Hepatic responses to inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase: a comparison of atorvastatin and simvastatin

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Abstract

We have compared the cellular responses to simvastatin (Simva) and atorvastatin (Atorva), two potent HMG-CoA reductase inhibitors. The two drugs exhibited similar IC50's for inhibition of either rat or human reductase, and single oral dosing in rats showed the compounds to be nearly equipotent at inhibiting hepatic cholesterol synthesis. Treatment of rats with Simva or Atorva in the feed for four days yielded comparable inductions of hepatic reductase activity and reductase protein. For example, 0.05% Simva induced reductase activity 27.3 ± 9.1 fold and 0.05% Atorva induced activity 26.9 ± 4.7 fold. This adaptive response was also studied in HepG2 cells, a human hepatoblastoma line, cultured for 24 h in delipidated serum and then for an additional 24 h with Simva or Atorva. Over a broad range (10 nM–10 μM), both drugs caused similar inductions of reductase activity, reductase protein, and reductase mRNA. Under all conditions, the drugs induced similar changes in the ratio of mRNA/protein suggesting that Simva and Atorva have similar effects on both transcriptional and post-transcriptional regulatory machinery. Moreover, reductase in cells treated with Simva or Atorva for 22 h responded similarly to subsequent challenge with 25-hydroxycholesterol. Finally, we measured the ability of the two reductase inhibitors to reduce ApoB secretion by HepG2 cells. Simva and Atorva at 0.5 μM inhibited ApoB secretion nearly identically, 38% and 42% respectively. We conclude that these two drugs induce similar adaptive responses in cells and that their actions are qualitatively and mechanistically identical. Human studies have shown that plasma is cleared of Atorva much more slowly than it is of Simva. The large pharmacokinetic difference in man, rather than some difference in mechanism, is the most likely explanation for the finding that the equipotent dose ratio for cholesterol lowering in humans of Simva to Atorva is about 2:1. © 1998 Elsevier Science B.V.

Keywords: HMG-CoA reductase inhibitor; Cholesterol lowering drug; Cholesterol synthesis; HepG2 cell

1. Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors lower cholesterol by first inhibiting cholesterol synthesis at the rate-limiting step [1]. A result of this inhibition is the reduction of a regulatory sterol pool which leads in turn to an upregulation of HMG-CoA reductase [2], other enzymes of cholesterol biosynthesis [3,4], and the LDL receptor [5,6]. The increases in activity of these proteins are largely mediated transcriptionally [7,8] and
by changes in the rate of degradation of HMG-CoA reductase [7,9]. The result of these changes is that HMG-CoA reductase inhibitors lower plasma cholesterol by increasing the uptake of LDL via the LDL receptor [10] and may also decrease secretion of cholesterol-bearing particles by the liver [10–13].

A structurally diverse group of HMG-CoA reductase inhibitors including fluvastatin [14], pravastatin [1,14], lovastatin [1,14], simvastatin [1,14], and atorvastatin [15] all lower plasma cholesterol and triglycerides in man and appropriate animals. In experimental animals, atorvastatin has shown greater potency in lowering both plasma cholesterol and triglycerides when compared with lovastatin or pravastatin [15–20]. These findings have prompted the speculation that atorvastatin may be unique among HMG-CoA reductase inhibitors [20], and here we seek evidence for any such unique characteristics.

In these studies on the mechanisms of action of these two drugs, we found that simvastatin and atorvastatin showed essentially identical ability to inhibit rat or human HMG-CoA reductase in vitro and to block cholesterol synthesis in the liver of rats after oral dosing. Additional studies sought to determine if there were differences in cellular adaptation to inhibition of HMG-CoA reductase caused by either agent. We found that both agents caused induction of reductase mRNA, induction of reductase protein, induction of reductase activity, reduction of the secretion of apoB containing lipoproteins, and these cellular adaptations were equivalent with atorvastatin and simvastatin. These findings have thus failed to reveal any qualitative or mechanistic differences between these potent HMG-CoA reductase inhibitors.

After oral dosing in man, the half-life of atorvastatin is 13–51.5 h [21–24] while the half-life of simvastatin is 2–3 h [25]. This difference in plasma half-life in humans, instead of some basic difference in mechanism of action, may account for the observation that equipotent dose of atorvastatin in man appears to be about 1/2 that of simvastatin [26,27].

2. Materials and methods

\( ^{3} \text{H}-\text{Acetate}, \text{Na salt (2.52 Ci/mmole) and D,L-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA (59.0 mCi/mmole) were obtained were Amersham.} \)

2.1. Animals

Animals were housed and cared for in keeping with the standards set forth in the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 86–23, 1985). All animal experimental protocols were reviewed and approved by the Merck Institutional Animals Care and Use Committee. Male Sprague-Dawley rats, 100–125 g, were obtained from Harlan Sprague Dawley, Indianapolis, IN. They were housed in a reverse lighting room with lights on from 3:00 p.m. to 3:00 a.m. and the lights off from 3:00 a.m. to 3:00 p.m. The animals were allowed at least a week to adjust to this lighting cycle before use. They were fed a standard laboratory chow, ad lib, except where noted.

2.2. Induction of HMG-CoA reductase in rat liver

Five groups of animals with four animals per group (175–225 g) were treated with simvastatin or atorvastatin in the feed. The groups were: a control group fed ground rodent chow, a group treated with 0.01% atorvastatin by weight in ground rodent chow, a 0.05% atorvastatin group, a 0.01% simvastatin group, and a 0.05% simvastatin group. After four days of feeding the animals were euthanized in the middle of the dark cycle, and liver sections removed. Approximately one gram sections were used in preparation of microsomes [28] which were in turn used for measurement of HMG-CoA reductase activity and protein mass measurements. A second one gram section was used for the preparation of RNA.

2.3. In vivo inhibition of cholesterol synthesis

Inhibition of cholesterol synthesis in vivo was assessed by gavage dosing the animals with simvastatin or atorvastatin and examining the effect on the incorporation of \( ^{3} \text{H}-\text{acetate} \) into hepatic cholesterol. The procedure followed was a modification of that of Edmond [29]. Animals, 200–250 g, were orally dosed with simvastin or atorvastatin within one hour of the mid-dark cycle time point. At times ranging between 30 min and 7 h post dosing, the animals were dosed subcutaneously with a 10 µCi dose of \( ^{3} \text{H}-\text{acetate} \) dissolved in 25 µl of saline. 30 min after dosing with the \( ^{3} \text{H}-\text{acetate} \), the animals were eutha-
nized, 5–6 g sections of the livers were removed, and were saponified at 70°C for 24 h in 20 ml of 40% NaOH:MeOH, 3:1. Non-saponifiables were extracted with heptane and the incorporation of 3H into the non-saponifiables fraction was determined.

2.4. Induction of HMG-CoA reductase in HepG2 cells

HepG2, a human hepatoblastoma cell line obtained from the ATCC, was maintained in 75 cm² flasks in the presence of minimal essential media (MEM) supplemented with penicillin, streptomycin, non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. The cells were plated out in 60 mm dishes and grown to near confluency for two or three days in MEM supplemented with 10% FBS. The cells were then grown for 24 h in MEM containing 10% delipidated FBS [30] and then switched to MEM with 10% delipidated FBS and also containing simvastatin or atorvastatin at various concentrations. Duplicate plates were used for HMG-CoA reductase activity, HMG-CoA reductase protein levels, and HMG-CoA reductase mRNA levels. For HMG-CoA reductase activity measurements, the HepG2 cell lysates were prepared by twice washing the cells in PBS, lysing the cells by incubating them at 0°C for 30 min in 0.5 ml of 50 mM KPO₄ pH 7.4 containing 5 mM DTT, 25 mM EDTA, 0.2 M KCl and 0.2% NP-40, and saving the supernatant after a 3 min 10 000 × g spin.

2.5. Determination of HMG-CoA reductase protein levels by immunoblotting

Washed HepG2 cells pellets or liver microsomes were treated with SDS sample buffer to give a solution 10 mg/ml in protein for liver microsomes or 1 mg/ml for HepG2 cell lysates and 0.62 M in Tris pH 6.8 with 3% SDS and 3% β-mercaptoethanol. The serial dilutions of the samples were loaded onto nitrocellulose membranes using the BioRad BIO-DOT® apparatus. These membranes were immunoblotted by using a 1/2000 dilution of rabbit anti-rat HMG-CoA reductase IgG and ECL western blotting kit from Amersham according to the Amersham protocols, with the exception that blocking of the membranes was done with 10% FBS rather than the supplied blocking reagent. Kodak XAR-5 film was exposed to the developed ECL blot for 10–15 s and the intensity of the bands was quantitated by scanning the developed films in a ISCO UA 5 absorbance detector equipped with a model 1312 Gel Scanner and the output peaks were integrated using a Spectra Physics SP4270 Integrator.

2.6. Determination of mRNA levels in HepG2 cells by RNase protection assays

Plasmid phRed-102 (ATCC) was used as template for the PCR amplification (30 cycles) of a 386 bp fragment corresponding to base 515 through 901 of HMG-CoA reductase coding sequence. The PCR product was phenol/chloroform/isoamyl alcohol extracted two times and ethanol precipitated. The product was digested with Kpn I and Sac I for 12 h and run on a 2% agarose TAE gel and purified from the matrix using the QIAEX II system (QIAGEN). The subcloned fragment is 308 base pairs, as HMG-CoA reductase contains a Kpn I site at position 592 of the coding region. This fragment was ligated into Kpn I/Sac I cut and purified pBluescript II KS (+). Pbluescript was chosen as a vector because the multiple cloning site is flanked by a both a T3 and T7 RNA polymerase recognition site to be used in vitro transcription. Labeled probe was synthesized using Ambion Maxiscript Kit per manufacturer’s instruction with 1 μg KpnI linearized phRedRNA and 50 μCi ³³P UTP (2000 Ci/mmol) and 2 μM cold UTP.

Total RNA was isolated from 60 mm dishes of HepG2 cells using TRIzol® (Gibco) reagent per manufacturer’s instructions. RNase protection was performed using Ambion RPA II kit per manufacturer’s instruction with 1.0 × 10⁵ cpm gel purified phRed RNA probe and 1.0 × 10⁴ cpm β-actin per 10 μg total RNA isolated from HepG2 cells. After digestion, the products were separated on 6% 8 M urea/TBE gel (Novex) and quantified using a phosphorimager.

2.7. Secretion of apoB from HepG2 cells

HepG2 cells were plated in 60 mm dishes at 1 × 10⁶ cells per plate in MEM media supplemented with Na pyruvate, amino acids and FBS as described above. After four days of growth, the cells were
transferred to unsupplemented OPTI-MEM media (Gibco) for 8 h. The cells were then incubated in 3 ml of OPTI-MEM media containing 800 μM Na oleate (diluted from a 15× stock in 10% BSA), 2 mM Na butyrate, and simvastatin or atorvastatin (diluted from 1000× stock solutions in 70% EtOH). After 24 h, 2 ml of the media was removed and centrifuged at 5000 × g for 5 min. The supernatants were frozen at −20°C and used for subsequent apoB determinations.

2.8. ApoB ELISA procedure

ApoB containing media samples were diluted 13-fold into Pierce Super-Block TBS containing 30% OPTI-MEM and 0.1% Tween 20. 50 μl aliquots of the diluted apoB containing samples were added to wells of Costar EIA/RIA flat bottom plates that had been precoated with murine monoclonal anti-ApoB IgG (Cal BioChem-Type 2) (coating was done at an antibody concentration of 6 μg/ml in 100 μl of 0.2 M bicarbonate buffer at pH 9.4) and blocked with Pierce Super-Block TBS. After a 45 min incubation, 50 μl of a 150 fold dilution of rabbit anti-ApoB polyclonal antibody (Cal BioChem) was added and incubation was continued for another 45 min. After the wells were washed 3 × with PBS containing 0.05% Tween 20, 100 μl of a 4000-fold dilution of horseradish peroxidase-linked donkey anti-rabbit IgG antibody (Pierce) was added and incubated for 30 min. Bound peroxidase activity was determined according to procedures described in the manual supplied by Pierce using OPD (o-phenylenediamine dihydrochloride) as the substrate. Endpoint measurements were made at 490 nm. Human plasma apoB from Cal Biochem was used as a standard in a range from 0.02 to 2.56 μg/ml.

2.9. Enzyme and protein assays

HMG-CoA reductase activity was assayed as described by Germershausen et al. [28]. Partially purified rat liver HMG-CoA reductase was prepared as described by Germershausen et al. [28]. Microsomes from cholesterol starved HepG2 cells was used as a source of human HMG-CoA reductase. Protein was determined using the Pierce coomassie protein reagent.

Fig. 1. Inhibition of human HepG2 cell HMG-CoA reductase (panels B and D) and rat liver HMG-CoA reductase (panels A and C) by simvastatin (panels C and D) and atorvastatin (panels A and B). HMG-CoA reductase was assayed as described in Section 2. The data was plotted, lines fit to the data as a hyperbola, and IC50’s determined using the program GraphPad Prism.
3. Results

3.1. Inhibition of HMG-CoA reductase

Simvastatin and atorvastatin were compared for their ability to inhibit rat liver HMG-CoA reductase and human HMG-CoA reductase from HepG2 cells (Fig. 1). Against the rat liver HMG-CoA reductase, simvastatin and atorvastatin were found to cause complete inhibition with similar curve shape and nearly identical IC₅₀ values (7.5 and 7.6 nM, respectively). Against the human enzyme prepared from the HepG2 cells, simvastatin, IC₅₀ = 10.8 nM, was slightly more potent than atorvastatin, IC₅₀ = 14.3 nM. The two inhibitors thus appear to be nearly equipotent as enzyme inhibitors regardless of whether the enzyme source is rat or human.

3.2. Inhibition of cholesterol synthesis in vivo in the rat

Simvastatin and atorvastatin were orally dosed in rats during the hour preceding the mid point of the dark cycle, the peak of their daily diurnal variation of cholesterol synthesis [31]. Cholesterol synthesis was measured at various points after this by giving the animals a dose of ³H-acetate and measuring the incorporation of the ³H into hepatic non-saponifiable lipids. Both simvastatin and atorvastatin caused strong inhibition of ³H incorporation into hepatic non-saponifiable lipids with similar potency (Fig. 2). The peak of simvastatin’s activity (30 min for 0.4 mg/kg and 1 h for 2 mg/kg) occurred earlier than that for atorvastatin (90 min for 0.4 mg/kg and 2 h for 2 mg/kg). The inhibition of both inhibitors was virtually gone by 7 h post dosing. The ability of equivalent doses of these compounds to inhibit cholesterol synthesis in the liver of rats in response to acute administration is thus very similar.

3.3. Induction of rat hepatic HMG-CoA reductase by simvastatin and atorvastatin

Liver cells alter their metabolic profile in response to HMG-CoA reductase inhibitors, exhibiting enhanced expression of LDL receptor, HMG-CoA reductase, and other enzymes [7]. To assess this adaptive response, simvastatin and atorvastatin were administered to rats in the feed for four days. The resulting levels of hepatic HMG-CoA reductase activity and immunoreactive protein levels are shown in Table 1. Both compounds caused large inductions of HMG-CoA reductase activity and protein mass. Since the microsomes isolated from treated animals may contain inhibitor that was not fully washed out, these values may underestimate the total amount of HMG-CoA reductase present. In keeping with this consider-

<table>
<thead>
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<th>Treatment</th>
<th>HMG-CoA reductase activity</th>
<th>Ref. to cont.</th>
<th>HMG-CoA reductance mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.13 ± 0.05</td>
<td>1.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Atorvastatin 0.01%</td>
<td>0.99 ± 0.44</td>
<td>7.6</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>Atorvastatin 0.05%</td>
<td>3.50 ± 0.61</td>
<td>26.9</td>
<td>93 ± 25</td>
</tr>
<tr>
<td>Simvastatin 0.01%</td>
<td>1.11 ± 0.13</td>
<td>8.5</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Simvastatin 0.05%</td>
<td>3.55 ± 1.18</td>
<td>27.3</td>
<td>60 ± 16</td>
</tr>
</tbody>
</table>

Diets were fed for 4 days.
Values are mean ± std. dev. N = 4 for each group.
ation, we observed an even greater induction of HMG-CoA reductase protein by using western blot estimation of enzyme mass (Table 1). When the compounds were mixed in the diet at 0.01%, the induction of enzyme activity was about 8-fold but induction of protein mass was about 13-fold for simvastatin and 18-fold for atorvastatin. When the compounds were mixed in the diet at 0.05%, induction of activity was about 27-fold and the inductions of HMG-CoA reductase protein mass was 60-fold for simvastatin and 95-fold for atorvastatin. None of the differences between atorvastatin and simvastatin were statistically significant. These data show that both atorvastatin and simvastatin induce adaptive changes in hepatic HMG-CoA reductase, that these changes are similar in magnitude, and that the changes exhibit similar dependence on the dose of the inhibitor.

3.4. Induction of HMG-CoA reductase activity in HepG2 cells

Cells respond to HMG-CoA reductase inhibitors with both increased HMG-CoA reductase mRNA and a decreased rate of reductase degradation [7–9]. Responses of hepatocytes in whole animals will depend on the time and dose of the inhibitor, changing plasma levels of the inhibitor, and diurnal variations in reductase mRNA. It is therefore difficult to obtain a reliable estimate of the contributions of transcriptional and post-transcriptional mechanisms to the changes in reductase levels induced by drugs in animals. To obviate these difficulties, we studied the regulation of HMG-CoA reductase by simvastatin and atorvastatin in HepG2 cells. HepG2 cells were exposed to a constant, fixed concentration of inhibitor for 24 h to allow attainment of steady state, then adaptive responses were quantitated. The results showed that over a three log dose range, simvastatin and atorvastatin both caused an induction of HMG-CoA reductase activity (Table 2). The inductions caused by simvastatin and atorvastatin were nearly identical in magnitude and dose-dependence.

To determine the mechanism underlying the induction of reductase by atorvastatin and simvastatin, cells were exposed to drug for 24 h, then reductase mRNA and enzyme mass were measured in parallel. Both inhibitors caused an ~2-fold induction in mRNA and a ~10-fold induction of protein mass (Fig. 3). These observations suggest that changes in post-transcriptional regulation may be more important in regulating reductase mass changes than changes in mRNA levels under the conditions chosen. They also suggest that atorvastatin and simvastatin are similar with respect to their effects on both the regulation of synthesis and the regulation of degradation of HMG-CoA reductase. To observe potential subtle differences in the adaptive mechanisms induced by atorvastatin and simvastatin, we calculated the ratio of protein induction vs. mRNA induction caused by the inhibitors. If one of the inhibitors had an effect on either mRNA or reductase degradation that was not shared, an effect on this ratio would be predicted. However, no differences were observed (Fig. 3(C)), and we conclude that atorvastatin and simvastatin induce similar effects on HMG-CoA reductase activity and induce quantitatively and qualitatively similar adaptive changes in HepG2 cells.

Table 2

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Simvastatin</th>
<th>Atorvastatin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>pmole/min/mg</td>
<td>% of control</td>
</tr>
<tr>
<td>0.009 μM</td>
<td>23 ± 4</td>
<td>177</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>132 ± 16</td>
<td>1060</td>
</tr>
<tr>
<td>1 μM</td>
<td>223 ± 42</td>
<td>1780</td>
</tr>
<tr>
<td>10 μM</td>
<td>301 ± 18</td>
<td>2410</td>
</tr>
</tbody>
</table>

Cells were cultured 24-48 in delipidated medium, the HMG-CoA reductase inhibitor was added to the level indicated, and after an additional 24 h incubation HMG-CoA reductase activity levels were determined as described in Section 2. Results are combined from two separate experiments and in each case the samples were done in duplicate and the range of the values is given by the ± value. Reductase activity in the absence of a HMG-CoA reductase inhibitor was 13 pmol/min/mg.
Fig. 3. Induction of HMG-CoA reductase protein and mRNA by Simvastatin or Atorvastatin in HepG2 cells. Confluent monolayers of HepG2 cells were incubated for 24 h in delipidated media and then switched to media containing various concentrations of simvastatin or atorvastatin for 24 h. Some of the dishes that were treated with 10 μM simvastatin or atorvastatin were challenged in addition with 10 μM 25-hydroxycholesterol for the last two hours of the 24 h incubation with simvastatin or atorvastatin. HMG-CoA reductase protein levels (Panel A) and HMG-CoA reductase mRNA levels (Panel B) were determined as described in Section 2. These values were used to determine the ratio of HMG-CoA reductase protein/mRNA (panel C).

Though atorvastatin and simvastatin induced equivalent adaptive changes in HepG2 cells, it remained possible that the drug-treated cells might exhibit differences in their ability to respond to a subsequent acute change in the regulatory sterol pool. To examine this possibility, HepG2 cells were exposed to 10 μM atorvastatin or simvastatin for 24 h. During the final two hours of the incubation, cells were challenged with 25-OH cholesterol, a compound well know for its ability down-regulate levels of HMG-CoA reductase by decreasing transcription and increasing degradation of HMG-CoA reductase protein [9]. As expected from previous studies, 25-OH cholesterol induced a ∼2-fold decline in levels of both reductase mRNA and protein and a very slight reduction in the mRNA/protein ratio (Fig. 3). Importantly, all of these alterations were equivalent in simvastatin-treated and atorvastatin-treated cells. Thus we could observe no difference in the sensitivity to exogenous sterols or the mechanism of response to sterols in cells treated with simvastatin or atorvastatin.

3.5. Inhibition of ApoB secretion by simvastatin and atorvastatin

Liver secretes triglyceride and cholesterol in the form of VLDL particles bearing apoB. Studies with simvastatin in man [11] and with atorvastatin and lovastatin in animals [12,13] have shown that these agents can inhibit the secretion of apoB from liver. The inhibition of the secretion of VLDL from the liver has been postulated to play a role in both the cholesterol and the triglyceride lowering effects of simvastatin and atorvastatin [11,12]. Previous studies have differed in their conclusions on whether limiting cholesterol and cholesterol ester production could affect apoB secretion from cell lines [32–36]. Two studies [32,33] found that either simvastatin or lovastatin could inhibit apoB secretion from HepG2 cells while two others found that fluvastatin [34] and pravastatin [35] did not. In preliminary studies using HepG2 cells in medium supplemented with oleate, we failed to observe an effect of simvastatin or atorvastatin on apoB secretion. However, when cells were cultured in OPTI-MEM medium supplemented with both oleate and butyrate, the secretion of apoB was enhanced >8-fold to 49.8 ± 4.5 μg/24 h/mg cell protein (mean ± S.D. with n = 6 for each group). Kaptein et al. [37,38] recently showed that butyrate can stimulate the secretion of ApoB from HepG2 cells. Butyrate has long been known to be an excellent precursor for the de novo synthesis of long chain fatty acids [39] and the enhanced secretion of
Apob in our incubations with oleate and butyrate in OPTI-MEM is likely due to increased synthesis of triglycerides. Under these optimized conditions, addition of either 0.5 \( \mu \text{M} \) simvastatin or 0.5 \( \mu \text{M} \) atorvastatin reduced secretion of apoB to 30.9 ± 4.1 \( \mu \text{g}/24 \text{ h}/\text{mg} \) (38% reduction) and 29.1 ± 4.8 \( \mu \text{g}/24 \text{ h}/\text{mg} \) (42% reduction) respectively. These studies thus indicate that simvastatin and atorvastatin produce similar responses not only with respect to inhibition of cholesterol synthesis but also with respect to their effects on the secretion of apoB.

4. Discussion

Here we have sought evidence for a either a qualitative or quantitative difference between the actions of atorvastatin and simvastatin in cells and laboratory animals. Despite suggestions in the literature [20], we were unable to define a clear difference by any of seven criteria: (1) Simvastatin and atorvastatin were found to be nearly equivalent as enzyme inhibitors of HMG-CoA reductase in vitro regardless of whether the enzyme was of rat or human origin (Fig. 1). (2) Single oral doses of simvastatin and atorvastatin caused similar inhibition of hepatic cholesterol synthesis (as measured by \(^3\)H incorporation into non-saponifiable lipids (Fig. 2)). (3) Four day dosing of simvastatin and atorvastatin in the feed of rats caused equivalent induction of hepatic HMG CoA reductase activity and protein (Table 1). (4) Constant levels of atorvastatin and simvastatin caused similar induction of HMG-CoA reductase activity in HepG2 cells. (5) The induction of HMG-CoA reductase protein, reductase mRNA, and the ratio of protein/mRNA was nearly identical in cells exposed to constant levels of simvastatin or atorvastatin (Fig. 3). (6) Cells exposed to atorvastatin or simvastatin showed equivalent changes in reductase mRNA and protein in response to a subsequent challenge with exogenous sterol. (7) Simvastatin and atorvastatin caused similar inhibition of the secretion of apoB-containing lipoproteins by HepG2 cells. In each of these tests, both the magnitude of the response and the dose dependence were similar for atorvastatin and simvastatin. These observations show that the inhibition of HMG-CoA reductase, the adaptive response to that inhibition, and the mechanisms of those responses for these two HMG-CoA reductase inhibitors are nearly identical under these experimental conditions.

Animals respond to HMG-CoA reductase inhibitors not only with reductions in plasma cholesterol but also with reductions in plasma triglycerides, and recent animal and clinical data indicate that simvastatin and atorvastatin induce similar alterations in triglyceride levels. The administration of equal doses of simvastatin and atorvastatin (2.5 \( \text{mg}/\text{kg} \)) to fat-fed rabbits showed that atorvastatin and simvastatin each caused strong reductions of both plasma cholesterol (45–60%) and triglycerides (37%) [16]. From additional studies in rats, rabbits, and guinea pigs, Krause and Newton [40] concluded that “triglyceride lowering is not due to some non-specific action of atorvastatin or dissociated from cholesterol synthesis, but instead is dependent upon inhibitory activity against HMG-CoA reductase.” Our studies showed that simvastatin and atorvastatin caused comparable reductions in ApoB secretion from HepG2 cells and are consistent with the above conclusion. Studies in man are further consistent with this conclusion. For example, a recent multicenter trial [26] in hypercholesterolemic patients with normal or near normal triglycerides (\( n = 140 \)) shows that simvastatin, 160 \( \text{mg} \), produces a median reduction in LDL cholesterol of 53% and a reduction in triglyceride of 33%, nearly identical to the LDL lowering of 52% and triglyceride lowering of 33% reported with atorvastatin, 80 \( \text{mg} \), [27] in a similar patient population (\( n = 189 \)). These and other clinical data also suggest that the equi-potent dose of simvastatin and atorvastatin in man is about 2:1.

We did observe a small difference in the kinetics of inhibition of cholesterol synthesis after single oral dosing of rats, with the effects of atorvastatin peaking shortly after those of simvastatin (Fig. 2). A major pharmacokinetic difference between blood levels of atorvastatin and simvastatin has been observed in man [21–25]. Plasma concentration of simvastatin (\( T_{1/2} = 2–3 \text{ h} \)) decline more rapidly than those of atorvastatin (\( T_{1/2} = 13–51.5 \text{ h} \)). Two consequences of the longer half-life of atorvastatin are that peripheral tissues may have greater exposure to drug, and effects on HMG-CoA reductase may be longer lived. The very long half-life of atorvastatin, rather than some mechanistic difference, appears to be a likely
explanation for the finding that atorvastatin achieves equivalent cholesterol lowering with a lower oral dose than with simvastatin.

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