Atorvastatin action involves diminished recovery of hepatic HMG-CoA reductase activity

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Abstract The effects of atorvastatin on the expression of the hepatic HMG-CoA reductase and LDL receptor genes were investigated in rats. Like the other statins, atorvastatin increased the rate of degradation and presumably cycling of the hepatic LDL receptor. In atorvastatin-treated rats, the half-life of the receptor was decreased by over 60%. Hepatic HMG-CoA reductase mRNA levels were increased about 3-fold by feeding a diet containing 0.04% atorvastatin while reductase protein levels were increased by as much as 700-fold. Apparent HMG-CoA reductase activity was not increased as much as protein levels. Washing experiments revealed that atorvastatin is more difficult to remove from microsomes than lovastatin. The results support the conclusion that the potent hypocholesterolemic action of atorvastatin involves decreased hepatic VLDL production due to effective inhibition of in vivo cholesterol biosynthesis resulting from diminished recovery of HMG-CoA reductase activity following drug treatment.**—Ness, G. C., C. M. Chambers, and D. Lopez.** Atorvastatin action involves diminished recovery of hepatic HMG-CoA reductase activity. *J. Lipid Res.* 1998. **39:** 75–84.

Supplementary key words atorvastatin • lovastatin • HMG-CoA reductase inhibitors • immunoblotting • Northern blotting • LDL receptor

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase are currently extensively used to lower serum cholesterol levels and improve the survival of individuals at risk of atherosclerotic vascular disease (1). Lovastatin was the first of these drugs to be used widely. These drugs lower serum cholesterol and LDL levels by 25 to 35% and have been shown to be effective in slowing the progression of coronary atherosclerosis (2, 3). In the Scandinavian Simvastatin Survival Study it was demonstrated that lowering serum cholesterol levels significantly improved the survival of coronary heart disease patients (4). Of 2221 patients treated with simvastatin, only 161 died within 5 years, while 212 out of 2223 patients receiving placebo died during this period. Thus, the 6-year probability of survival increased from 87.6% to 91.3%. In these studies (4), mean total cholesterol levels were lowered 25%. Perhaps further cholesterol lowering will provide additional benefit.

One of the characteristic responses of cells and tissues to the family of HMG-CoA reductase inhibitors known as the statins is a large compensatory induction of the reductase (5, 6). This induction is felt to be due to relief of the normal feedback regulation of the enzyme. Thus the inhibition of whole body cholesterol biosynthesis is not as great as might have been expected (7). This finding has focused attention on the role of LDL receptors in lowering serum cholesterol levels in response to treatment with these drugs (8). It is thought that hepatic LDL receptors are increased in subjects treated with the statins (8).

In a recent study of the effects of lovastatin, pravastatin, fluvastatin, and rivastatin on hepatic LDL receptor expression in rats (6), we found, surprisingly, that none of these statins increased hepatic LDL receptor protein levels. They did, however, increase the levels of LDL receptor mRNA and the rate of receptor protein degradation. These findings suggested that the statins might act to increase the rate of removal of serum LDL by increasing the rate of cycling of the receptors (6). The relative potency of the statins correlated with their effects on increasing the rate of hepatic LDL receptor protein degradation.

Recently, it was reported that atorvastatin, a new statin, is able to lower serum cholesterol levels by as much as 60% in patients given 80 mg daily (9). Initial studies of the mechanism of action of atorvastatin suggested that it acts mainly by decreasing production of apoB-containing lipoproteins (10). Interestingly, it was re-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; FH, familial hypercholesterolemia; SDS, sodium dodecyl sulfate.

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cently reported that administration of atorvastatin to guinea pigs did not increase the activity of microsomal HMG-CoA reductase (11). Clearly, a drug that does not cause a compensatory induction of the reductase would be expected, intrinsically, to be more effective at lowering serum cholesterol levels.

Based on these observations, we hypothesized that the greater cholesterol-lowering action of atorvastatin may be due to its greater effect on hepatic LDL receptor protein degradation (cycling) and its lack of compensatory induction of hepatic HMG-CoA reductase. Thus, we examined the effects of atorvastatin, in comparison with lovastatin, on hepatic LDL receptor mRNA levels, protein levels, and rate of receptor protein degradation and on HMG-CoA reductase mRNA levels, protein levels, and activity. We found little difference between the two drugs on the rate of LDL receptor protein degradation. Surprisingly, we found that the compensatory induction of HMG-CoA reductase protein by atorvastatin was even greater than that caused by lovastatin. However, reductase activity was lower because atorvastatin was more difficult to wash off of microsomal HMG-CoA reductase. Its greater serum cholesterol lowering action may relate to this property. This finding may also explain the previous report (11) claiming that atorvastatin does not increase HMG-CoA reductase activity in guinea pigs.

MATERIALS AND METHODS

Materials

Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, $NADP^+$, HMG-CoA, cycloheximide, and silica gel G were purchased from Sigma (St. Louis, MO). The nick translation kit was purchased from Boehringer Mannheim (Indianapolis, IN). [^{14}C]RS HMG-CoA, [α^{32}P]dCTP, Gene Screen Plus membrane, and PVDF Plus membrane were purchased from DuPont/New England Nuclear (Boston, MA). Tri Reagent was purchased from Molecular Research Center (Cincinnati, OH). Oligo dT cellulose was obtained from Fisher Scientific (Atlanta, GA). The ECL Western blotting kit was purchased from Amersham (Arlington Heights, IL). A peptide antisera to the rat LDL receptor was generated in rabbits as previously described (12). Antisera to the catalytic domain of rat HMG-CoA reductase was generated in rabbits as previously described (13). The sources of the cDNAs for the LDL receptor, HMG-CoA reductase, and β-actin have been given previously (14). Lovastatin was a generous gift from Merck (Rahway, NJ). Atorvastatin was kindly provided by Parke-Davis, Warner-Lambert (Ann Arbor, MI).

Animals

Male Sprague-Dawley rats weighing 100–150 g, 35–42 days of age, were purchased from the University of South Florida Animal colony. They were housed in a reverse-cycle light-controlled room with the lights on from 3 pm to 5 am and off from 5 am to 3 pm. The animals were fed ground Purina Rodent Laboratory chow and water ad libitum. Lovastatin and atorvastatin were mixed with the diet using a Glen Mills mixer at the indicated concentrations and fed to the rats for 3 days. These young animals express relatively high levels of hepatic HMG-CoA reductase and LDL receptors and are rather resistant to developing hypercholesterolemia. As the animals age or are rendered hypothyroid, their serum cholesterol levels more than double (15, 16), their expression of hepatic HMG-CoA reductase and LDL receptors decrease markedly (17), and they lose their resistance to hypercholesterolemia (16). When thyroidectomized rats are given a replacement dose of triiodothyronine, their ratio of LDL to HDL cholesterol decreases from 1.0 to 0.28 (G. C. Ness, D. Lopez, W. P. Newsome, P. Cornelias, C. A. Long, and H. J. Harwood, unpublished observations). Rats differ from hamsters, rabbits, and mice in their initial LDL to HDL ratio, yet they are quite responsive. We have recently shown that several statins markedly increased hepatic LDL receptor mRNA levels, rates of receptor protein degradation, and receptor cycling in rats (6).

In contrast with the responses of animals with higher LDL levels, chow-fed rats exhibit very little if any serum cholesterol-lowering in response to statins (18). Rats do, however, exhibit marked reductions in plasma triglyceride levels in response to the statins that correlate with the efficacy of these drugs in LDL animal models (18). It has been concluded that chow-fed rats are a useful model in evaluating the effects of HMG-CoA reductase inhibitors.

The rats were fed diets containing lovastatin or atorvastatin for 3 days in order to provide sufficient time for changes in hepatic LDL receptor and HMG-CoA reductase gene expression to reach a new steady state. The length of time required for this depends on the half-life of each protein. In the case of the LDL receptor, the half-life is about 8 h (6). In the case of HMG-CoA reductase, the half-life is about 2.5 h (19). In a previous study, we demonstrated that a new steady state is reached for HMG-CoA reductase within 72 h (20).

Determination of cholesterol levels

Liver and serum cholesterol levels were determined by reverse phase HPLC analysis. Portions of liver (100

mg) or serum (100 μl) were saponified in 1 ml of 20% (w/v) potassium hydroxide in 66% (v/v) methanol in a boiling water bath for 30 min. About 20,000 cpm of [^{3}H]cholesterol (4 pmol) was added as a recovery standard. After cooling to room temperature, the samples were extracted four times with 2 ml of petroleum ether each time. The combined extracts were taken to dryness under a stream of argon. The residue was dissolved in 1 ml of methanol. A sample of 100 μl of this preparation was resolved on a Spheri-5, RP-18, 5 μ reverse phase column using 100% methanol as the solvent (21). The elution was monitored at A_{210}. Identification of the cholesterol peak and determination of its mass were carried out by comparison to a cholesterol standard. The recoveries of [^{3}H]cholesterol ranged from 80 to 97%. Liver cholesterol levels are expressed as mg/g while serum cholesterol levels are given in terms of mg/dl.

Northern blotting analysis

Rat liver RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform extraction method (22). Rats were killed by decapitation. A midline incision was made and a 0.2-g portion of liver was cut from a lobe within 10 sec and placed in 2 ml of room temperature TRI Reagent (Molecular Research Center, Cincinnati, OH). This was immediately homogenized using a Polytron homogenizer at full speed for 10 sec. After standing at room temperature for 5 min, the sample was mixed vigorously with 0.4 ml of chloroform. After centrifugation at 12,000 *g* for 15 min at 4°C, the RNA pellet was washed with 75% ethanol and then air-dried. The RNA pellet was dissolved in 1 ml of DEPC-treated water and heated to 60°C for 5 min. An equal volume of 1 m NaCl, 0.02 m Tris-HCl, pH 7.4, 0.002 M EDTA and 0.2% (w/v) SDS was added. Poly A^+ RNA was isolated by oligo (dT) cellulose chromatography. Relative levels of hepatic LDL receptor and HMG-CoA reductase mRNA were determined by Northern blotting analysis as previously described (14) using rat cDNA probes. β-Actin was used as an internal standard.

Immunoblotting analysis

Rat liver membranes essentially free of lysosomes were prepared as previously described (23). Quickly excised liver was homogenized in 10 volumes of cold 0.25 m sucrose using a Potter-Elvehjem type homogenizer with a motor-driven serrated Teflon pestle. The homogenate was centrifuged twice at 16,000 *g*. The upper two-thirds of the final supernatant was centrifuged at 100,000 *g* for 1 h to pellet the microsomes. This pellet was resuspended in one-half of the original volume and centrifuged again at 100,000 *g*. Additional washes of microsomes were carried out using 10 ml of 0.25 m sucrose for microsomes isolated from 1 g of liver. Membrane samples were subjected to SDS-PAGE on 7.5% slab gels containing 0.1% (w/v) SDS. Prestained molecular weight markers were applied to one lane. The separated proteins were electrophoretically transferred to PVDF Plus membranes in 25 mm Tris-HCL, pH 8.3, 192 mm glycine, and 20% (v/v) methanol. Membranes were blocked with 5% Carnation nonfat dry milk and then incubated at room temperature for 1 h with a 1:1500 dilution of LDL receptor antisera (12) or a 1:1000 dilution of HMG-CoA reductase antisera (23). Immunoreactive protein was detected using the ECL kit with a horseradish peroxidase-conjugated second antibody. Multiple exposures ranging from 10 sec to 1 min were made. Hepatic LDL receptor protein migrated as a 160-kDa band while HMG-CoA reductase was detected at a size of about 100 kDa. Levels of immunoreactive protein were quantitated by scanning with a laser densitometer. The values are expressed as arbitrary units or as relative levels compared to the control.

Determination of LDL receptor protein half-life

The half-life of hepatic LDL receptor protein was determined by measuring the amount of immunoreactive protein remaining as a function of time after injecting rats with the protein synthesis inhibitor, cycloheximide. Rats were injected subcutaneously with 250 μg of cycloheximide per 100 g at the fourth hour of the dark period and killed 1 to 4 h later. The injection of cycloheximide was given 3 days after placing the animals on diets containing either 0.02% lovastatin or 0.02% atorvastatin. Levels of LDL receptor immunoreactive protein were determined by immunoblotting analysis. The half-life was calculated from semilog plots of LDL receptor immunoreactive protein remaining as previously described (6). The half-life of hepatic LDL receptor protein in normal rats is about 8 h (6). In normal rats not injected with cycloheximide, LDL receptor levels remain constant during a 24 h period (24).

HMG-CoA reductase activity

HMG-CoA reductase activity was measured in hepatic microsomes using [^{14}C]HMG-CoA and an NADPH-regenerating system as previously described (25). The product, [^{14}C]mevalonate, was converted to the lactone and isolated by thin-layer chromatography (25). The recovery averaged 70% as determined with [^{3}H]mevalonate. Microsomal protein concentrations were determined by a biuret procedure (26). Enzyme activities were expressed as nmol/min per mg of microsomal protein and then compared to zero time controls.

Washing of atorvastatin and lovastatin from liver microsomes in vitro

Liver microsomes, 14 mg of protein resuspended in 1 ml of 0.25 M sucrose, were incubated for 10 min at 37°C with 100 μg of either lovastatin or atorvastatin. After the incubations, aliquots were removed from each sample for assays of protein and enzyme activity. The microsomes were washed by homogenization (Dounce homogenizer) in 10 ml 0.25 M sucrose followed by centrifugation at 100,000 *g* for 1 h. Samples were washed three times. Aliquots were removed from each sample after each washing step and used for determination of enzyme activity and protein. Microsomes that were not incubated with inhibitor but washed in an identical manner were used as controls.

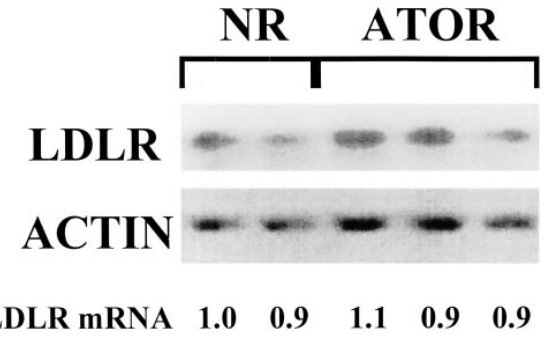

Fig. 2. A typical Northern blot of hepatic LDL receptor mRNA from rats fed a normal chow diet (NR) or one supplemented with 0.04% atorvastatin (ATOR). The blot was probed for LDL receptor (LDLR) and then for β-actin (ACTIN), which served as an internal standard.

RESULTS

Cholesterol levels

The effects of lovastatin and atorvastatin on serum and hepatic cholesterol levels are presented in **Fig. 1**. Liver cholesterol levels were significantly decreased by 30–40%, while serum cholesterol levels were only slightly reduced. These changes occurred after 3 days with 0.04% of the drug added to the diet, which is equivalent to a 40 mg/kg dose. Previously it was reported that liver cholesterol levels were not decreased in rats given 100 mg/kg of atorvastatin for 2 weeks (18). The difference between these findings and the present observations could relate to the duration of treatment, mode of administration of the drug, time elapsed since last drug treatment, or the time at which the animals were killed in relation to the diurnal variation in HMG-CoA reductase activity.

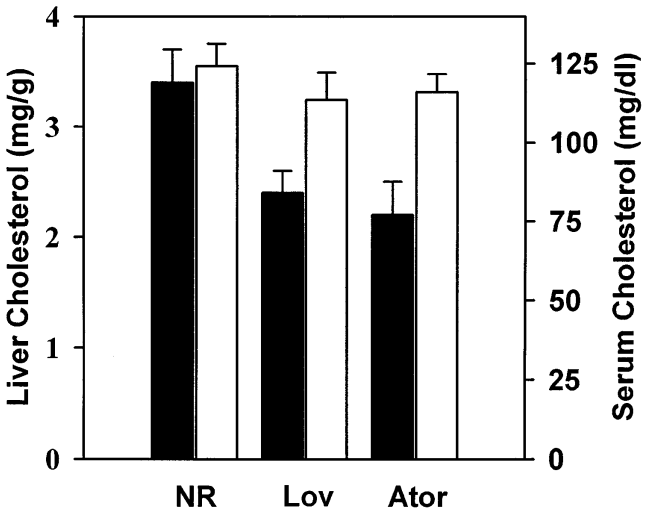

Fig. 1. Serum (□) and liver (■) cholesterol levels in rats fed chow diets (NR), chow containing 0.04% lovastatin (LOV), or chow containing 0.04% atorvastatin (ATOR). Values are presented as means ± SD for 9 NR rats, 8 LOV rats, and 6 ATOR rats. Liver cholesterol levels were significantly reduced, $P < 0.01$, in both LOV and ATOR rats compared to NR rats.

LDL receptor response

Conflicting reports have appeared concerning whether atorvastatin treatment causes up-regulation of hepatic LDL (apoB/E) receptors. In studies of guinea pigs, a modest increase was reported (11), while studies using rabbits or miniature pigs (10, 27) showed no increase in the number of receptors. In these studies (10, 11, 27), LDL receptor number was determined by measuring LDL binding to hepatic membranes. No previous studies have been reported in which measurements of the effects of atorvastatin on hepatic LDL receptor mRNA or immunoreactive protein levels have been made.

As shown in **Fig. 2** and **Fig. 3**, treatment with 0.04% atorvastatin had no effect on hepatic LDL receptor mRNA levels. In contrast, treatment with this dose of lovastatin significantly increased hepatic LDL receptor mRNA levels (Fig. 3). The levels of hepatic LDL receptor immunoreactive protein were determined by immunoblotting analysis. As shown in **Fig. 4**, treatment with either a high (0.04%) or low (0.002%) dose of atorvastatin did not increase hepatic LDL receptor immunoreactive protein levels. Similar treatment with lovastatin also failed to increase hepatic LDL receptor protein levels (Fig. 4). This agrees with our previous finding that lovastatin, rivastatin, and pravastatin all fail to increase hepatic LDL receptor protein levels despite

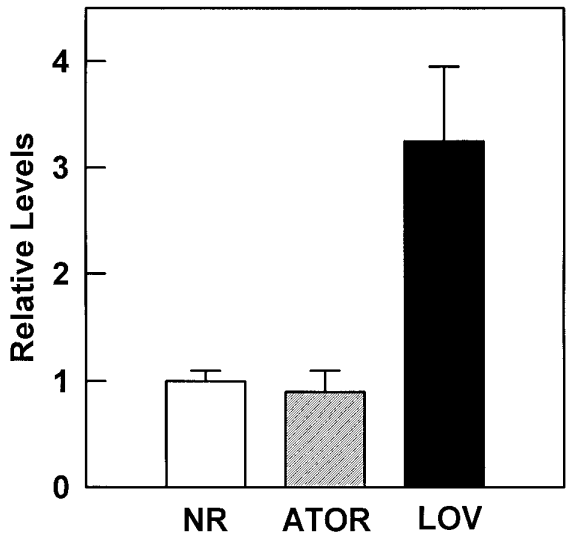

Fig. 3. Comparison of the effects of 0.04% lovastatin (LOV) and 0.04% atorvastatin (ATOR) treatment on hepatic LDL receptor mRNA levels. Values are presented as means ± SD, for chow-fed normal, for 6 NR (■), 6 ATOR (▩) and 4 LOV (□)-treated rats. LDL receptor mRNA levels were significantly increased, $P < 0.01$, in LOV-treated rats compared to NR rats.

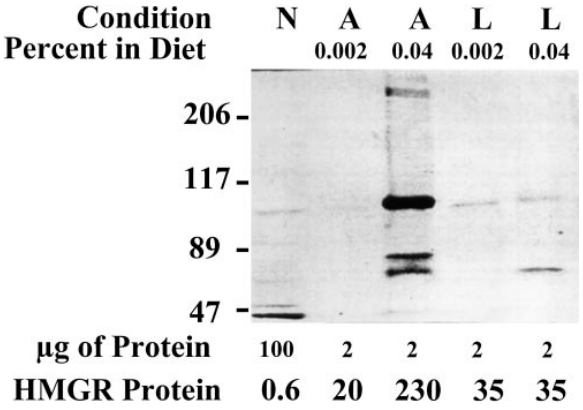

Fig. 5. Hepatic HMG-CoA reductase immunoreactive protein levels in microsomes isolated from rats fed a normal chow diet (N), a diet supplemented with 0.002 or 0.04% atorvastatin (A), or a diet supplemented with 0.002% or 0.04% lovastatin (L). A typical immunoblot is presented. Molecular weight markers are given on the left. The amount of microsomal protein applied to each lane is given at the bottom of the lanes. Quantitation of HMG-CoA reductase protein by densitometric scanning in arbitrary units per 100 µg of microsomal protein is given below each lane.

the fact that they all increase LDL receptor mRNA levels (6).

We have recently demonstrated that inhibitors of cholesterol biosynthesis cause an increase in the rate of turnover of hepatic LDL receptor protein (6). It was postulated that this results in an increased rate of cycling of hepatic LDL receptors which provides for an enhanced rate of uptake of LDL from the serum (6). Thus, the affect of 0.04% atorvastatin on the half-life of the hepatic LDL receptor was determined. The half-life was found to be 2.8 ± 0.2 h for four determinations. This value was not significantly different from the 3.9 ± 0.6 h half-life observed in four sets of rats treated with 0.04% lovastatin (6). It represents a reduction of over 60% in the half-life of the hepatic LDL receptor. Thus, atorvastatin appears to increase the turnover and cycling rate of the hepatic LDL receptor to a similar degree as lovastatin and, like the other statins, does not cause an increase in hepatic LDL receptor protein levels.

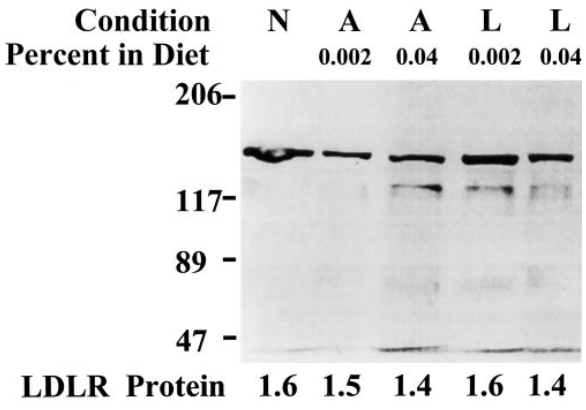

Fig. 4. Hepatic LDL receptor immunoreactive protein levels in microsomes isolated from rats fed a normal chow diet (N), a diet supplemented with 0.002 or 0.04% atorvastatin (A) or a diet supplemented with 0.002 or 0.04% lovastatin (L). A typical immunoblot is presented. Molecular weight markers are given on the left. Each lane contained 100 µg of microsomal protein. Quantitation of LDL receptor protein by densitometric scanning in arbitrary units per 100 µg of microsomal protein is presented below each lane.

HMG-CoA reductase mRNA and protein levels

In view of the recent report (11) that atorvastatin does not increase HMG-CoA reductase activity when administered to guinea pigs, we wished to know whether it affected levels of immunoreactive reductase protein. As can be seen in **Fig. 5**, atorvastatin treatment dramatically increased levels of hepatic HMG-CoA reductase protein. To determine whether this also involved an increase in mRNA levels, Northern blotting analyses were performed. As shown in **Fig. 6**, hepatic HMG-CoA reductase mRNA levels were significantly increased by atorvastatin treatment. However, the magnitude of this increase, about 3-fold (**Fig. 7**), was not nearly as great as the extent of induction of reductase protein which was as much as 700-fold. Also as shown in Fig. 7, atorvastatin does not increase hepatic HMG-CoA reductase mRNA levels as much as an equivalent dose

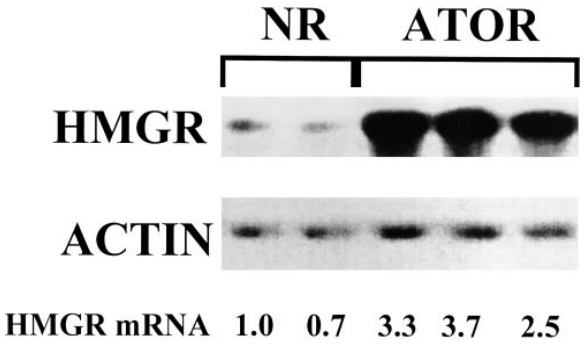

Fig. 6. A typical Northern blot of hepatic HMG-CoA reductase mRNA from rats fed a normal chow diet (NR) or a diet supplemented with 0.04% atorvastatin (ATOR). The blot was probed for HMG-CoA reductase (HMGR) and then for β-actin (ACTIN), which served as an internal standard.

of lovastatin. This suggests a large post-transcriptional affect. The greater increase in reductase protein levels seen in rats treated with atorvastatin could reflect a higher degree of in vivo inhibition of cholesterol biosynthesis. It has been reported that atorvastatin is 4.5-fold more potent than lovastatin at inhibiting cholesterol biosynthesis in primary rat hepatocytes (28).

Persistence of the induction of hepatic HMG-CoA reductase protein

It is known that the induction of HMG-CoA reductase protein by lovastatin is rather short-lived as the elevated levels return to normal within 12 h after removal of rats from a diet containing this drug (29). We wished to know whether the apparently greater induction of hepatic HMG-CoA reductase protein by atorvastatin might be due to a slower rate of metabolism of this drug or to formation and metabolism of active metabolites. Thus we carried out the experiment depicted in **Fig. 8**. Rats were placed on diets containing either 0.04% lovastatin or atorvastatin for 3 days and then switched to a normal diet. The decline in hepatic HMG-CoA reductase protein was monitored by immunoblotting analysis. As can be seen, the rate of fall of HMG-CoA reductase protein in the atorvastatin-treated animals was not slower than that in the lovastatin-treated group. Actually the apparent half-life in the atorvastatin group was 3 h while in the lovastatin group it was 6.5 h. Thus a slower rate of decline in HMG-CoA reductase protein does not appear to underlie its greater effectiveness.

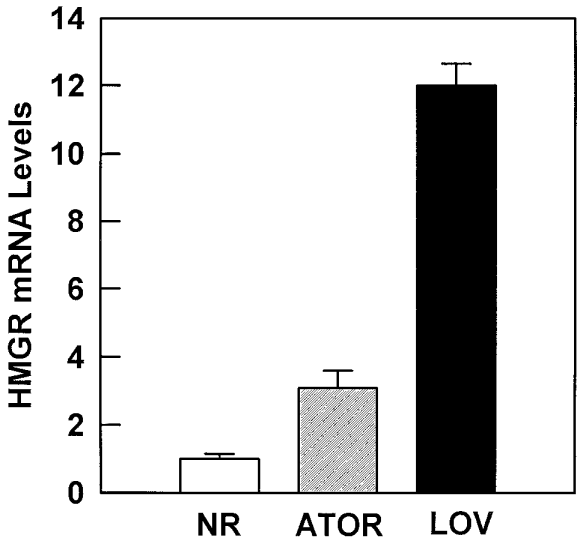

Fig. 7. Comparison of the effects of 0.04% lovastatin (LOV) and 0.04% atorvastatin (ATOR) treatment of hepatic HMG-CoA reductase mRNA levels. Values are presented as means ± SD for 6 chow-fed normal, NR (■), 6 ATOR (▩) and 4 LOV (□)-treated rats. For ATOR compared with NR, $P < 0.01$. For LOV compared with NR, $P < 0.001$.

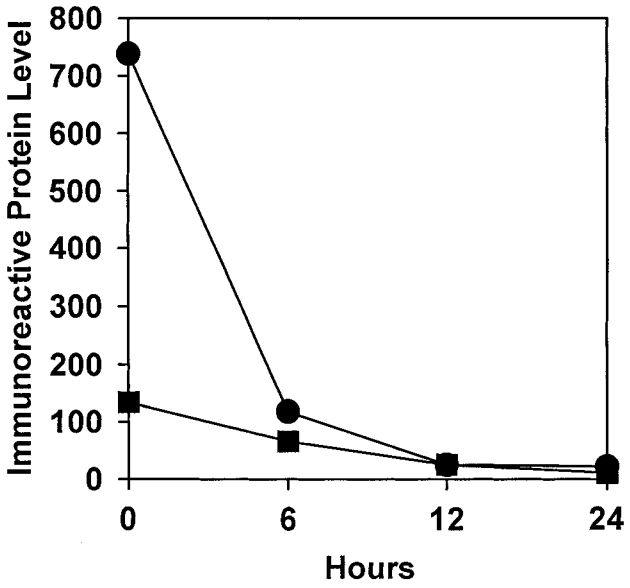

Fig. 8. Decline in hepatic HMG-CoA reductase immunoreactive protein levels in rats treated with 0.04% atorvastatin or 0.04% lovastatin as a function of time after switching the animals to a normal chow diet. ATOR (●) and LOV (■).

Effect of atorvastatin dose on levels of hepatic HMG-CoA reductase protein and activity

In view of the apparently greater induction of hepatic HMG-CoA reductase protein by atorvastatin than by lovastatin, we wished to compare the effects of dose of these two drugs. As shown in **Fig. 9A**, low levels of the two drugs caused similar degrees of induction. At higher doses, a greater induction was observed with atorvastatin. As can be seen in Fig. 9B compared with

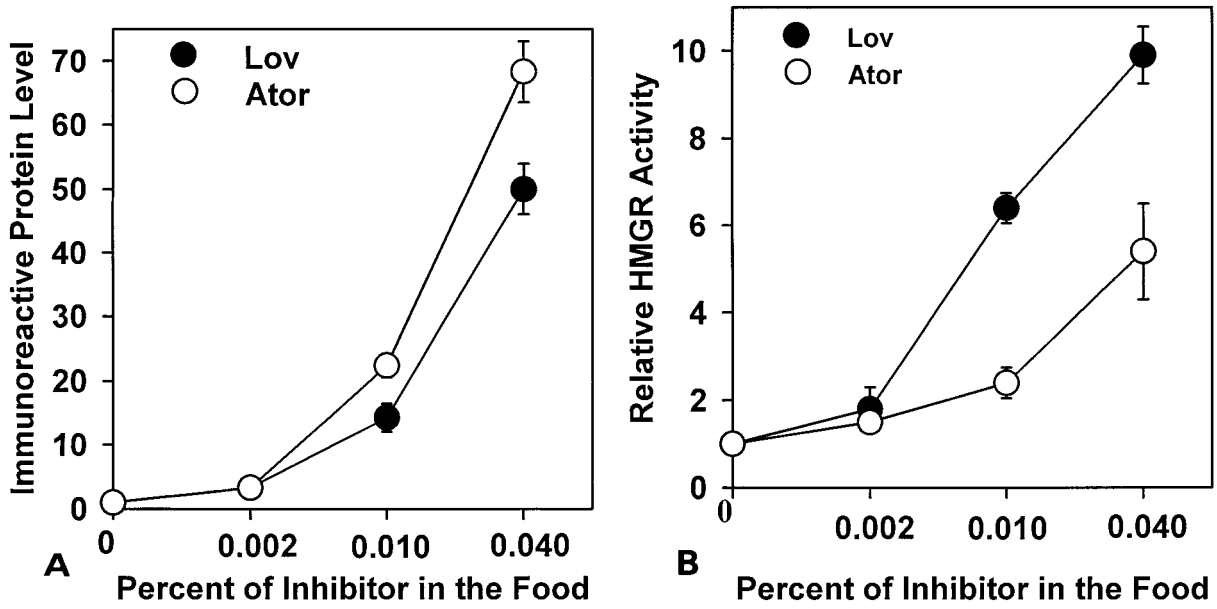

Fig. 9. Effect of dose of atorvastatin or lovastatin on hepatic HMG-CoA reductase immunoreactive protein and enzyme activity levels. In A, relative levels of immunoreactive reductase protein are presented. In B, relative levels of enzyme activity are presented.

Fig. 9A, less HMG-CoA reductase activity in proportion to the amount of enzyme protein was present in hepatic microsomes isolated from animals on diets supplemented with atorvastatin. This observation could mean that a less catalytically efficient form was produced in the presence of atorvastatin or that a significant amount of atorvastatin remains bound to the enzyme despite the fact that the microsomes were washed.

Effect of washing hepatic microsomes

Hepatic microsomes isolated from rats fed diets supplemented with either 0.04% lovastatin or 0.04% atorvastatin were subjected to one or two additional washes in order to remove bound inhibitor. As can be seen in **Fig. 10**, a much greater increase in enzyme activity was observed in microsomes isolated from rats fed a lovastatin-supplemented diet than in the case of animals fed atorvastatin-supplemented diets. This is consistent with atorvastatin being a tighter binding inhibitor. It could also reflect the formation of an active tight-binding metabolite generated from atorvastatin. In order to attempt to distinguish between these possibilities, lovastatin and atorvastatin were added to hepatic microsomes isolated from a rat fed normal chow and the effects of washing were compared. As can be seen in **Fig. 11**, the rate at which HMG-CoA reductase activity was restored in lovastatin treated microsomes was significantly faster than in atorvastatin-treated microsomes, i.e., a greater percentage of activity was restored after three washes. The fact that atorvastatin was more difficult to remove from microsomal HMB-CoA reductase than lovastatin suggested that it might bind more tightly to the enzyme or have a slower rate of dissociation from the enzyme. Alternatively, atorvastatin may have a greater affinity for the microsomal membrane leading to a higher local concentration of drug in the vicinity of the enzyme and

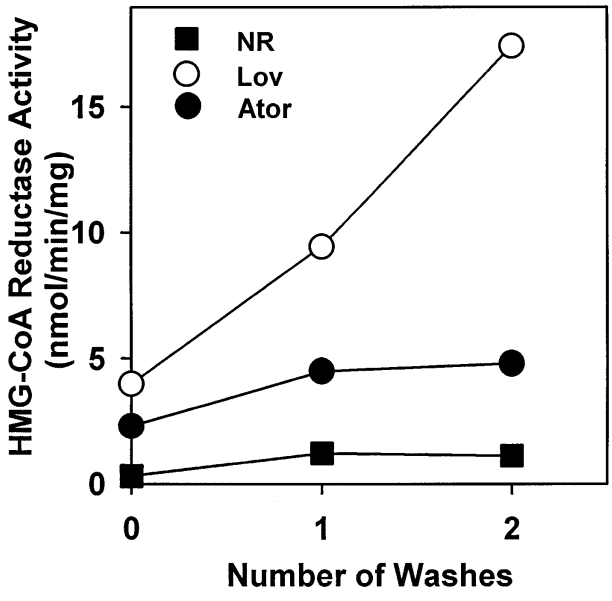

Fig. 10. Effect of washing microsomes isolated from liver of rats fed a chow diet containing 0.04% lovastatin (LOV) or 0.04% atorvastatin (ATOR).

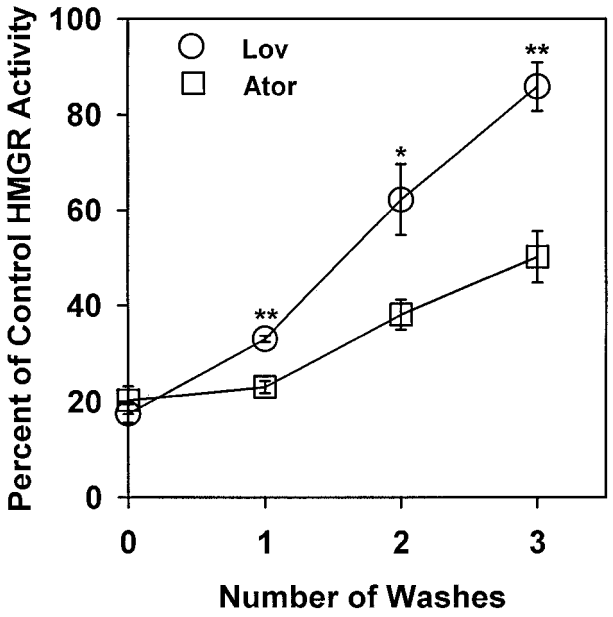

Fig. 11. Restoration of HMG-CoA reductase activity by washing of hepatic microsomes treated with 100 μg/ml of either lovastatin or atorvastatin. Values are means ± SD for 4 microsomal preparations. *Differs significantly from corresponding atorvastatin value at $P < 0.05$. **Differs significantly from corresponding atorvastatin value at $P < 0.01$.

therefore leading to prolonged inhibition and a relative inability to remove the drug from the enzyme. Either of these mechanisms would allow it to more effectively inhibit cholesterol biosynthesis in vivo, resulting in a greater lowering of serum cholesterol levels. It is also possible that atorvastatin might be a slow binding-tight binding inhibitor. If this should be the case, time-dependent inhibition should be observed. This was tested by incubating microsomes with a dose of atorvastatin that was known to cause 50% inhibition for zero to 20 min prior to starting the reaction by the addition of HMG-CoA. No difference was found in the extent of inhibition. Thus atorvastatin is not a slow binding inhibitor.

DISCUSSION

The possibility that the greater cholesterol-lowering action of atorvastatin, compared to some other statins, might be due to a greater increase in the rate of hepatic LDL receptor cycling reflected by an increased rate of receptor degradation (6) was examined. It was found that the increased rate of hepatic LDL receptor turnover caused by atorvastatin did not differ significantly from that caused by an equivalent dose of lovastatin. Thus it is unlikely that this explains its greater effectiveness. It has recently been reported that administration of simvastatin, pravastatin, or atorvastatin to patients with heterozygous familial hypercholesterolemia (FH) significantly lowered serum LDL cholesterol levels (30). In 21 such FH patients treated with 80 mg of atorvastatin daily, LDL cholesterol levels were decreased an average of 54% (30). Plasma mevalonate levels were decreased 58.8% in these same patients; indicating a similar decrease in in vivo cholesterol biosynthesis. It has also been demonstrated that the statins, including atorvastatin, effectively lower plasma LDL cholesterol in LDL receptor-deficient mice (C. L. Bisgaier, personal communication). These findings suggest that inhibition of cholesterol biosynthesis per se may play a major role in the hypocholesterolemic action of this class of drugs.

In view of the previous report that atorvastatin does not increase hepatic HMG-CoA reductase activity when administered to guinea pigs (11), the effects on hepatic HMG-CoA reductase mRNA, protein and activity were examined in these young male rats. It was found that like the other statins (6), atorvastatin caused a large compensatory induction of hepatic HMG-CoA reductase immunoreactive protein levels. Actually, atorvastatin treatment caused a greater induction than lovastatin (Figs. 5 and 8). Surprisingly, the increase in reductase mRNA levels caused by atorvastatin treatment was considerably less than that caused by lovastatin (Fig. 7). Despite the larger increase in immunoreactive protein levels caused by atorvastatin, the increase in enzyme activity was less (Fig. 9). Further washes of the microsomes increased the enzyme activity; however, this affect was much less in microsomes isolated from rats treated with atorvastatin (Fig. 10). In experiments in which either lovastatin or atorvastatin was directly added to the microsomes, it was confirmed that atorvastatin itself is more difficult to remove from the microsomes (Fig. 11). Thus atorvastatin may exert a greater cholesterol-lowering action due to a more pronounced inhibition of HMG-CoA reductase and cholesterol biosynthesis.

A somewhat surprising finding was that atorvastatin caused less induction of hepatic HMG-CoA reductase mRNA than lovastatin despite causing a larger induction of reductase protein (Figs. 5 and 7). The increase in mRNA levels was only 3-fold while the increase in immunoreactive protein levels was as much as 700-fold. This suggests that considerable post transcriptional regulation occurred. This is also true for the lovastatin induction. HMG-CoA reductase mRNA levels only increased 10- to 20-fold by lovastatin while immunoreactive protein levels increased over 200-fold (Figs. 5 and 7). In part, this post transcriptional regulation may be due to stabilization of HMG-CoA reductase protein

(19). It is also possible that altered translational efficiency could play a role. We have recently shown that dietary cholesterol acts to decrease translational efficiency by decreasing the portion of HMG-CoA reductase mRNA associated with actively translating polysomes (31).

It will be of interest to determine whether the apparently slow release of atorvastatin from microsomal HMG-CoA reductase also occurs in animals with high LDL levels such as hamsters. Hamsters have much lower levels of HMG-CoA reductase (32). Thus, it is possible that a lesser amount of a tight-binding inhibitor such as atorvastatin would exert a greater effect.

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