Combined Analysis of Pharmacokinetic and Efficacy Data of Preclinical Studies with Statins Markedly Improves Translation of Drug Efficacy to Human Trials


TNO (The Netherlands Organization for Applied Scientific Research), Delft, The Netherlands

Received August 16, 2013; accepted September 17, 2013

ABSTRACT
Correct prediction of human pharmacokinetics (PK) and the safety and efficacy of novel compounds based on preclinical data, is essential but often fails. In the current study, we aimed to improve the predictive value of ApoE*3Leiden (E3L) transgenic mice regarding the cholesterol-lowering efficacy of various statins in humans by combining pharmacokinetic with efficacy data. The efficacy of five currently marketed statins (atorvastatin, simvastatin, lovastatin, pravastatin, and rosuvastatin) in hypercholesterolemic patients (low-density lipoprotein ≥ 160 mg/dl) was ranked based on meta-analysis of published human trials. Additionally, a preclinical combined PK efficacy data set for these five statins was established in E3L mice that were fed a high-cholesterol diet for 4 weeks, followed by 6 weeks of drug intervention in which statins were supplemented to the diet. Plasma and tissue levels of the statins were determined on administration of (radiolabeled) drugs (10 mg/kg p.o.). As expected, all statins reduced plasma cholesterol in the preclinical model, but a direct correlation between cholesterol lowering efficacy of the different statins in mice and in humans did not reach statistical significance ($R^2 = 0.11, P < 0.57$). It is noteworthy that, when murine data were corrected for effective liver uptake of the different statins, the correlation markedly increased ($R^2 = 0.89, P < 0.05$). Here we show for the first time that hepatic uptake of statins is related to their cholesterol-lowering efficacy and provide evidence that combined PK and efficacy studies can substantially improve the translational value of the E3L mouse model in the case of statin treatment. This strategy may also be applicable for other classes of drugs and other preclinical models.

Introduction
The pharmaceutical industry is facing a huge challenge in marketing new medicines, mainly because of decreased research and development productivity and the decreasing number of truly innovative new medicines approved by the US Food and Drug Administration (FDA) (Booth and Zemmel, 2004). For example, the number of new molecular entities (NMEs) that were approved by the FDA during 2000–2005 was 50% lower compared with the preceding 5 years. Although actual costs may vary between different classes of medicines, the average investment needed to bring an NME to the market is estimated to be approximately $1.8 billion and is rising rapidly (Paul et al., 2010). Interestingly, the number of drug candidates successfully reaching phase 1 has increased in recent years, partly a result of better preclinical characterization and improved absorption, distribution, metabolism, excretion, and toxicity properties. Nevertheless, attrition in late-stage drug development (phases 2 and 3) is still high and therefore remains the most important determinant of the overall research and development efficiency. For example, the phase 2 success rates for NMEs have fallen from 28% (2006–2007) to 18% (2008–2009), and the prospect of successfully progressing through phase 2 is currently 50%. Importantly, attrition in phase 2 is caused mainly by insufficient efficacy of the newly developed drug, accounting for 51% of the drug failures (Kola and Landis, 2004; Arrowsmith, 2011, 2012). The importance of correctly predicting the efficacy of novel drug candidates and demonstrating the disease-modifying potency of those candidates, especially in the early stage preclinical phases, are therefore crucial. Although the translation from mouse to human proves to be difficult as a result of interspecies differences in efficacy or pharmacokinetics, humanized mouse models are being developed to improve such translation.

The apolipoprotein E (ApoE)*3Leiden (E3L) transgenic mouse model is a well-established humanized model for (familial) hyperlipidemia and cholesterol-induced atherosclerosis (van
Importantly, the intensity of a drug studies could substantially improve the translational value of PK studies combined with efficacy. We report for the first time a systematic link between the hepatic uptake of statins and their cholesterol-lowering profile in mice. To this end, we set up a preclinical study combining PK and efficacy data. This is also obvious from clinical studies that demonstrate the strong correlation between statin efficacy and hepatic uptake (Kleemann et al., 2003; Zadelaar et al., 2007). E3L mice develop atherosclerotic lesions with all the characteristics of human vascular pathology (Zadelaar et al., 2007). The E3L mouse model is a widely used model for studying the differential effects of cholesterol-lowering drugs (e.g., statins, fibrates, niacin) (van Vlijmen et al., 1998; Kleemann et al., 2003; Zadelaar et al., 2007; Verschuren et al., 2005, 2012).

Statins are lipid-lowering drugs that are widely prescribed to reduce the risk of primary and secondary coronary heart disease. Statins reduce plasma cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and by enhancing hepatic uptake of LDL cholesterol via upregulation of LDL receptor expression (Liao and Laufs, 2005). It is estimated that about 50 million people take statins everyday worldwide. The currently marketed statins used in this study belong to the second-generation (lovastatin, simvastatin, and pravastatin) and third-generation (atorvastatin and rosuvastatin) statins, with the latter having a higher affinity to inhibit HMG-CoA reductase and inhibit the enzyme for a longer duration (Kleemann and Kooistra, 2005). This is also obvious from clinical studies that demonstrate that only atorvastatin and rosuvastatin can reduce plasma LDL-cholesterol (LDL-C) by more than 40% in patients with hypercholesterolemia (Weng et al., 2010). The therapeutic equivalence of statins to reduce LDL-C in humans was recently reviewed in this meta-analysis by Weng et al. (2010), providing a ranking order from most to least potent of rosuvastatin > atorvastatin > simvastatin > lovastatin > pravastatin. Importantly, the intensity of a drug's effect, besides its affinity for the drug target (e.g., receptor or enzyme), is determined mainly by the drug concentration at the site of action. Since HMG-CoA reductase is expressed mainly in the liver (hepatocytes), the efficacy of statins to reduce plasma cholesterol levels is largely dependent on the pharmacokinetics and local concentration of these drugs in the liver. For example, it has been demonstrated that patients carrying a variant of the hepatic uptake transporter, OATP1B1*15 (Asn130Asp and Val174Ala), which is known to have a strongly reduced hepatic uptake transporter, OATP1B1*15 (Asn130Asp and Val174Ala), which is known to have a strongly reduced

Materials and Methods

Systematic Review of the Cholesterol-Lowering Abilities of Statins in Humans. To review systematically the abilities of five commonly used statins (atorvastatin, pravastatin, simvastatin, lovastatin, and rosuvastatin) known to reduce plasma cholesterol levels in patients with hyperlipidemia, we selected all clinical trials published between 1966 and 2009 that were previously also reviewed by Weng et al. (2010) and the Oregon Health Resources Commission (Beth Smith et al., 2009). These meta-analyses used the following selection criteria to include the studies: 1) only randomized controlled trials with 2) head-to-head comparisons of at least two statins were included. Trials needed to be based on 3) human subjects older than 18 years 4) who used statins as monotherapy to treat hyperlipidemia. The studies had to 5) report the primary data, 6) needed to last for at least 4 weeks, 7) and had to be published in English. As additional selection criteria, we included only trials in which patients with baseline plasma LDL-C levels >160 mg/dl (i.e., cut-off for hypercholesterolemia) (National Cholesterol Education Program, 2002) were selected, resulting in 77 selected studies (see Supplemental Material Note 1).

Animals. E3L mice, bred by The Netherlands Organisation for Applied Scientific Research (TNO), were housed and handled according to institutional guidelines complying with Dutch and European legislation. For this study, we used female heterozygous E3L transgenic mice (8–12 weeks of age), characterized by enzyme-linked immunosorbent assay for the presence of human apoE*3 in the serum (van Vlijmen et al., 1998). All animal experiments were approved by the Institutional Animal Care and Use Committee of TNO.

Chemicals and Reagents. The following drugs were supplemented to the diet: atorvastatin (Lipitor; atorvastatin-calcium; Pfizer, New York, NY), pravastatin (Selektine; pravastatin-sodium; Bristol-Myers Squibb, New York, NY), lovastatin (L472225; Toronto Research Chemicals, Toronto, ON, Canada), simvastatin [Zocor; simvastatin (lacton); Merck Sharp & Dohme, Whitehouse Station, NJ], and rosuvastatin (Crestor; rosuvastatin-calcium; AstraZeneca, London, UK). For the pharmacokinetic experiments, we used atorvastatin calcium and rosuvastatin calcium from Sequoia Research Chemicals (Pangbourne, UK) and simvastatin, lovastatin, and pravastatin sodium from Toronto Research Chemicals. [3H]Atorvastatin (740 GBq/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [3H]Rosuvastatin (40.7 GBq/mmol) and [3H]pravastatin (136.9 GBq/mmol) were custom synthesized by Moravek Biochemicals (Brea, CA).

Study Design. During a 4-week run-in period, all animals (except for the Chow-control group, n = 12) received a semisynthetic high-fat cholesterol (HFC) diet containing 40.5% sucrose, 15% cocoa butter, and 1% cholesterol (all w/w) (4021.04; Abdiets, Woerden, The Netherlands). After randomization into 11 groups (n = 8 per group) matched for age, body weight, plasma cholesterol, and triglyceride levels, the mice received the HFC diet alone (control group) or HFC supplemented with either atorvastatin (0.003 or 0.008% w/w), pravastatin (0.03 or 0.05% w/w), lovastatin (0.05 or 0.07% w/w), simvastatin (0.03 or 0.06% w/w), or rosuvastatin (0.0025 or 0.005% w/w) for 6 weeks (Table 1). Blood samples were taken after a period of 3 and 6 weeks of treatment. Animals were kept in a temperature-controlled environment with 12-hour light/12-hour dark cycle and received food and water ad libitum. Body weight and food intake were monitored during the study.

Analysis of Plasma Lipids. Blood was sampled after 4 hours of fasting into EDTA-containing cups by tail bleeding, and plasma was isolated. Immediately after isolation, total plasma cholesterol (TC), total plasma triglyceride (TG) levels were measured with commercially available enzymatic kits according to the manufacturer's protocols (Roche Diagnostics, No-1489437 for total cholesterol; Roche Diagnostics, No-1488872 for triglyceride; Roche Diagnostics, Indianapolis, IN).
Ultraperformance Liquid Chromatography-Mass Spectrometry Analysis of Simvastatin. Fractions of the livers (~200 mg) were sonicated twice in acetonitrile. Lysates were centrifuged 10 minutes at 14,000 rpm, and 5 μl of the supernatant was injected into the ultraperformance liquid chromatography-mass spectrometry (MS) for analysis (BEI Acquity column; C18, 50 × 2.1 mm). Samples were eluted at flow rate of 0.5 ml/min at room temperature with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The following mobile-phase conditions were used: 0–1 minute 98% A and 2% B (isocratic); 1–4 minutes linear 98 to 20% A and 2 to 80% B; 4–5 minutes 20% A and 80% B (isocratic); 5–5.1 minutes linear 20 to 98% A and 80 to 2% B; 5.1–6 minutes 98% A and 2% B (isocratic). The mass spectrometer was operating in selective reaction mode using turbo spray ionization in positive ion mode, with a capillary voltage of 5.5 kV and a spray temperature of 450°C. The multiple reaction monitoring transitions were determined from MS spectra and appeared to be MS2 = 437.5 for the precursor ion of simvastatin acid and m/z 285.3 for the product ion. Peak identification and quantification were performed using Masslynx software version 4.1 (Waters Corporation, Milford, MA).

Data and Statistical Analysis. ED30 values of statin efficacy in human and mouse were determined by linear regression with a 95% confidence interval (CI) (Liengme, 2010). Unless otherwise specified, the two-sided unpaired Student’s t test was used throughout the study to assess the statistical significance of differences between two sets of data. Differences were considered statistically significant when P < 0.05.

Results

Comparison of Cholesterol-Lowering Effects of Statins in Humans. The cholesterol-lowering effects of the different statins in patients with hypercholesterolemia are shown in Fig. 1 and Supplemental Material Note 1. There was a clear linear dose-effect relation, with the exceptions of atorvastatin and rosuvastatin at the highest dose of 80 mg/day, which might be due to saturation of HMG-CoA reductase inhibition (Supplemental Material Note 2). We also observed a clear and significant correlation between the reduction in plasma levels of TC and LDL-C after statin treatment (R^2 = 0.83, P < 0.001; data not shown). Based on linear regression, we calculated the ED30 [i.e., effective dose (milligrams per day)] to reduce plasma TC by 30% for the individual statins and found that these were, in order from strong to weak: rosuvastatin [1.79 mg/day (CI: 0.15–3.66)], atorvastatin

Plasma and Tissue Pharmacokinetic Experiments. The groups of mice that were treated with the highest dose of statin were used for analysis of pharmacokinetics. The evening before the PK study, diets of all mice were switched to the HFC control diet to allow washout of the statin (half-time of orally dosed statins ≤5 hours in mice) (Lau et al., 2006; Peng et al., 2009; Tiwari and Pathak, 2011; Zhu et al., 2011; Iusuf et al., 2012). Subsequently, after 2 hours of fasting, mice were dosed with a 10 mg/kg concentration of the different statins (10 μl/g body weight of drug solution; 1 mg/ml in saline finally in 5% DMSO). After food intake of 12 20.8

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW at t = 0</th>
<th>Food Intake^a</th>
<th>Statin Intake^b</th>
<th>Statin Intake^b</th>
<th>Plasma TC at t = 0</th>
<th>Plasma TG at t = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow control</td>
<td>12</td>
<td>20.8 ± 0.61</td>
<td>3.72 ± 0.14</td>
<td>—</td>
<td>—</td>
<td>2.83 ± 0.21</td>
<td>2.83 ± 0.48</td>
</tr>
<tr>
<td>HFC-control</td>
<td>8</td>
<td>20.3 ± 1.11</td>
<td>2.70 ± 0.20</td>
<td>—</td>
<td>—</td>
<td>15.1 ± 3.49</td>
<td>2.41 ± 0.77</td>
</tr>
<tr>
<td>HFC-0.003% Atorvastatin</td>
<td>8</td>
<td>20.9 ± 0.82</td>
<td>2.61 ± 0.21</td>
<td>0.08</td>
<td>3.76</td>
<td>15.1 ± 1.89</td>
<td>2.49 ± 0.90</td>
</tr>
<tr>
<td>HFC-0.006% atorvastatin</td>
<td>8</td>
<td>21.8 ± 1.39</td>
<td>2.65 ± 0.13</td>
<td>0.21</td>
<td>10.1</td>
<td>15.1 ± 3.52</td>
<td>2.52 ± 0.41</td>
</tr>
<tr>
<td>HFC-0.03% pravastatin</td>
<td>8</td>
<td>20.9 ± 1.57</td>
<td>2.58 ± 0.18</td>
<td>0.77</td>
<td>37.2</td>
<td>14.9 ± 2.35</td>
<td>2.28 ± 0.58</td>
</tr>
<tr>
<td>HFC-0.04% pravastatin</td>
<td>8</td>
<td>21.0 ± 1.76</td>
<td>2.55 ± 0.18</td>
<td>1.02</td>
<td>48.7</td>
<td>15.2 ± 2.81</td>
<td>2.45 ± 0.58</td>
</tr>
<tr>
<td>HFC-0.05% lovastatin</td>
<td>8</td>
<td>20.6 ± 1.21</td>
<td>2.51 ± 0.11^*</td>
<td>1.26</td>
<td>60.9</td>
<td>15.0 ± 2.21</td>
<td>2.37 ± 0.40</td>
</tr>
<tr>
<td>HFC-0.07% lovastatin</td>
<td>8</td>
<td>20.9 ± 1.11</td>
<td>2.53 ± 0.28</td>
<td>1.77</td>
<td>84.7</td>
<td>14.8 ± 3.03</td>
<td>2.37 ± 0.50</td>
</tr>
<tr>
<td>HFC-0.03% simvastatin</td>
<td>8</td>
<td>20.7 ± 1.03</td>
<td>2.60 ± 0.13</td>
<td>0.78</td>
<td>37.7</td>
<td>15.1 ± 2.01</td>
<td>2.41 ± 0.38</td>
</tr>
<tr>
<td>HFC-0.06% simvastatin</td>
<td>8</td>
<td>21.5 ± 0.85</td>
<td>2.60 ± 0.19</td>
<td>1.56</td>
<td>72.7</td>
<td>14.9 ± 2.71</td>
<td>2.72 ± 0.34</td>
</tr>
<tr>
<td>HFC-0.0025% rosuvastatin</td>
<td>8</td>
<td>21.5 ± 0.77</td>
<td>2.59 ± 0.09</td>
<td>0.06</td>
<td>3.01</td>
<td>15.0 ± 2.50</td>
<td>2.57 ± 0.49</td>
</tr>
<tr>
<td>HFC-0.005% rosuvastatin</td>
<td>8</td>
<td>21.0 ± 0.89</td>
<td>2.55 ± 0.25</td>
<td>0.13</td>
<td>6.08</td>
<td>15.2 ± 2.84</td>
<td>2.71 ± 0.68</td>
</tr>
</tbody>
</table>

Note: BW, body weight; HFC, high-fat cholesterol diet.

^aAverage over the 6-week treatment period.

^bBased on body weight at t = 0.

^cP < 0.01 compared with HFC-control group; data are presented as mean or mean ± S.D.

Note: For all experiments, samples were sacrificed after 120 minutes by CO2, followed by cardiac puncture, blood was sampled by tail bleeding after 60 and 90 minutes, and mice were used: 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in 0.1% trifluoroacetic acid (solvent B). The following mobile-phase conditions were used: 0–5 minutes linear 98 to 20% A and 2 to 80% B; 5–6 minutes 98% A and 2% B (isocratic); 6–7 minutes linear 20 to 98% A and 80 to 2% B; 7–8 minutes 98% A and 2% B (isocratic). The mass spectrometer was operating in selective reaction mode using turbo spray ionization in positive ion mode, with a capillary voltage of 5.5 kV and a spray temperature of 450°C. The multiple reaction monitoring transitions were determined from MS spectra and appeared to be MS2 = 437.5 for the precursor ion of simvastatin acid and m/z 285.3 for the product ion. Peak identification and quantification were performed using Masslynx software version 4.1 (Waters Corporation, Milford, MA).

HPLC Analysis of Lovastatin and Simvastatin. Levels of lovastatin acid and simvastatin acid in plasma and liver were measured by HPLC analysis based on the method previously described by Zhang and Yang (2007). In brief, 50 μl of methanol and 200 μl of acetonitrile were added to 100 μl of plasma or liver homogenate (homogenized in ice-cold saline using a ULTRA-TURRAX; Sigma-Aldrich, St. Louis, MO). After thoroughly vortexing, the samples were centrifuged for 10 minutes (14,000 rpm at room temperature), and 20 μl of the supernatant was injected into the column (Hypersil BDS column; 250 × 4.6 mm, 5 μm) for analysis. Samples were eluted at flow rate of 1.0 ml/min at 20°C with 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). The following mobile-phase conditions were used: 0–1 minute linear from 100 to 60% A and 0 to 40% B; 1–2 minutes linear from 60 to 30% A and 40 to 70% B; 2–3 minutes linear from 30 to 0% A and 70 to 100% B; 3–5 minutes linear from 70 to 30% A and 30 to 70% B; 5–6 minutes linear from 30 to 0% A and 70 to 100% B (isocratic). Ultraviolet chromatograms were obtained at 238 nm. Peak identification and quantification were performed using Lablogic Laura software (South Yorkshire, UK).
Comparing Lipid-Lowering Effects of Statins in E3L Mice. E3L mice treated with a HFC diet containing 1% cholesterol for 4 weeks showed on average a 5.3-fold increase in plasma total cholesterol levels compared with the chow-control reference mice. After this 4-week run-in period to achieve steady-state conditions, mice were randomized into 11 groups (n = 8 per group) based on age, body weight, plasma cholesterol levels, and triglyceride levels, and the mice received the HFC diet (control group) or supplemented with different doses of statin during an intervention period of 6 weeks (Table 1). Body weights and food intake of the E3L mice during the intervention period are presented in Table 1 and Fig. 2. The average body weights of the mice did not differ between the different groups. The food intake was comparable between all treatment groups (∼2.6 g/day on average), except the group receiving 1.26 mg of lovastatin per day, of which the food intake was 2.5 g/day (p < 0.05; one-way analysis of variance, followed by Dunnett’s multiple comparison test; Table 1). The average food intake in the chow-control group was 3.7 g/day. The effects of the different statins on plasma TC levels after 6 weeks of treatment in E3L mice are presented in Table 2 (individual data in Supplemental Material Note 3). With the exception of simvastatin, all statins decreased plasma TC levels by at least 30% at the highest tested dose. Based on the food intake, we calculated the statin intake (in milligrams per day) per group and plotted these against the percentage reduction in TC compared with the HFC-control group (Fig. 3). Linear regression was used to calculate the ED₃₀ for plasma TC reduction in E3L mice for the individual statins (extrapolation of the data was used to estimate the ED₃₀ for simvastatin). ED₃₀ values (in milligrams per day) were in order from strong to weak: rosuvastatin [0.11 mg/day (CI: 0.10–0.12)], atorvastatin [0.14 mg/day (CI: 0.12–0.16)], pravastatin [0.97 mg/day (CI: 0.90–1.03)], lovastatin [1.53 mg/day (CI: 1.51–1.54)], and simvastatin [3.34 mg/day (CI: 2.84–4.05)] (Fig. 3). The effects of the different statins on plasma TG levels after 6 weeks of treatment in E3L mice are presented in Table 2 (individual data are presented in Supplemental Material Note 4). Rosuvastatin, pravastatin, and lovastatin decreased plasma TG levels in E3L mice (62, 63, and 59% upon treatment of E3L mice with 0.13 mg of rosuvastatin per day, 1.02 mg of pravastatin per day, and 1.77 mg of lovastatin per day, respectively), as has been observed before (Kleemann et al., 2003; Delsing et al., 2005; van der Hoorn et al., 2007). Atorvastatin and simvastatin, however, did not statistically change plasma TG levels in E3L mice, which is also in agreement with previous observations (van De Poll et al., 2001; Wang et al., 2002; Delsing et al., 2003).

Pharmacokinetic Analysis of Different Statins in E3L Mice. To study putative differences in pharmacokinetics and tissue distribution between the various statins used in this study, E3L mice were orally dosed with a bolus injection of 10 mg/kg of the (radiolabeled) drug. Concentrations of the statin in plasma and liver are presented in Figs. 4 and 5, respectively. Plasma exposure as determined by the area under the curve (AUC) was 373 (pravastatin), 101 (simvastatin), 36.6 (atorvastatin), 9.77 (rosuvastatin), and 4.78 minutes
x µg/ml (lovastatin). It is noteworthy that liver levels did not correlate with the plasma levels of the different statins and were, from high to low, 14.7 (atorvastatin), 9.81 (pravastatin), 4.58 (rosuvastatin), 3.49 (lovastatin), and 1.56 µg/g (simvastatin) 30 minutes after administration. Liver-to-plasma ratios of the individual statins 30 and 120 minutes after oral administration are presented in Table 3, demonstrating the highest liver-to-plasma ratios for rosvastatin and the lowest for simvastatin.

For the three statins that were analyzed by liquid scintillation counting ([3H]atorvastatin, [3H]pravastatin, and [3H]rosuvastatin), we determined drug levels in the stomach, intestine (tissue plus contents), colon (tissue plus contents), and kidney as well (Supplemental Material Note 5). Drug levels in the stomach were comparable between the three statins (~50 µg/g 30 minutes after dosage). Compared with rosvastatin and atorvastatin, pravastatin showed the lowest level of drug in the small intestine (including contents), concomitantly with the highest plasma AUC and highest level of drug present in the kidneys. These data suggest that pravastatin has the highest oral bioavailability of these statins in E3L mice.

Comparison of the Efficacy of Different Statins between Humans and E3L Mice. The cholesterol-lowering efficacy of the different statins used in this study was compared between humans and E3L mice. To do so, we plotted the calculated ED30 values of the statins for humans against the ED30 values obtained for E3L mice, before and after correcting for pharmacokinetic differences (plasma AUC or average liver concentration) between the statins in mice (Fig. 6). Importantly, after correcting for the average liver concentrations in E3L mice, we found a significant correlation between the efficacy of statins in humans and E3L mice (R² = 0.89, P < 0.05; Fig. 6C). The hepatic extraction of statins in humans is 80% for simvastatin, 70% for atorvastatin and lovastatin, 65% for rosvastatin, and 45% for pravastatin (Neuvonen et al., 2006). When correspondingly taking these differences into account, the correlation between human and mouse studies was even further increased (R² = 0.99, P < 0.001; Fig. 6D).

**Discussion**

The E3L transgenic mouse model exhibits a human-like plasma lipid profile in response to high-fat diet feeding and develops atherosclerotic lesions that resemble their human counterparts (van Vlijmen et al., 1994; Zadelaar et al., 2007; Verschuren et al., 2012). This mouse model is a valid and is still widely used mouse model for studying the cholesterol-

### TABLE 2

Plasma TC and TG levels in E3L mice fed a HFC diet after 6 weeks of treatment with different statins

Data are presented as mean ± S.D. Time (t) = 6 after 6-week intervention period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reductiona (%)</th>
<th>Plasma TC at t = 6 (mM)</th>
<th>Plasma TG at t = 6 (mM)</th>
<th>Reductiona (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>—</td>
<td>2.03 ± 0.26</td>
<td>2.59 ± 0.35</td>
<td>—</td>
</tr>
<tr>
<td>HFC</td>
<td>—</td>
<td>16.4 ± 3.03</td>
<td>2.28 ± 0.62</td>
<td>—</td>
</tr>
<tr>
<td>HFC + 0.08 mg/day atorvastatin</td>
<td>—</td>
<td>11.5 ± 1.04***</td>
<td>29.4 ± 6.39</td>
<td>2.52 ± 0.56</td>
</tr>
<tr>
<td>HFC + 0.21 mg/day atorvastatin</td>
<td>—</td>
<td>8.55 ± 1.81***</td>
<td>47.8 ± 11.1</td>
<td>2.35 ± 0.68</td>
</tr>
<tr>
<td>HFC + 0.77 mg/day pravastatin</td>
<td>—</td>
<td>13.1 ± 1.35***</td>
<td>20.1 ± 8.27</td>
<td>1.25 ± 0.18***</td>
</tr>
<tr>
<td>HFC + 1.02 mg/day pravastatin</td>
<td>—</td>
<td>10.9 ± 1.13***</td>
<td>33.2 ± 6.87</td>
<td>0.86 ± 0.14***</td>
</tr>
<tr>
<td>HFC + 1.26 mg/day lovastatin</td>
<td>—</td>
<td>12.9 ± 1.39***</td>
<td>21.1 ± 8.48</td>
<td>2.13 ± 0.55</td>
</tr>
<tr>
<td>HFC + 1.77 mg/day lovastatin</td>
<td>—</td>
<td>10.5 ± 1.65***</td>
<td>36.0 ± 10.1</td>
<td>0.94 ± 0.18***</td>
</tr>
<tr>
<td>HFC + 0.78 mg/day simvastatin</td>
<td>—</td>
<td>16.1 ± 2.59</td>
<td>7.06 ± 10.3</td>
<td>2.55 ± 0.34</td>
</tr>
<tr>
<td>HFC + 1.56 mg/day simvastatin</td>
<td>—</td>
<td>14.7 ± 2.87</td>
<td>14.1 ± 12.3</td>
<td>2.40 ± 0.96</td>
</tr>
<tr>
<td>HFC + 0.06 mg/day rosvastatin</td>
<td>—</td>
<td>12.2 ± 0.95**</td>
<td>25.5 ± 5.77</td>
<td>1.41 ± 0.18**</td>
</tr>
<tr>
<td>HFC + 0.13 mg/day rosvastatin</td>
<td>—</td>
<td>10.5 ± 0.58***</td>
<td>36.1 ± 3.56</td>
<td>0.86 ± 0.12***</td>
</tr>
</tbody>
</table>

—, no difference.

*compared with average TC or TG levels in the HFC-control group at t = 6.

**P < 0.01 compared with HFC control group.

***P < 0.001 compared with HFC control group.
lowering and pleiotropic effects of (newly developed) drugs (e.g., statins, fibrates, ezetimibe, olmesartan). There is continuously increasing need by the pharmaceutical industry to predict correctly the efficacy of novel drug candidates in early stage preclinical phases. We therefore aimed to map the predictive value of the E3L mouse model with respect to the cholesterol-lowering efficacy of a set of statins. Importantly, since the pharmacokinetics, and especially the concentration at the target site, codetermines the efficacy of drugs, we hypothesized that combining pharmacokinetic with efficacy data can increase the predictability of drug efficacy in preclinical models. To this end, the efficacy profile of five currently marketed statins was determined in E3L transgenic mice and compared with their efficacy in humans based on data obtained from a comprehensive meta-analysis of human trials. Besides efficacy, we also determined the pharmacokinetics and tissue distribution of these drugs in E3L mice. These results show that oral bioavailability and target organ concentrations varied markedly between the statins. We demonstrate that consideration of these differences improves the prediction of the efficacy of the drugs in preclinical model and facilitates the translation of preclinical data to situation in humans.

Fig. 3. Comparison of the cholesterol-lowering effect of different statins after 6 weeks of treatment in E3L mice fed with a HFC diet. ED₃₀ values represent the effective dose (milligram per day) to reduce plasma TC by 30% (in case of simvastatin, the value had to be extrapolated). Data are presented as the percentage reduction of plasma TC compared with the HFC control group (mean ± S.D.). CI, 95% confidence interval.
mice, partly even beyond and independent of the cholesterol-lowering effect. This latter effect might be explained by the more pleiotropic effects of statin drugs (e.g., anti-inflammatory effects) in the vasculature and the liver and is in line with observations in humans (Liao and Laufs, 2005). In the current study, we concentrated on the cholesterol-lowering effects of the statins in the preclinical E3L model. The observed ED$_{30}$ values of atorvastatin and rosvastatin

Fig. 4. Plasma levels of atorvastatin, pravastatin, lovastatin, simvastatin, and rosuvastatin in E3L mice orally dosed with 10 mg/kg. All data are presented as means ± S.D. (n = 4). LLQ, lower limit of quantification (i.e., 0.1 μg/ml for lovastatin).

Fig. 5. Liver levels of atorvastatin, pravastatin, lovastatin, simvastatin, and rosuvastatin in E3L mice 30 and 120 minutes after receiving an oral dose of 10 mg/kg. All data are presented as means ± S.D. (n = 4). For very low bars, the measured value is also presented above the bar.
(0.14 and 0.11 mg/day, respectively) in this study are highly comparable to those found in studies by Verschuren et al. (atorvastatin: \(\sim0.13\) mg/day) and Delsing et al. (rosuvastatin: \(\sim0.09\) mg/day) that were carried out more than 7 years ago using the same mouse model and comparable dietary conditions (Delsing et al., 2005; Verschuren et al., 2005). This clearly demonstrates the validity and reproducibility of the E3L transgenic mouse model for these types of research questions.

We determined plasma exposure and liver uptake of the different statins in E3L mice that were exposed to statin therapy for 6 weeks after an oral bolus injection of 10 mg/kg. It has been shown before that rosuvastatin is more efficiently taken up by hepatic cells than are pravastatin and simvastatin (Nezasa et al., 2002). Here, we also observed more than 30-fold increased liver-to-plasma ratios of rosuvastatin compared with simvastatin and pravastatin, albeit in the study by Nezasa et al. (2002), the statins were dosed at 5 mg/kg i.v. Furthermore, in line with results from DeGorter et al. (2012), liver-to-plasma ratios of rosuvastatin were at the same order of magnitude as that of atorvastatin, with higher liver concentrations of atorvastatin compared with rosuvastatin, but more than 2-fold lower plasma exposure of rosuvastatin compared with atorvastatin. Again, in the referred study, the statins were dosed differently (1 mg/kg i.v.) than in the current study, indicating that comparable studies are the lacking. In the present study, we clearly demonstrate a high bioavailability (i.e., plasma exposure after oral administration) of pravastatin in mice compared with the other statin drugs. To the best of our knowledge, this has never been published before. Notably, this finding is substantiated by the relative low levels of remaining pravastatin in the small intestine and colon 30 and 120 minutes after dosing (Supplemental Material Note 5). The high plasma exposure, but more importantly the relatively high liver uptake of...
pravastatin in E3L mice, might explain the rather high cholesterol-lowering efficacy of pravastatin compared with the other two second-generation statins, lovastatin and atorvastatin. In contrast, simvastatin, which also has a rather high plasma exposure (101 min μg/ml) in E3L mice, but the lowest hepatic uptake, was the least effective. These results for the first time systematically link the hepatic transport of statins to their cholesterol-lowering efficacy in a valuable mouse model of hyperlipidemia.

The therapeutic equivalence of statins in humans was recently reviewed in a meta-analysis by Weng et al. (2010), providing a ranking order from most to least potent statins when looking at the plasma LDL-cholesterol lowering effect: rosuvastatin > atorvastatin > simvastatin > lovastatin > pravastatin. We added 16 clinical head-to-head trials that were published between 2006 and 2009 (references from Beth Smith et al., 2009) and compared the total cholesterol-lowering properties of the above mentioned statins (since we also determined the total cholesterol levels in the E3L mice). We found a ranking in potency of statins in humans with hypercholesterolemia that was fully consistent with the analysis of Weng et al. (2010). Determination of ED₅₀ values enabled us to compare directly the efficacy of these statins in E3L mice relative to humans. Interestingly, rosuvastatin and atorvastatin, which belong to the newer third-generation synthetic statins (McTaggart et al., 2001), are by far the most potent statins in humans as well as in the E3L mouse model. However, the efficacy of simvastatin, pravastatin, and lovastatin in humans is remarkably less well predicted by just looking at the potency of these statins in E3L mice. Only after taking into account the differences in liver uptake of these statins in E3L mice, we found a significant correlation between the cholesterol-lowering efficacy of these statins in E3L mice and patients with hypercholesterolemia. The fact that just correcting for differences in liver accumulation of the various statins in E3L mice is already sufficient to gain a good correlation suggests that the relative differences in liver uptake between these statins in humans are minor compared with those in E3L mice. Hepatic extraction of simvastatin, lovastatin, atorvastatin, and rosvastatin is reported to be comparable in humans (65–80%), whereas that of pravastatin is slightly lower (45%) (Neuvonen et al., 2006, 2008). When taking into account these minor differences in hepatic extraction of the studied statins, we retrieve an even better correlation between the efficacy of the drugs in humans and E3L mice (Fig. 6D).

In conclusion, in this study, we provide evidence that a combined PK-efficacy study substantially improves the translational value of the E3L mouse model in case of the cholesterol-lowering effect of statin treatment. We hypothesize that a similar strategy could be used to increase the clinical predictability of drug treatment of other classes of drugs in various preclinical (disease) models.

Acknowledgments
The authors thank I. van Scholl (TNO Triskelion, Zeist, The Netherlands) for the analyses of simvastatin in liver homogenates using UPLC-MS.

Authorship Contributions
Participated in research design: Steeg, Kleemann, Wortelboer, DeGroot.

Conducted experiments: Steeg, Duyvemooroo, Offerman, Jansen. Performed data analysis: Steeg, Duyvemooroo. Wrote or contributed to the writing of the manuscript: Steeg, Kleemann, Wortelboer, DeGroot.

References


**Address correspondence to:** Dr. Evita van de Steeg, TNO, Utrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands. E-mail: evita.vandesteeg@tno.nl.

---

**Note:** The text above is a natural representation of the document's content.