)</td>
<td>−41% (−33, −65)</td>
<td>−14% (+2, −35)</td>
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<td>LDL baseline (mM)</td>
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<td>3.3 (1.6–6.1)</td>
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<td>LDL change during study (min, max)</td>
<td>−7% (+6, −19)</td>
<td>−65% (−48, −78)</td>
<td>−21% (+3, −58)</td>
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<td>HDL baseline (mM)</td>
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<td>HDL change % during study (min, max)</td>
<td>0% (+10, −21)</td>
<td>0% (+20, −40)</td>
<td>−12% (+18, −31)</td>
</tr>
</tbody>
</table>

Discussion

Here we show that atorvastatin has a large impact on the hepatic gene expression of SLCO2B1, ABCB1, and ABCG2 in humans. A 3- to 4-fold induction of the SLCO2B1 and ABC genes was observed.

TABLE 1
Demographic data of the study cohort

Number of women in premenopausal age (28–41 years old), postmenopausal women (55–76 years old), and men (32–68 years old). For age, BMI, cholesterol (chol.), LDL, and HDL, the numbers show median values and minimum (min) and maximum (max) within parentheses in the different treatment arms.
after 4 weeks’ daily treatment with 80 mg of atorvastatin. No previous in vivo studies have investigated the impact of statins on hepatic gene expression of these transporters. In human hepatocytes, atorvastatin exposure was associated with approximately 2-fold induction of ABCB1 and SLCO1B1 genes (Hoffart et al., 2012). Similarly, other in vitro studies investigating the expression profile of these genes after

Fig. 1. Relative expression of (A) CYP2C9, (B) CYP3A4, (C) SLCO1B1, (D) SLCO2B1, (E) ABCB1, (F) ABCG2 in the liver obtained from individuals treated with placebo (n = 9), atorvastatin (ATV) (n = 10), or fluvastatin (FLU) (n = 10) for 4 weeks. Boxes show mean, 95% confidence interval (CI), and minimum and maximum values. ATV treatment increased the mRNA levels of ABCB1, ABCG2, and SLCO2B1. FLU decreased the mRNA levels of ABCB1.
Atorvastatin Induces Expression of Membrane Transporters

A limitation of this study is that fluvastatin was given at a low dose (20 mg), whereas atorvastatin was given at a high dose (80 mg). Even though both are in the accepted therapeutic dose range (20–80 mg/day for fluvastatin and 10–80 mg/day for atorvastatin), it is possible that a higher dose of fluvastatin would have had an effect on the transporters studied herein. Both active atorvastatin and its inactive lactone metabolite are substrates of SLCO and ABC transporters, whereas fluvastatin is a poor substrate because of its hydrophobicity (Hochman et al., 2004; Chen et al., 2005; Kameyama et al., 2005). Our results indicate that atorvastatin induces several steps in its own elimination, which may further contribute to the interindividual variability in disposition and therapeutic efficacy of atorvastatin, as previously suggested (Hoffart et al., 2012). In addition to the genes studied here, the expression and activity of other drug metabolizing enzymes may be involved in the elimination of statins. Recent studies indicate that UDP-glucuronosyltransferases (UGTs) play an important role in the clearance of atorvastatin (Riedmaier et al., 2010; Stormo et al., 2013). Unfortunately, no material is left to perform additional gene studies. It is also important to address that the ultimate outcome is the activity of these enzymes/transporters and that the gene expression not always corresponds to the enzyme activity. For CYP3A4, there is a strong correlation between mRNA levels and enzyme activity, whereas for CYP2C9 only weak correlations could be discerned (Rodriguez-Antona et al., 2001). It is likely that the enzyme activities of the proteins studied here would correlate to a higher degree with LDL reduction than can be shown when studying the mRNA levels.

In a meta-analysis of 18 prospective, randomized controlled trials, the highest rate of muscular adverse effects was associated with use of atorvastatin and the lowest risk with use of fluvastatin (Silva et al., 2006). Pharmacokinetic differences may partly explain this observation. The half-life of atorvastatin is 15–30 hours, compared with fluvastatin, which has a half-life of only 0.5–2.3 hours. The statins are metabolized by different cytochromes. Fluvastatin is metabolized by CYP2C9 (Toda et al., 2009), whereas atorvastatin is metabolized mainly by CYP3A4. Consequently, the co-use of other pharmacologic agents affecting the activity of CYP3A4 is associated with increased risk of myotoxicity. Many patients not tolerating atorvastatin (or any other CYP3A-metabolizing statin) may escape side effects when turning to fluvastatin.

The interindividual variation in the hepatic gene expression of CYP2C9 and CYP3A4 appears to be large, as shown in other studies (Furuya et al., 1991; Wortham et al., 2007). No upregulation of CYP2C9 or CYP3A4 occurred in the statin-treated subjects, in contrast to previously in vitro studies showing that atorvastatin upregulates cytochrome enzymes in primary hepatocytes (Kocarek et al., 2002; Monostory et al., 2009). However, in these experiments, very high statin concentrations (10–30 μM) were used, compared with concentrations of 1–10 nM achieved in human serum during statin treatment (Bjorkhem-Bergman et al., 2011). On the other hand, the large variability in cytochrome expression between the subjects in this study, in addition to the lack of data about hormonal therapy in women and the few samples analyzed, warrant caution in interpretation of the results. Previous studies have shown that the gene expression and activity of CYP3A are higher in women than in men (Wolbold et al., 2003; Bjorkhem-Bergman et al., 2013). The reason for this sex difference is not clear, but a sex-specific hormonal pattern has been suggested to be involved (Sarkar et al., 2003; Isoherranen and Thummel, 2013; Papageorgiou et al., 2013). In line with this sex-related difference, we saw reduced CYP3A4 expression in postmenopausal women, who have lower estrogen levels than premenopausal women. However, we did not see any difference in CYP3A4 expression between men and women in this study, probably a result of the limited number of subjects. In contrast to CYP3A4, CYP2C9 has been shown to be downregulated by estradiol (Mwinyi et al., 2011), consistent with our results showing lower levels of hepatic CYP2C9 expression in premenopausal women than in men (Fig. 2).

Large variability was seen in the statin-induced effect between the subjects (Table 1). Some studies have shown that genetic variations in SLCO1B1 and ABCG2 may affect the efficacy of statins (Generaux et al., 2011; Rodrigues et al., 2011), which could potentially explain the variability observed here. However, it should also be noted that others have failed to find any associations with these genetic variations and response to statins (Deshmukh et al., 2012). It is interesting that our correlation analysis of drug transporter–specific mRNA levels

**Fig. 2.** Relative mRNA expression of (A) CYP2C9 and (B) CYP3A4 in premenopausal women (n = 8, age 28–41 years), postmenopausal women (n = 10, age 55–76 years), and males (n = 11, age 32–68 years). Boxes shows mean, 95% confidence interval (CI) and minimum and maximum values. CYP2C9 was higher in men compared with premenopausal women, whereas CYP3A4 is significantly higher in premenopausal women compared with postmenopausal women.
revealed that the gene expression of efflux (ABCB1 and ABCG2), as well as the uptake drug transporter SLC02B1, was negatively correlated with the percent reduction in LDL after statin treatment. The finding that both influx and efflux transporters are upregulated is difficult to interpret, and they would be expected to cancel out each other; however, the results suggest that the upregulation of efflux transporters is dominant since there is a negative correlation with LDL reduction, indicating lower intracellular concentrations of statins in the hepatocytes.

The concomitant regulation of expression of SLC02B1 and ABCB1/G2 transporters indicates that these genes are regulated to a large extent by the same hepatic transcription factors. Atorvastatin has been suggested to activate pregnane X receptor (PXr) and constitutive androstane receptor (CAR), two nuclear receptors responsible for inducing transcriptional activity of various genes involved in absorption, distribution, metabolism, and excretion of drugs in hepatocytes (Monostory and Dvorak, 2011; Hoffart et al., 2012). Moreover, a decrease in cholesterol results in an activation of sterol regulatory element binding protein 2 (SREBP-2) (Brown and Goldstein, 2009), resulting in an increase in the gene expression of HMGCoo reductase and LDL receptor in the atorvastatin samples (Parini et al., 2008). It is possible that the sterol regulatory element binding protein 2 regulates the expression of the transporters (Kobayashi et al., 2013). However, further studies are warranted to elucidate the mechanism behind the atorvastatin-specific regulation of these genes in the liver.

In conclusion, we have for the first time demonstrated an induction of treatment with atorvastatin on efflux and uptake transporters in the human liver that may potentially counteract the efficacy of the treatment. Our findings may cast light on the mechanisms of therapeutic problems with adverse reactions and drug interactions in statin treatment.

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References


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