

# The Toxicology of HMG–CoA Reductase Inhibitors: Prediction of Human Risk

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## ABSTRACT

The discovery that 3-hydroxy-3-methylglutaryl coenzyme A reductase was a rate-determining step in the biosynthesis of cholesterol led to the discovery of inhibitors of this enzyme. To support the development of these agents (statins) as potential hypocholesterolemic drugs, a variety of preclinical studies were conducted in several animal species. Not unexpectedly due to the central role played by mevalonic acid and its products including cholesterol in development and maintenance of cellular homeostasis, administration of high dosage levels of these agents led to the expression of a broad variety of adverse effects in many different tissues. Using the tools of toxicologic pathology and classical risk assessment, these varied toxicities were evaluated by many groups relative to the conditions of use in human therapy and a perspective was developed on potential human risk. These approaches of mechanism-based risk assessment predicted that most of the adverse effects observed in animals would not be seen under conditions of human use and supported the successful introduction of one of the most important classes of human medicines.

*Keywords.* Statin toxicology; mechanism-based risk assessment; HMG-CoA reductase inhibitors; prediction of human toxicity.

## INTRODUCTION

The relationship of elevated plasma levels of cholesterol to cardiovascular disease and the associated mortality and morbidity has been suspected for decades. Some of the earliest data documenting the relationship between elevated serum cholesterol concentration and vascular disease came from rabbit cholesterol feeding studies in the early 1900s (Anitschkow, 1913). Several human studies begun in the 1950s (Framingham, Seven Countries' Study), demonstrated a firm link between elevated plasma LDL cholesterol levels and coronary heart disease mortality (Kannel et al., 1976; Keys, 1971; Lipid Research Clinics Program (LRCP), 1984). Based on data that was emerging from these long-term human studies, the NIH Consensus Conference in 1984 concluded that lowering plasma cholesterol with diet and drugs would reduce the risk of coronary heart disease (NIH Consensus Conference, 1984). At this time, however, the only nondietary regimens that were available to treat high cholesterol were either only moderately effective (bile acid binding resins, fibrates) or associated with significant adverse effects (niacin).

Through the pioneering work of Brown and Goldstein (1986), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) was identified as the rate determining step in the biosynthesis of cholesterol (see Figure 1). The first naturally occurring fungal metabolite that inhibited this rate determining enzyme was compactin (mevastatin) discovered by A. Endo at Sankyo in 1976 (Endo et al., 1976). Shortly after this in 1978, scientists at Merck published the structure of a similar molecule derived from a different fungal organism that was a slightly more potent inhibitor of this important enzyme (Alberts et al., 1980). (See Figure 2.) The discovery of lovastatin led to the search for synthetic analogues of this

molecule. This search resulted in the discovery of simvastatin from the Merck laboratories in 1985 (Hoffman et al., 1986). As shown in Figure 3, simvastatin (like lovastatin) exists in the lactone form and is hydrolyzed to the open hydroxy-acid form which is the active form responsible for inhibition of the enzyme.

Since the introduction of these molecules, many other members of this class of statins have been studied and several have been introduced commercially. The structures of compounds that have been made available commercially are shown in Figure 4. Pravastatin was first isolated as a urinary metabolite of compactin in dogs and its introduction was followed by fluvastatin, atorvastatin, cerivastatin, and most recently rosuvastatin. Cerivastatin was removed from the market in 2000 as a result of significant toxicity (myopathy) that will be described in subsequent sections of this paper.

As this new class of agents was being studied preclinically to support introduction into clinical use, a broad spectrum of toxicity was seen in a variety of target tissues in several animal species. As more molecules have been evaluated in a similar battery of preclinical studies, it has become clear that there is a relatively consistent profile of toxicity in animals of agents in this class (see Table 1). Despite this impressive profile of toxicity in animals, statins have become one of the most widely used and effective agents for the treatment of lipid disorders and have clearly been demonstrated to significantly reduce cardiovascular mortality associated with hyperlipidemias (Tobert, 2003).

In the sections that follow, this preclinical profile will be discussed in more detail with particular attention to how the relative importance of these findings for potential human risk was evaluated.

As statins inhibit an enzyme that is essential not only for cholesterol biosynthesis but also for the synthesis of several other nonsterol precursors essential for normal cellular function (Figure 1), it was hypothesized early on that some or all of the toxicity seen at high exposure levels in animals could

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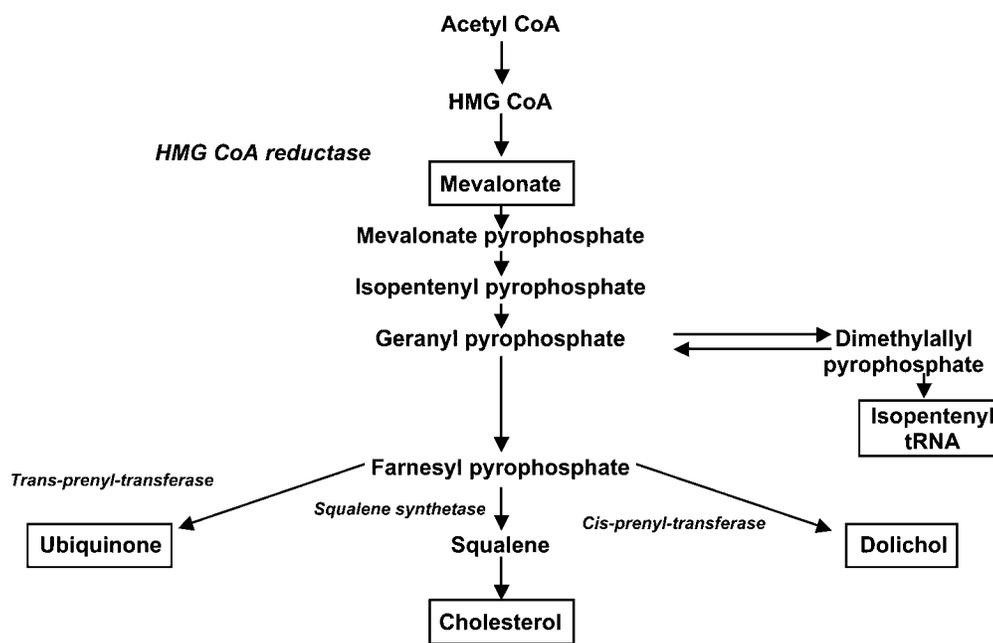


FIGURE 1.—Cholesterol biosynthetic pathway.

be related to an exaggeration of the desired biochemical effect (MacDonald et al., 1988). This hypothesis will be used to facilitate the discussion of this broad spectrum of toxicity in the sections that follow by dividing the findings into the three broad classifications shown in Table 2.

#### Effects That Are Clearly Related to Exaggerated Biochemical Effect

**Nonneoplastic Hepatic Effects:** Hepatic effects of statins range in severity from frank hepatocellular necrosis observed in rats and rabbits as well as hamsters to several characteristic morphological alterations in rats (Corsini et al., 1996; MacDonald, 1988; Gerson, 1989; Kornbrust et al., 1989). At high dosage levels in dogs treated with some HMG-CoA reductase inhibitors, significant evidence of liver pathology is observed (Walsh and Rockwell, 1999; Hartman et al., 1996). With other reductase inhibitors, elevations in serum transaminase activity were observed in dogs in the absence of any morphologic abnormalities in the liver or other tissues (MacDonald et al., 1988; Gerson et al., 1989; Gerson et al., 1991).

Rabbits are uniquely sensitive to HMG-CoA reductase inhibitors demonstrating significant liver toxicity and mortality after administration of high doses (MacDonald et al., 1988; Gerson et al., 1989; Kornbrust et al., 1989). Rapid induction of hepatocellular necrosis can be induced in this species as evidenced by significant increases in serum transaminase activity, alkaline phosphatase activity, and morphologic appearance of the liver. Coadministration of mevalonic acid, the product of the enzyme inhibited by the statins can prevent or attenuate this hepatic toxicity (Table 3; Kornbrust et al., 1989). The unique sensitivity of this species may be related to the low endogenous levels of HMG-CoA reductase activity in the hepatocytes of rabbits compared to that of other species (Kornbrust et al., 1989).

The relationship of the hepatic morphologic abnormalities in rats treated with lovastatin to inhibition of mevalonic acid synthesis was described by MacDonald et al. (1988). Characteristic foci of cellular alteration (basophilic and esophilic), periportal atypia (characterized by cytoplasmic and nuclear polymorphism, an increase in the number of multinucleated hepatocytes, and tinctorial variability of hepatocytes), and

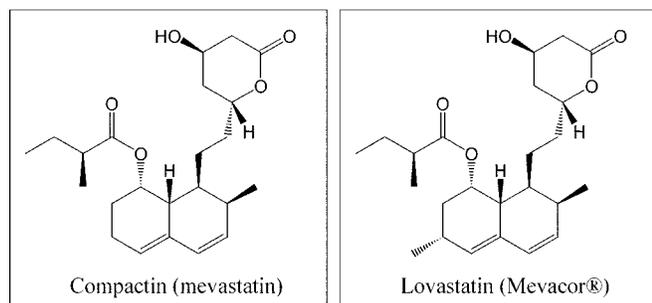


FIGURE 2.—Structures of compactin and lovastatin.

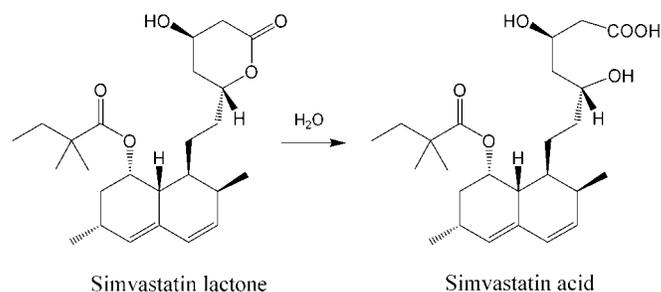


FIGURE 3.—Structure of simvastatin and pathway of conversion to active open acid form.

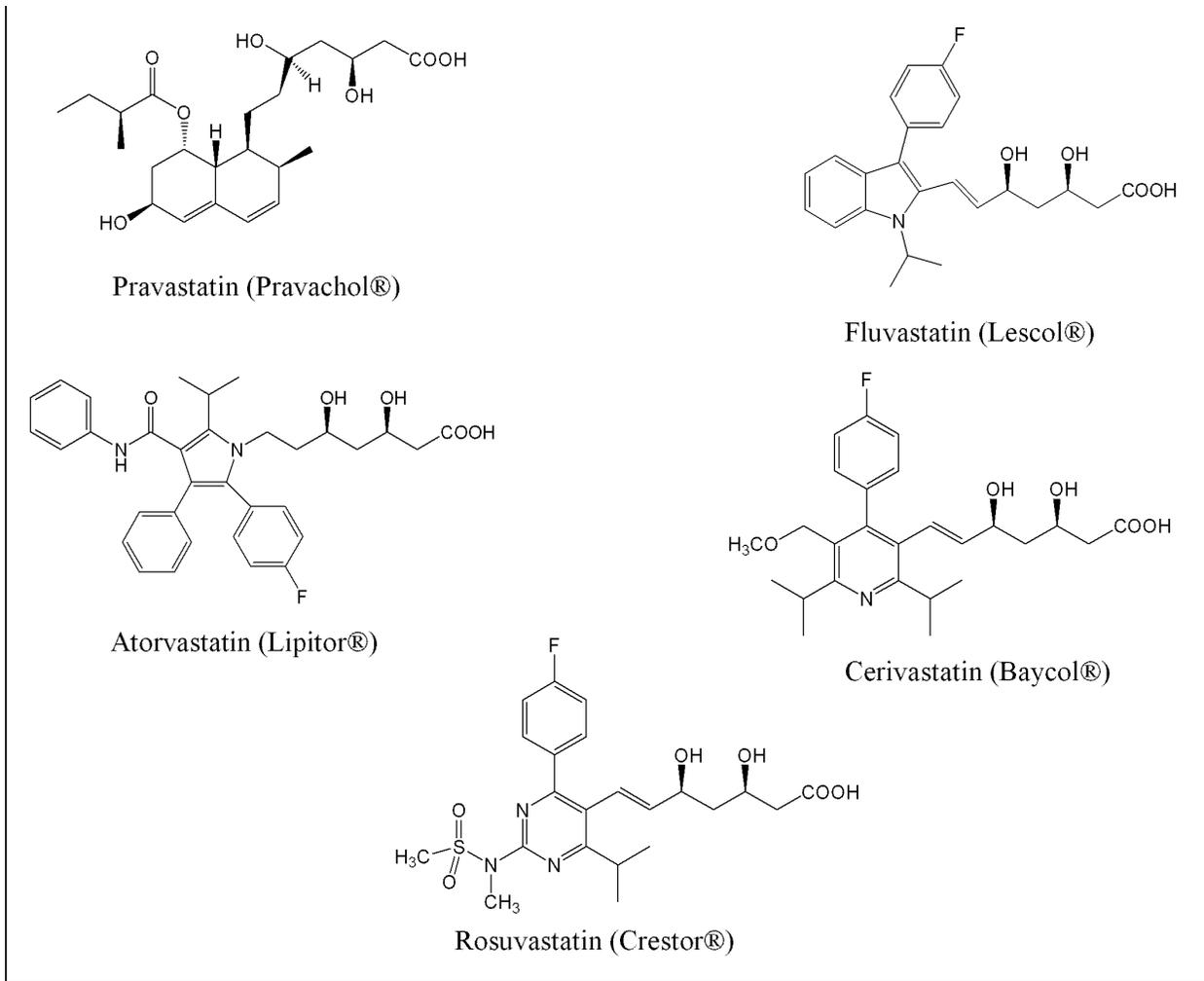


FIGURE 4.—Structures of additional statins made commercially available.

bile duct hyperplasia were seen in the livers of rats treated with high doses of lovastatin or simvastatin (see Figures 5 through 7). As shown in Table 4, the incidence of these changes was reduced to control levels by supplementation with a regimen of mevalonic acid.

Examination of the areas of hepatocellular atypia in the periportal regions of the liver lobule with electron microscopy or immunohistochemistry showed marked proliferation of smooth endoplasmic reticulum, the membrane to which HMG-CoA reductase is bound (Singer et al., 1984; Li et al.,

1988). Immunohistochemical studies demonstrated marked increases in HMG-CoA reductase positive hepatocytes in the periportal region of the liver lobule after short-term administration of high doses of lovastatin or simvastatin to rats (Figures 8, 9; Singer et al., 1984). These morphologic changes represent the significant induction of the enzyme in hepatocytes that has been demonstrated biochemically (Li

TABLE 1.—Target organs of statins in preclinical species.

Target organ	Atorva	Ceriva	Fluva	Lova	Prava	Rosuva	Simva
Liver	✓	✓	✓	✓	✓	✓	✓
Gallbladder	✓	—	✓	✓	—	✓	✓
Kidney	—	✓	—	✓	✓	✓	✓
Muscle	✓	✓	✓	✓	✓	✓	✓
Forestomach	✓	✓	✓	✓	✓	✓	✓
Lymphatic system	✓	✓	✓	✓	✓	✓	✓
CNS	✓	✓	—	✓	✓	✓	✓
Eye (lens)	—	✓	✓	✓	—	✓	✓
Testes	✓	✓	✓	✓	—	✓	✓
Thyroid gland	—	—	✓	—	✓	—	✓

(From von Keutz and Schluter, 1998).

TABLE 2.—Classification of adverse effects seen in nonclinical studies based on relationship to desired biochemical effect.

Category	Examples
Effects that are clearly related to exaggerated biochemical effects	<ul style="list-style-type: none"> <li>– Hepatic toxicity in rodents and rabbits; elevated transaminase activity in dogs</li> <li>– Renal, gallbladder lesions, rabbits, and dogs</li> <li>– Rodent forestomach lesions</li> </ul>
Effects that are probably related to exaggerated biochemical effects	<ul style="list-style-type: none"> <li>– Fetal effects</li> <li>– Myopathy in rodents</li> <li>– Cataracts in rats and dogs</li> <li>– Testicular effects in dogs</li> </ul>
Effects that are of uncertain relationship to biochemical mechanism of action	<ul style="list-style-type: none"> <li>– Central nervous system lesions in dogs</li> <li>– Increased incidence of tumors in rodents</li> </ul>

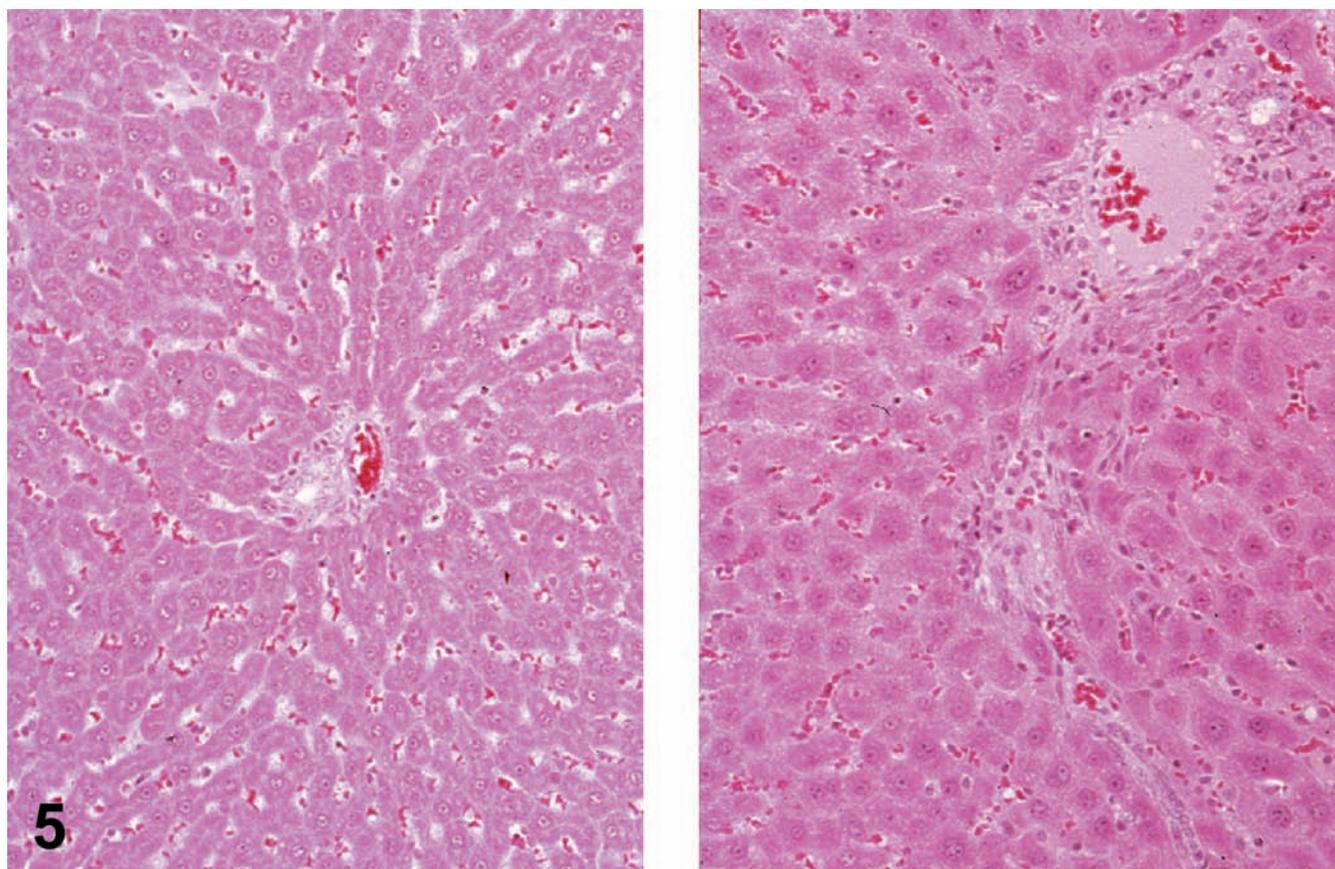


FIGURE 5.—Histologic appearance of rat liver. Control section from rat liver (left panel, H & E). Right panel shows section of liver from rat treated with simvastatin, 180 mg/kg/day for 13 weeks. Hepatocellular atypia in periportal region is apparent along with bile duct hyperplasia.

et al., 1988). Coadministration of mevalonic acid reversed the ultrastructural changes seen in the hepatocyte (Li et al., 1988) and prevented the induction of this enzyme as seen by immunohistochemistry. These studies demonstrate that in response to exposure to HMG-CoA reductase inhibitors, a marked induction of the enzyme is observed in the rat liver. This marked induction may explain, in part, the lack of sustained decreases in serum cholesterol concentration in this species after administration of statins (Endo et al., 1979).

High doses of the HMG-CoA reductase inhibitors atorvastatin or fluvastatin given to beagle dogs can result in hepatocellular degeneration or necrosis, inflammation and necrosis of gallbladder epithelium and evidence of bile duct hyper-

plasia (Corsini et al., 1966; Hartman et al., 1996; Walsh et al., 1996). With other statins, administration to dogs for extended periods of time (up to 2 years) with high doses results in significant elevations of serum transaminase activity in the absence of evidence of hepatic morphologic changes detected either by light or electron microscopy (MacDonald et al., 1988; Gerson et al., 1989, 1991). These alterations in serum transaminase activity can be transient in nature even with continued administration, can result in significant spikes in activity that return to normal with continued administration, and occur without any clinical evidence of hepatic toxicity (MacDonald et al., 1988). Although not as well characterized as changes in rats and rabbits, these alterations in

TABLE 3.—Effect of mevalonic acid coadministration on simvastatin-induced hepatic damage in rabbits.

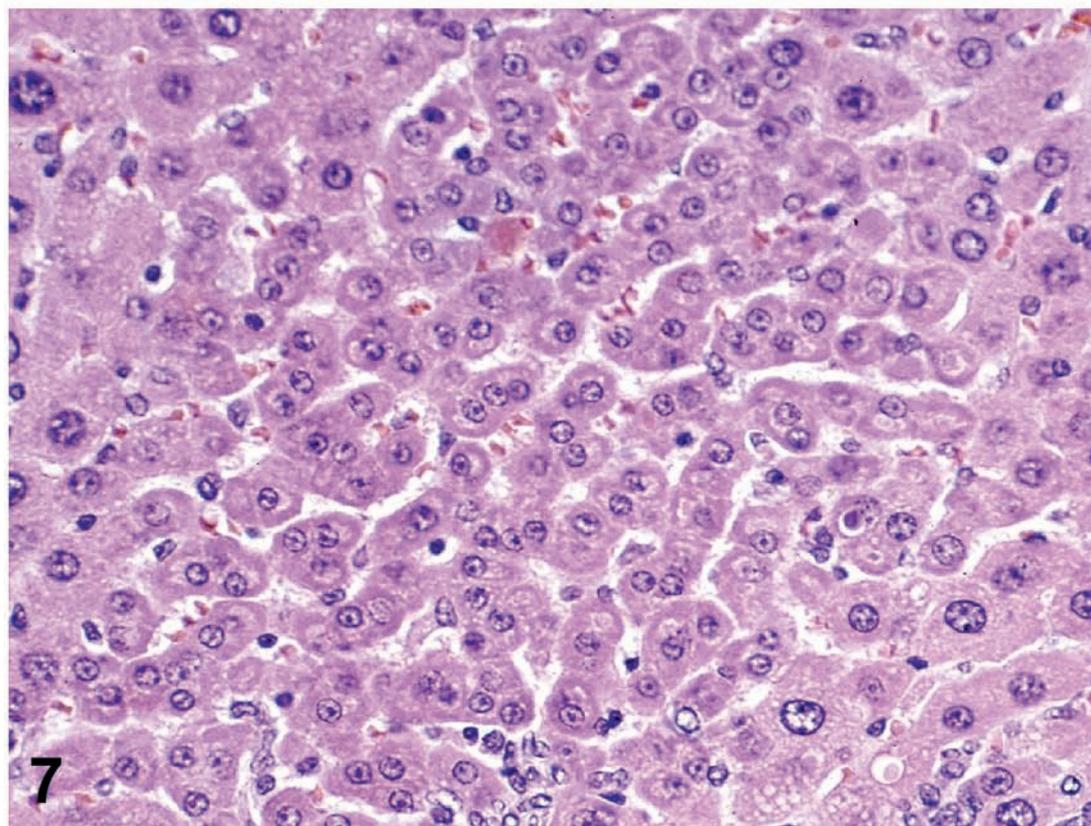
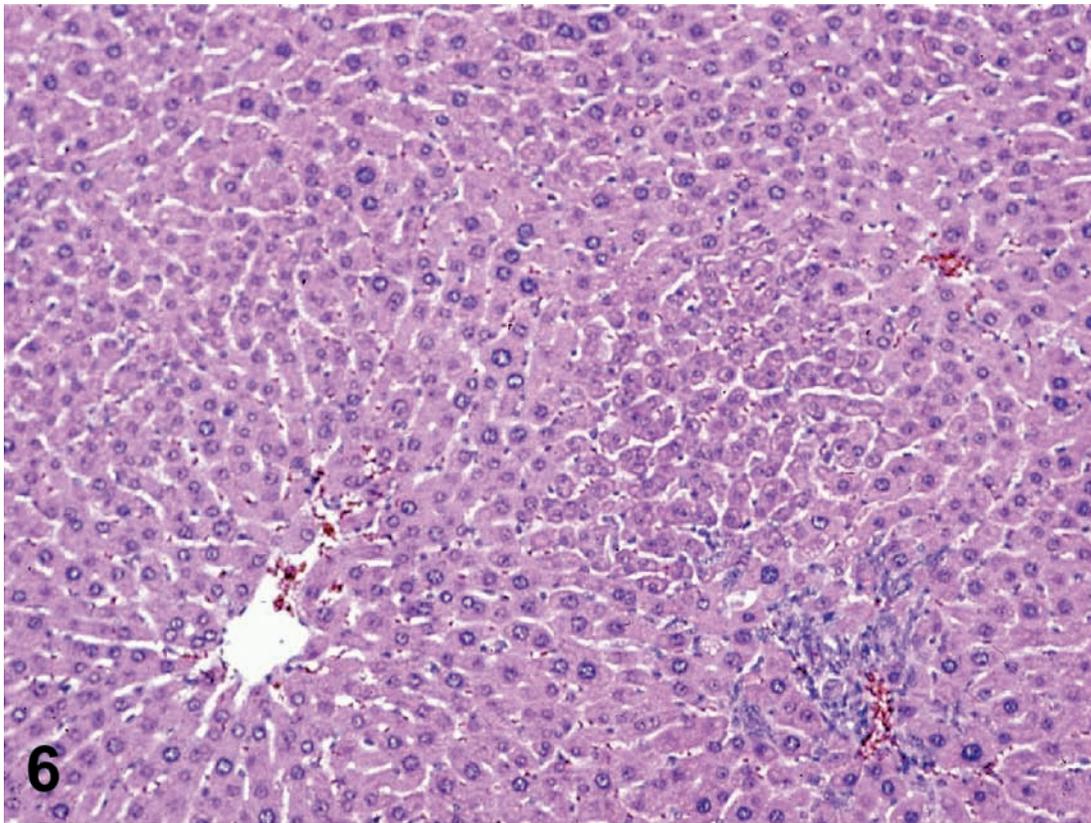
Treatment group	dosage level (mg/kg)	AST (IU/L)	ALT (IU/L)	AP	Hepatic necrosis (incidence)
Control	—	14	21	64	0/6
Simvastatin alone	90	967	397	132	3/6 <sup>a</sup>
Simvastatin + Mevalonic acid (40 mg/kg/day)	90	37	41	46	0/6

<sup>a</sup>Histologic grade was moderate to marked in affected animals. Dosing period was for 6 consecutive days. (From: Gerson R. J. et al. (1989). *Am J Med* 87, 28S–38S.

TABLE 4.—Incidence of hepatic morphologic abnormalities in rats treated with lovastatin and mevalonic acid.

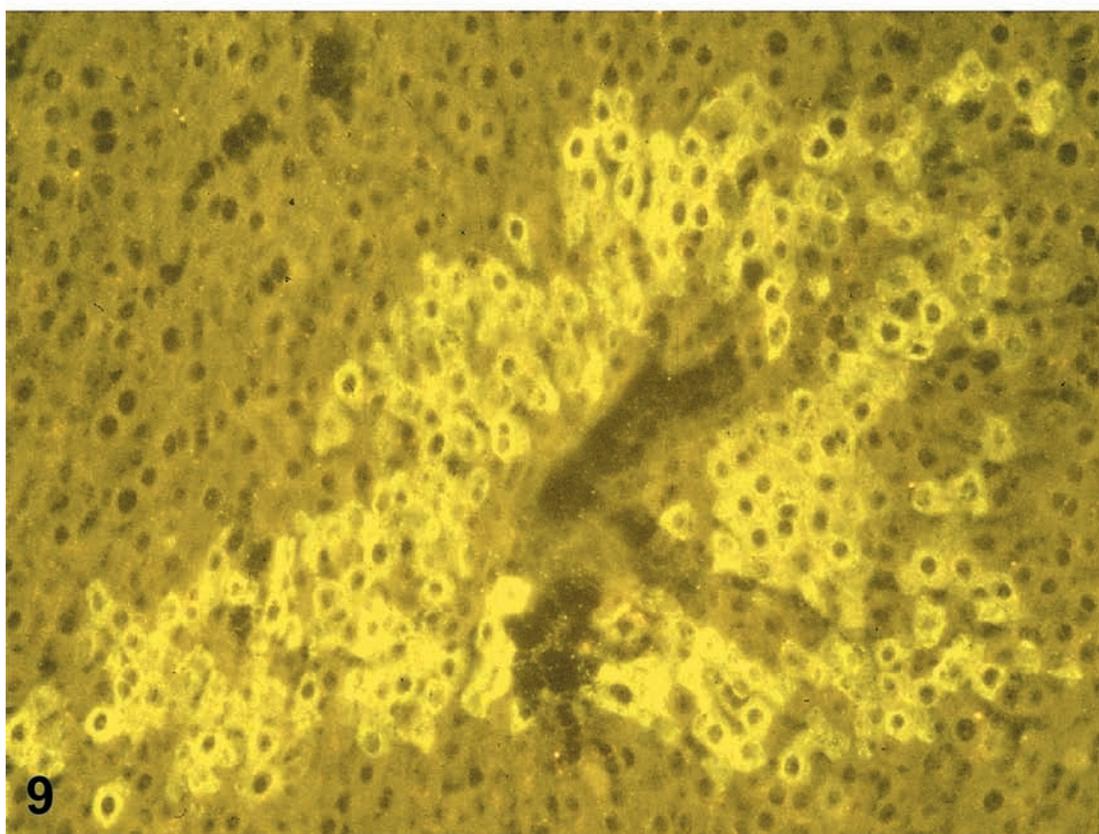
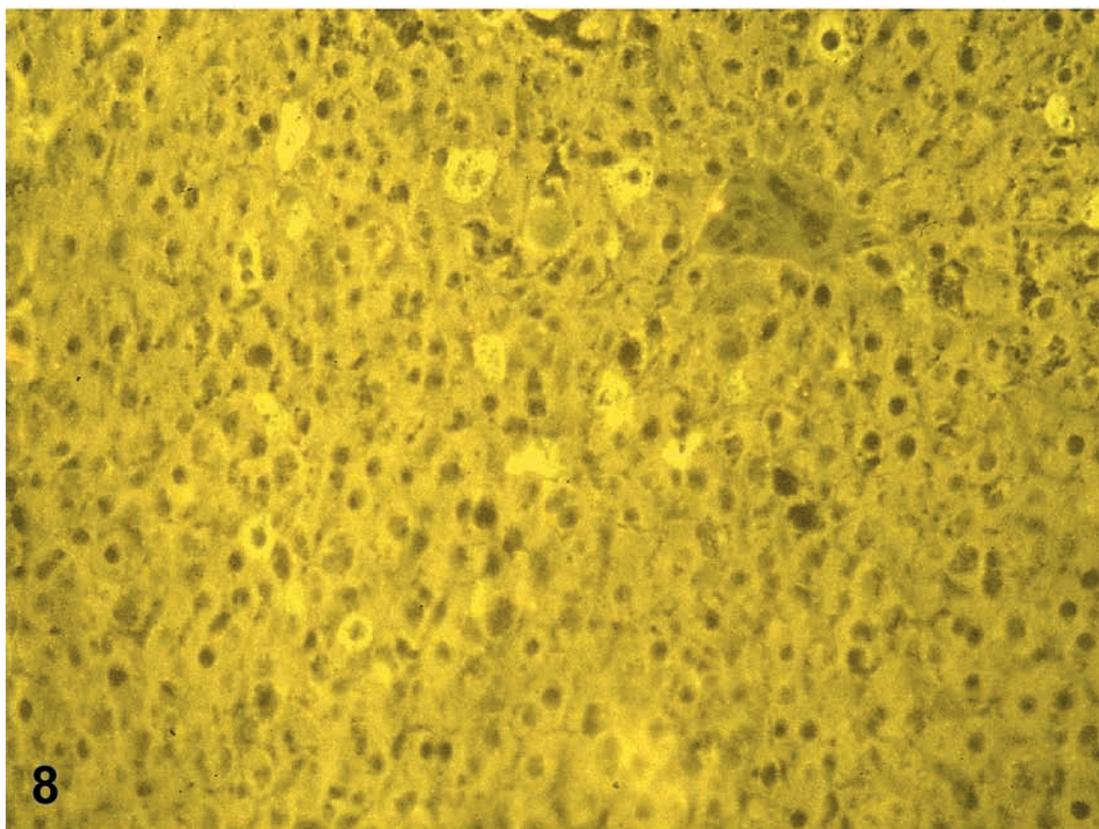
Hepatic morphologic abnormality	Control	Mevalonic acid (0.5%, diet)	Lovastatin (200 mpk, bid)	Lovastatin (200 mpk, bid) + mevalonic acid
Focal basophilic cellular alteration	1	1	15	1
Focal eosinophilic cellular alteration	1	1	12	1
Cellular atypia	3	2	21	3
Bile duct hyperplasia	8	7	23	7

Numbers show number of animals with indicated abnormality; n = 15/sex/group. From: MacDonald J. S. et al. (1988). *Am J Cardiol* 62, 16J–27J.



Figures 6–7.

FIGURE 6.—Basophilic focus of cellular alteration in rat liver. Section taken from liver of rat treated with lovastatin, 1600 mg/kg/day for 14 weeks (H & E). Evidence of hepatocellular atypia seen surrounding basophilic focus. 7.—High magnification of rat hepatocellular basophilic focus (H & E).



Figures 8–9.

FIGURE 8.—Control rat liver section treated with rabbit anti-rat monoclonal antibody against HMG-CoA reductase and stained with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. HMG-CoA reductase positive hepatocytes are seen scattered randomly around portal triad. 9.—Section of liver from rat treated with simvastatin, 100 mg/kg/day, for 14 days and treated as in Figure 8. Note intense staining of HMG-CoA reductase positive hepatocytes in periportal region.

TABLE 5.—Percent incidence of confirmed ALT elevations (3× upper limit of normal) in patients.

Drug	Placebo	10 mg	20 mg	40 mg	80 mg
Atorvastatin	—	0.2	0.2	0.6	2.3
Fluvastatin	—	—	0.2	1.5	2.7
Lovastatin	0.1	—	0.1	0.9	1.5

From: Tolman, K. G. (2000). *Am J Cardiol* **85**, 15E–19E; data taken from package inserts.

serum transaminase activities can be ameliorated though not completely prevented by coadministration of mevalonic acid (unpublished observation).

#### Clinical Hepatic Safety Data

Because of the results described previously in rabbits, rats, and dogs, along with data from clinical trials showing an apparent dose-related elevation in serum transaminase activity in patients, lovastatin, (the first statin to be approved for treatment of hypercholesterolemia) carried label warnings for liver toxicity with recommendations for periodic monitoring of serum transaminase activity. Extensive experience with this agent and those subsequently introduced in this therapeutic class provided very little evidence suggestive of direct drug-induced hepatotoxicity (Tobert et al., 2003; Tolman, 2000). As shown in Table 5 (from Tolman, 2000) there is a dose-related increase in confirmed ALT elevations (to 3 times the upper limit of normal) in patients taking statins. These elevations are usually asymptomatic and reversible even with continuation of therapy. Most studies have shown that less than 1% of patients require cessation of therapy. Biopsies have revealed no consistent histological presentation of hepatic toxicity and there is rare progression to overt clinical hepatitis. Tolman reviewed the cases of hepatotoxicity associated with treatment of lovastatin reported to Merck's Worldwide Adverse Database (WAES) (Tolman, 2000). They report 232 cases of acute hepatitis in 24 million patient-years of treatment for a rate of 9.6 per million patient years. Five cases of acute liver failure were reported in this same database for a rate of 0.2 per 10 per million patient years. Importantly, biopsy data available for 57 of the 230 cases of acute hepatitis revealed no consistent pattern of hepatotoxicity. A broad variety of pathologies were evident in these cases with no single morphologic abnormality predominant suggesting multiple (nondrug-related) etiologies.

It is becoming apparent that subtle elevations (of 2 to 3 times the upper limit of normal) are seen with all lipid lowering therapies including the nonabsorbed resins (Lipid Research Clinics Program, 1984). While the mechanism for this effect is not yet known, it has been speculated that the increased serum activity reflects alterations to the hepatocellular membrane (e.g., enhanced permeability) that permit leakage of these intracellular proteins (Tolman, 2000). This effect appears to be directly related to the magnitude of effect on serum lipids. These serum enzyme changes appear to be a pharmacodynamic effect rather than a representation of cellular and particularly liver toxicity.

#### Additional Findings in Animals That Represent Exaggerated Biochemical Activity

In addition to the effects on the liver described above, administration of HMG-CoA reductase inhibitors at high

dosage levels to animals is associated with several other effects that are thought to be mediated through inhibition of mevalonic acid synthesis. These include effects on the rodent forestomach and effects on the kidney of rabbits and gallbladder of rabbits and dogs.

In addition to the hepatotoxic effects described in rabbits, degeneration of proximal tubular epithelial cells in rabbit kidneys is also observed at high dosage levels. As with the effects on the liver described above, supplementation of rabbits with mevalonic acid prevents the nephrotoxicity observed. Results from these experiments do not, however, make it clear whether these nephrotoxic insults with simvastatin and lovastatin are a direct result of drug-induced toxicity or a secondary to drug-induced hepatotoxicity (Kornbrust et al., 1989).

Similarly, necrosis of gallbladder epithelium was observed in rabbits receiving simvastatin and this change, too, was prevented by mevalonate co-administration. Although no similar direct evidence is available from pharmacologic intervention studies with mevalonate, fluvastatin and atorvastatin produce similar effects in dog gallbladder epithelium (Corsini et al., 1996; Hartman et al., 1996; Walsh et al., 1996) and can be presumed to have similar etiology.

A final drug-induced adverse effect that is common to statins and likely mechanism-based is hyperplasia, hyperkeratosis and submucosal edema in the squamous epithelium of the nonglandular forestomach in mice and rats (Corsini et al., 1996; MacDonald et al., 1986; Gerson et al., 1988; Singer et al., 1991; Kloss et al., 1991). Although the mechanism for this effect on this specific subpopulation of gastric epithelium in rodents is not clear, it is characterized by the proliferation of squamous epithelial cells accompanied by submucosal edema and hyperkeratosis. This effect is confined to squamous epithelium in the forestomach and is usually not seen in squamous epithelium lining the esophagus in the rodent or in other species. No similar changes are observed in the glandular mucosa of the rodent stomach. This change has the appearance of local irritation but is not produced by structurally similar but biochemically inactive isomers of active agents suggesting that this effect is mediated through inhibition of HMG-CoA reductase (Kloss et al., 1991). Induction of HMG-CoA reductase and corresponding proliferation of smooth endoplasmic reticulum in forestomach keratinocytes (similar to what was observed in rat hepatocytes) was demonstrated in rats receiving lovastatin or its hydroxyacid form (Singer et al., 1991). While these changes seem to be mechanism-based, they appear to require a very high local concentration of active drug. Indeed, parenteral administration failed to produce similar lesions at this site (Gerson et al., 1989). The available data indicate that drug concentrations achieved in forestomach epithelium are greater than those seen in other tissues (Gerson et al., 1989). Importantly, the absence of effect in squamous epithelium lining the G.I. tract in other regions in the rodent as well as other species indicate that this is unlikely to predict potential human risk.

#### Findings With Statins That Are Probably Related to An Exaggerated Biochemical Effect of the Drugs

Several additional adverse events observed at high dosage levels in animal studies are generally considered to be a result of an exaggerated biochemical effect of this class of agents although the evidence for this is less compelling than

that described previously. The effects included in this category are fetal changes in rats (which appear to be secondary to mechanism-based maternal toxicity), myopathy in rats, cataracts in rats and dogs, and testicular degeneration observed in dogs.

**Fetal Effects:** A priori, it would seem logical that the developing fetus might be particularly sensitive to the pharmacological effect of statins. Cholesterol plays a central role in biosynthesis of hormones and is an essential lipid in cellular membranes. In addition to cholesterol, HMG-CoA reductase is responsible for the synthesis of nonsterol intermediates essential for cellular function (Figure 1). Thus, it was not surprising when decreased body weights and skeletal malformations were first observed in fetuses of rats and mice given lovastatin during pregnancy (MacDonald et al., 1988). Similar findings were noted when mevinnolinic acid (the pharmacologically active form of lovastatin) was administered to pregnant rats. Mevalonate supplementation prevented or markedly attenuated the fetal effects associated with mevinnolinic acid treatment (Minsker et al., 1983). Subsequently it was shown that fluvastatin delayed fetal skeletal development in rats and rabbits, and, at maternally toxic doses, produced malaligned thoracic vertebrae in rats (Lescol Product Insert). Simvastatin, pravastatin, and atorvastatin were not teratogenic in rats or rabbits as described in their respective product inserts. Given the fetal findings with lovastatin and fluvastatin, all HMG-CoA reductase inhibitors were considered teratogenic and, in the USA, are currently classified as pregnancy category X.

More recent rodent data shed some doubt on the link between statin administration and fetal risk. Decreased maternal body weight often results in fetal skeletal variations and malformations. Typically, rodents display a loss in body weight or decreased body weight gain during the first 2 weeks of statin administration. Forestomach toxicity is thought to mediate the statin-associated body weight effects. The forestomach effects that are most pronounced during the first few weeks of dosing, subside with continued treatment. Researchers at Merck used two approaches to circumvent the forestomach toxicity and elucidate the role of maternal toxicity in statin-associated skeletal abnormalities (Wise et al., 2000; Lankas et al., 2004).

If lovastatin was administered orally 14 days before mating through gestation day 20 or administered subcutaneously on gestation days 6 through 20, the increased incidence of skeletal abnormalities (variations or malformations) was prevented or markedly attenuated. The conclusion that fetal effects are secondary to maternal (forestomach) toxicity is consistent with the work of (Minsker et al., 1983) since mevalonate supplementation also prevents the forestomach toxicity.

#### Review of Human Pregnancy Data

Clinical evidence of teratogenicity in humans is limited. The product inserts of all marketed statins indicate that rare reports of congenital abnormalities have been noted following treatment of the mother with statins. In particular, the inserts cite 1 report of severe congenital bony deformity, tracheo-esophageal fistula and anal atresia in a baby born to a woman given lovastatin and dextroamphetamine sulfate during the first trimester of the pregnancy.

TABLE 6.—Incidence of skeletal muscle degeneration in female rats.

Treatment	Alone	Plus cyclosporine (10 mg/kg/day)
Lovastatin		
200 mg/kg/day	1/12	7/14
600 mg/kg/day	1/3	nt
900 mg/kg/day	2/3	nt
1200 mg/kg/day	3/3	nt
Pravastatin		
200 mg/kg/day	0/11	8/13
600 mg/kg/day	2/3	3/3
1200 mg/kg/day	2/2	nt
Simvastatin		
100 mg/kg/day	0/8	5/10
150 mg/kg/day	0/4	3/4
180 mg/kg/day	4/15	nt

From: Smith P. F. et al. (1992). *European Heart J* 13(Suppl. B), 2–6.  
nt = not tested.

Postmarketing surveillance by Merck examined the outcome of 134 pregnancies reported in women taking lovastatin or simvastatin between 1987 and 1998 (Manson et al., 1996). The percentages of normal outcomes (85%), congenital abnormalities (4%), spontaneous abortions (8%), fetal deaths/still births (1%), and miscellaneous adverse outcomes (2%), were not higher than those expected in the general population. Although the small sample size limited the power of the analysis, these data suggest that therapeutic doses of simvastatin and lovastatin were not associated with adverse pregnancy outcomes.

#### Myopathy

The myotoxic potential of HMG-CoA reductase inhibitors was first identified in a compassionate use clinical protocol involving coadministration of lovastatin, cyclosporine A, and gemfibrozil in a cardiac transplant patient (Norman et al., 1988; Tobert, 2003). Subsequent to this observation, a series of studies in rats demonstrated myopathy either with very high doses of HMG-CoA reductase inhibitors alone or at lower doses in the presence of cyclosporine A. The myotoxicity in rats is characterized by myofibrillar necrosis and interstitial edema and infiltration by inflammatory cells (see Figure 10; Smith et al., 1991a, 1992). The incidence of skeletal muscle degeneration in female rats treated with lovastatin, pravastatin, or simvastatin is shown in Table 6. As can be seen from these data, a significant incidence of skeletal muscle degeneration is not observed until very high dosage levels of these HMG-CoA reductase inhibitors are given. When non-toxic dosage levels of the statins are coadministered with cyclosporine A, however, the incidence of myotoxicity increases dramatically. This increase in myotoxic potential in the presence of cyclosporine A was directly related to an increase in systemic exposure to these drugs (see Table 7). It was demonstrated that a plausible explanation for this increase in

TABLE 7.—Systemic exposure to drugs in rat myotoxicity model.

Treatment	AUC (ug eq × hr/ml)		C <sub>max</sub> (ug eq/ml)	
	No CsA	+CsA	No CsA	+CsA
Lovastatin 100 mpk	2.32	ND	0.35	2.27
Pravastatin 100 mpk	1.08	8.52	0.47	4.28
Simvastatin 50 mpk	0.82	1.51	0.34	0.47

Cyclosporine A (CsA) administered concomitantly at a dose of 10 mg/kg.  
From: Smith P. F. et al. (1992). *European Heart J* 13(Suppl. B), 2–6.  
ND = not determined.

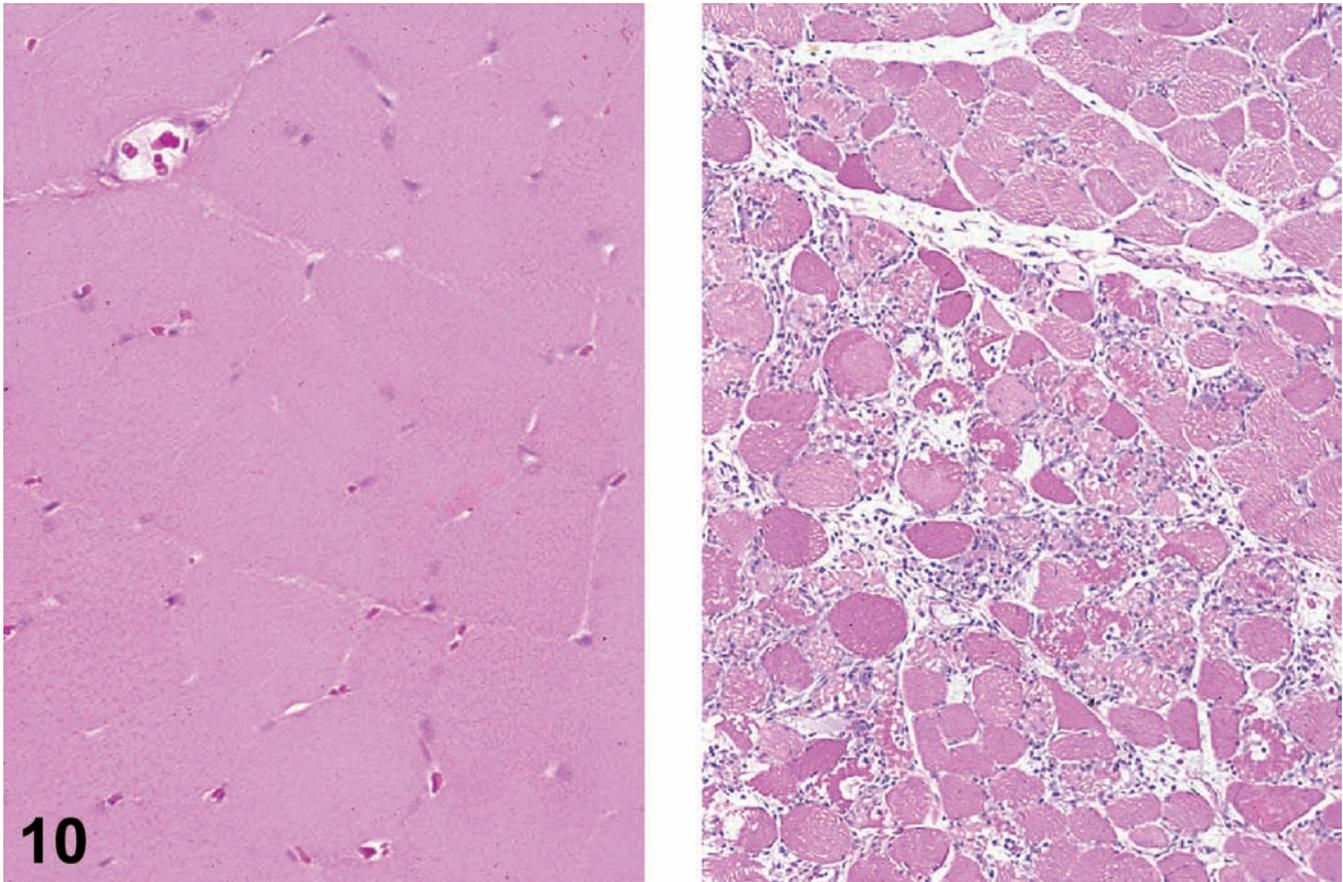


FIGURE 10.—Histologic appearance of rat skeletal muscle. Section from control rat on left showing normal appearance of myocytes (H & E). Section on right from soleus muscle of rat treated with pravastatin (200 mg/kg bid) for 14 days in combination with cyclosporine A, 10 mg/kg/day. Evidence of cellular necrosis, interstitial edema, and cellular infiltration are seen. Note selective nature of damage with marked difference in appearance of neighboring muscle bundles. (From Smith et al., 1991.)

systemic exposure was inhibition of the biliary clearance of the statin by cyclosporine A. These authors also demonstrated that co-administration of mevalonic acid could ameliorate the myotoxicity although it was hypothesized that this protection may not have been a result of a direct effect at the level of the myocyte but rather amelioration of the cyclosporine A-mediated inhibition of statin clearance.

While the risk of myopathy is clearly related to the extent of systemic exposure, the mechanism of the myotoxic event is not known with certainty. It is plausible to consider that, as HMG-CoA reductase inhibitors block the synthesis of mevalonic acid and subsequent synthesis of ubiquinone (a molecule essential for mitochondrial electron transport), the myotoxic event may be associated with a primary effect on the mitochondria. This is supported by histochemical studies showing that the myotoxic effect is selective for primarily type II glycolytic fibers which have the lowest mitochondrial density in the muscle bundle (Smith et al., 1991a). Other investigators have suggested that since the intracellular pool of ubiquinone is rather large and would not explain the differential sensitivity of different cell types (Goldstein and Brown, 1990), an alternative explanation is needed. Myotoxicity has been demonstrated *in vitro* using neonatal rat skeletal myocytes. These studies have provided evidence that the inhibition of

posttranslational modification of several specific cellular proteins (geranyl-geranylation) may be the cellular biochemical mechanism of myotoxicity (Masters et al., 1995; Flint et al., 1997).

#### *Incidence of Myopathy in Clinical Experience*

Since the original observation of rhabdomyolysis in a cardiac transplant patient receiving coadministration of cyclosporine and other drugs, the incidence of myopathy has been extensively studied in clinical populations. Myopathy is characterized by increases in creatine kinase (CK) activity greater than 10 times the upper level of normal accompanied by symptoms of myalgia or muscle weakness. These data suggest (with the exception noted below), that the incidence of myopathy is probably similar for all lipid-lowering drugs and is rarely seen with monotherapy (Evans and Rees, 2002; Hodel, 2002; Tobert, 2003). These studies further indicate that the incidence of myopathy is dose-related and, in the early stages, is generally reversible with cessation of treatment. The severe cases of myopathy (rhabdomyolysis with or without associated acute renal failure) are generally restricted to patients experiencing very high plasma levels of these agents. Significant increases in systemic exposure to drug occur in elderly patients with chronic renal failure or

following coadministration of multiple other agents that alter statin clearance from plasma.

While the overall incidence of fatal rhabdomyolysis in patients in the United States receiving statin therapy is very low, the incidence of rhabdomyolysis in patients taking cerivastatin appeared to be about 10 fold higher than that in patients on other statins. The reasons for this significantly increased incidence are still not known with certainty. There were 31 deaths in the United States from drug-associated rhabdomyolysis and associated acute renal failure in patients taking cerivastatin and, of these patients, 12 were also taking gemfibrozil. (Farmer, 2001). The reasons for this unique interaction are not known. Efforts to understand the effects on pharmacokinetics and metabolism of cerivastatin indicate that in human liver microsomes, gemfibrozil is a more potent inhibitor of glucuronidation as well as CYP2C8 and CYP3A4-mediated oxidative pathways of cerivastatin metabolism than was seen with either atorvastatin or simvastatin (Prueksaritanont et al., 2002). It has also been shown that cerivastatin is a substrate for the transport protein OATP 2 which may play a role in some of the drug-drug interactions (e.g., with cyclosporine A) that lead to fatalities. The relative affinities of other statins for these transporters has not been carefully studied, however (Shitara et al., 2003) so it cannot be stated with certainty at this point that this is the cause of the uniquely high reported toxicity for this drug. As a result of this marked increased incidence in rhabdomyolysis, cerivastatin was withdrawn from the market in 2001.

#### Cataracts

Relatively early in the development of lovastatin, lenticular opacities were observed in dogs receiving high dosage levels of this drug (MacDonald et al., 1988). These opacities first appeared in the posterior subcapsular region as increasing density of the suture lines. With continued treatment, these opacities progressed to full lenticular cataracts. Exploratory work in animals revealed several important characteristics of these lens opacities. As shown in Figure 11, there was no apparent relationship between the effect on serum cholesterol and the appearance of cataracts in dogs. There was, however, an important relationship between the extent of systemic exposure to these agents and the appearance of changes in the lens. This was shown dramatically by a comparison of the incidence of cataracts observed in dogs receiving simvastatin or the open hydroxy acid form of this drug where relatively similar dose levels of these drugs produced essentially equivalent reductions in mean serum cholesterol concentration (Gerson et al., 1989). The open acid form, however, produces a significantly higher systemic exposure as measured by AUC. This increase in AUC of approximately an order of magnitude results in an increase in cataract incidence of approximately 8-fold.

A final important observation from these early preclinical studies with simvastatin and lovastatin was that the incidence of cataracts did not increase beyond a certain percentage despite an increase in the duration of dosing (MacDonald et al., 1988; Gerson et al., 1989). A series of studies with simvastatin in dogs supported the hypothesis that the target cell population for HMG-CoA reductase inhibitors in this species that yields cataracts was the anterior epithelium in the cortex of the lens (Gerson et al., 1990). These studies demonstrated

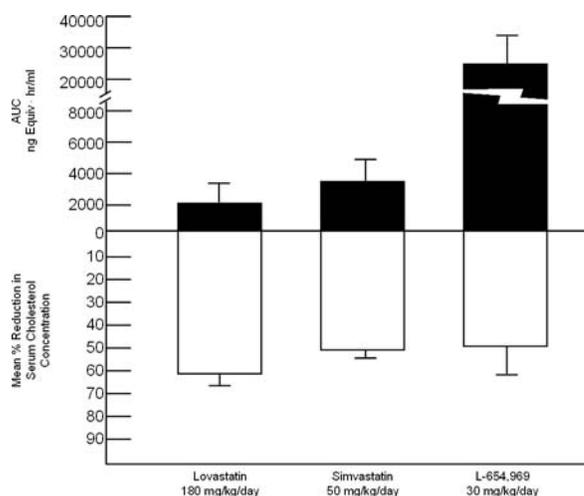


FIGURE 11.—Comparison of plasma levels of HMG-CoA reductase inhibitors in dogs with reduction in cholesterol concentration. Plasma concentrations expressed as area under the plasma concentration vs. time curve (AUC, 0–24) measured as ng equivalents X hour/ml. Values shown represent mean values  $\pm$  SD,  $n = 8$  dogs/group. Corresponding reductions in serum cholesterol concentration in the same groups of dogs are shown in the bottom portion of the graph. Values expressed as mean percent reduction from pretest values  $\pm$  SD,  $n = 8$  dogs per group. L-654,969 is the open hydroxy acid form of simvastatin. (Data from MacDonald et al., 1988.)

no abnormal sterols or effect of drug on cholesterol content in lens tissue from affected and unaffected dogs. These observations provided strong evidence that the etiology of statin-induced cataracts was different from those produced in animals by previous inhibitors of cholesterol synthesis (triparanol, MER 29; U18666A) (Kirby, 1967; Alcalá et al., 1985). In addition to showing no difference in lens cholesterol content between clear or cataract-containing lenses, these studies showed no relationship between drug content and clear or cataract-containing lenses. The increase over time of drug content in the lens is expected given the nature of the growth of this tissue. It is also known that as lens fibers form and develop and progress toward the nucleus, they lose many of the normal cellular functions (Cenedella, 1996). The available data support the hypothesis that administration of HMG-CoA reductase inhibitors yields a steady-state concentration of drug in the target cells of the anterior epithelium of the lens. This offers an explanation for why continued drug administration past a sensitive period does not yield a continuing increase in the incidence of cataracts despite a continuing increase in drug deposition in the total lens fibers (Gerson et al., 1990; MacDonald, 1995). Supporting the hypothesis that this effect is related to inhibition of HMG-CoA reductase is work suggesting that the cataractogenic effect of lovastatin in cultured rat lens can be prevented by incubation with geranyl pyrophosphate, a mevalonate by-product (Cheng et al., 2002).

The exposure multiples achieved at the no-effect dose for cataracts in dogs predicted the absence of cataracts in patients being treated with these drugs (Gerson et al., 1990). This has been born out in careful evaluations in patients. When statins were initially introduced into clinical use, the results of dog studies dictated careful and periodic eye examinations (Tobert, 2003). Because of careful evaluations

over many years of treatment with lovastatin and simvastatin, these evaluations are no longer required as there is no indication of an increased incidence of cataracts in patients treated with these statins (Schmidt et al., 1990; Chylak et al., 1993; Schmidt et al., 1994; Harris et al., 1995; Pedersen et al., 1996).

#### *Testicular Effects*

A low incidence of testicular changes has been noted in dogs given statins (MacDonald, 1988; Gerson, 1989; Walsh et al., 1996; von Keutz and Schluter, 1998). The findings include decreased testicular weights, atrophy/degeneration of seminiferous epithelial cells, disruption of spermatocyte maturation and an increased incidence of multinucleated giant cells. Sertoli cells and Leydig cells were not affected. Preclinical studies have shown that serum testosterone and luteinizing hormone levels were not affected by administration of lovastatin, simvastatin or cerivastatin to dogs. In clinical studies, with the exception of a slight decrease in sperm motility, indicators of testicular function (including sperm counts and morphology and serum androgen levels) were not altered in men treated with lovastatin or simvastatin (Farnsworth et al., 1987; Purvis et al., 1992).

Due to the low incidence and sporadic nature of the findings, the testicular changes have been difficult to reproduce and investigate with mevalonate supplementation. Indeed, many of these histopathologic changes have also been observed in naïve beagle dogs (Rehm, 2000). The relationship of these findings to the pharmacologic effect of statins remains to be determined.

#### FINDINGS WITH STATINS OF UNCERTAIN RELATIONSHIP TO BIOCHEMICAL MECHANISM OF ACTION

The final category into which findings in animals have been grouped for discussion here is that for which the relationship between the observed adverse effect and the biochemical mechanism of action of the drug is uncertain. Included in this category are CNS findings in dogs and tumors in rodents.

#### *Central Nervous System Toxicity*

High dosage levels of all of the HMG-CoA reductase inhibitors that have been made available commercially (with the exception of fluvastatin as well as others not commercialized) have produced CNS toxicity in dogs (respective package inserts and Summary Basis of Approval documentation). In most cases this was characterized by vascular endothelial degeneration and hemorrhage (Berry et al., 1988; Walsh et al., 1996). With pravastatin, this effect was observed consistently at high doses (25 mg/kg/day and greater; 7–8-fold greater than human therapeutic AUC). At lower doses with this drug, dogs were asymptomatic for as long as 18 months on treatment before displaying CNS signs and cerebral hemorrhage on postmortem examination (pravastatin FDA Summary Basis of Approval).

These lesions were most extensively characterized by Berry in dogs treated with lovastatin (Berry et al., 1988). The predominate effect appears to be on vascular endothelial cells with degenerative lesions in optic nerve thought to be secondary to ischemia resulting from the vascular effect. The Wallerian-like axonal degeneration observed in dogs receiving 60 mg/kg per day or greater of lovastatin may also have

been secondary to drug-induced ischemia although alternative explanations could not be excluded. The relationship of these changes to inhibition of HMG-CoA reductase and the subsequent alterations of cellular metabolism do not appear to have been further elucidated.

#### *Tumors in Mice and Rats*

An increase in the spontaneous background rate of tumors in mice and rats has been reported in a variety of tissues in all members of this therapeutic class that have been taken to global registration (Table 8; respective FDA Summary Basis of Approval documents and package inserts). No data have been reported which indicate that any of the members of this class have activity in *in vitro* or *in vivo* genotoxicity assays indicating no mutagenic or clastogenic potential. The preponderance of evidence suggests that the increase in tumors in rats and mice is a result of a nongenotoxic mechanism.

An increase in the incidence of hepatocellular tumors was observed in rats receiving lovastatin along with an increase in the incidence of pulmonary adenomas and forestomach squamous papillomas (MacDonald et al., 1988). In addition, a 95% incidence of hepatocellular adenomas and carcinomas was seen in male mice treated with simvastatin along with an increase in pulmonary adenomas and Harderian gland adenomas (Simvastatin, U.S. FDA Summary Basis of Approval, 1992). The profile of tumors is clearly different among members of this class despite the fact that all induce an increase in the incidence of one or more tumor types (Robinson et al., 1994, respective FDA Summary Basis of Approval documents).

The mechanism for the increase in the incidence of these spontaneously occurring neoplasms in rodents is not clear. That there is a drug-related effect at relatively low systemic exposure multiples to those used therapeutically in people cannot be disputed (for example, a 95% incidence of hepatocellular adenomas or adenocarcinomas in male mice receiving simvastatin at a dose multiple of 3-fold to the usual human therapeutic dose (Simvastatin, U.S. FDA Summary Basis of Approval, 1992). As discussed before, HMG-CoA reductase inhibitors produce profound effects in rodent livers that are not seen in livers from other species. Although no data are

TABLE 8.—Carcinogenicity of statins in rodents.

Target Organ – Tumor Type	Atorva	Ceriva	Fluva	Lova	Prava	Rosuva	Simva
Liver							
– Adenoma/carcinoma	M	M	—	M/R	R	M	M/R
Thyroid							
– Adenoma/carcinoma	—	—	R	—	—	—	R
Forestomach							
– Papilloma	—	—	M/R	M	M	—	M
Lung							
– Adenoma	—	—	—	M	—	—	M
Lymphatic system							
– Malignant lymphoma	—	—	—	—	M	—	—
Harderian gland							
– Adenoma	—	—	—	—	—	—	M
Uterus							
– Polyps	—	—	—	—	—	R	—
Muscle							
– Rhabdomyosarcoma	R	—	—	—	—	—	—
– Fibrosarcoma	M	—	—	—	—	—	—

M = mouse, R = rat.

Information obtained from respective FDA Summary Basis of Approval documents.

available to clearly identify causative factors, it is not difficult to extrapolate from the observed effects of HMG-CoA reductase inhibitors on the rodent hepatocyte to the changes in cell function, cell membrane structure, and/or cell-to-cell communication which may impact control and regulation of proliferative responses.

The mechanism of increase of some of the tumor types observed in rodents have been relatively well established. For example, the increase in thyroid follicular cell adenoma associated with simvastatin administration to rats is clearly related to a secondary effect on thyroxin metabolism resulting from direct hepatic effects of the drug (Smith et al., 1991b). The mechanisms behind increases in the incidence of other tumor types and other tissues is not known.

Statins have been available for the treatment of hypercholesterolemia since 1986. The millions of patient days of therapy that have been achieved since this introduction have not been associated with any reported increase in the incidence of any particular tumor type in patients. This anecdotal evidence, however, must be considered relative to the comparatively short period of time (approximately 18 years) that this class of agents has been available. For a compound which induces an increase in tumor incidence in rodents by clearly nongentoxic mechanisms, this would have to be considered to be a minimal period of time to result in an increase in the incidence of tumors in patients. Thus, while the increase in the spontaneous incidence of tumors in 2-year rodent bioassays after treatment with statins has drawn some to suggest that this class of agents should only be reserved for patients at high risk of heart disease (Newman and Hulley, 1996), the consensus of the field is that these rodent data should not prevent the use of these agents in patients who have been demonstrated to benefit from this therapy (Dalen and Dalton, 1996).

#### *Clinical Adverse Events*

The most important clinical adverse events have been discussed in the sections previously. Certainly the most clinically important adverse event associated with HMG-CoA reductase inhibitor administration has been myopathy and the occasional associated rhabdomyolysis. Additionally, the incidence of acute hepatitis associated with drug administration has been very rare despite the relatively common increase in the incidence of serum transaminase activity in patients on therapy with this class of agents.

Of the other adverse side effects reported with statins, the next most prominent appears to be a potential effect on peripheral nerves (Gaist et al., 2002; Muscari et al., 2002). In one study, a retrospective evaluation of statin users suggested a 4- to 14-fold increased risk of idiopathic polyneuropathy particularly in long-term statin users (Gaist et al., 2002). At the present time, the mechanistic basis of this reported increase in peripheral neuropathy is not known (Backes et al., 2003).

Early reviews of clinical trial data with statins revealed a significant increase in the incidence of death from suicide, accidents, and violence and it was suggested that these adverse events could be related to either treatment with HMG-CoA reductase inhibitors or significant cholesterol lowering. Subsequent carefully controlled studies of this potential association, however, have not revealed any relationship between

treatment with statins and these cognitive disorders (Muldoon et al., 2000; Tomlinson et al., 2002).

None of the other significant adverse effects observed in animals treated with HMG-CoA reductase inhibitors has been reproduced in extensive clinical experience with these agents.

#### DISCUSSION AND CONCLUSION

The data displayed in Table 1 show clearly that the first principle of toxicology has been satisfied in these nonclinical studies with statins: the potential adverse effect profile of these chemical entities has clearly been fully expressed. In addition, these nonclinical studies provided significant information on dose-response and dose-exposure relationships evaluating systemic exposure to these agents at toxic and nontoxic dosage levels in multiple species. Within this classical toxicological risk evaluation framework, then, an understanding of probable mechanism of adverse effect provides a powerful additional tool to provide prospective for potential human risk. Demonstration that an adverse effect is mechanism-based gives added assurance of lack of hazard at lower exposure near the therapeutic range.

The data in Figure 12 illustrate this principle. Much of the significant toxicity observed in nonclinical studies was seen in dogs treated with statins. A dose of simvastatin of 50 mg/kg per day produced the greatest incidence of adverse effects observed in this species after treatment with this drug (Gerson et al., 1989). A dose of 10 mg/kg per day, however, for the same treatment period was not associated with any evidence of significant adverse effect. The systemic exposure expected at human therapeutic concentrations, then, can be compared to the exposure seen at the no-effect dose (10 mg/kg per day) or at the effect dose (50 mg/kg per day). These data support the risk assessment that human adverse events would be unlikely with monotherapy resulting in the expected low systemic exposure.

The mechanistic studies that have been conducted in nonclinical experiments with statins have identified a number of adverse effects that are clearly a result of an exaggeration of the desired biochemical effect of these drugs (inhibition of mevalonate and/or cholesterol biosynthesis). Early work in this field suggested that quantitative differences in toxicity seen in animal studies may be related to differences in tissue distribution (Kaga et al., 1990; Kamaii et al., 1992; Ziegler et al., 1992). This was referred to (in liver) as a tissue

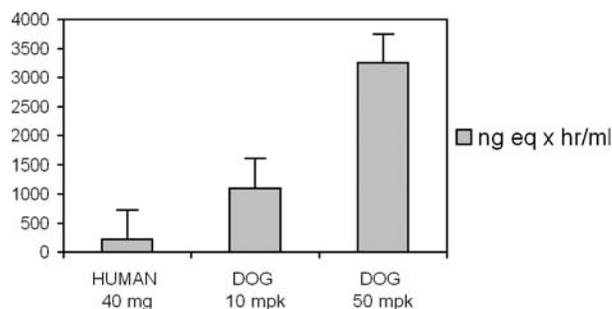


FIGURE 12.—Mean 24-hour plasma levels in dogs and humans after administration of simvastatin at the indicated doses (expressed as AUC, ng equivalents X hr/ml). Bars represent means of at least 7 subjects each. (Data from Gerson et al., 1989.)

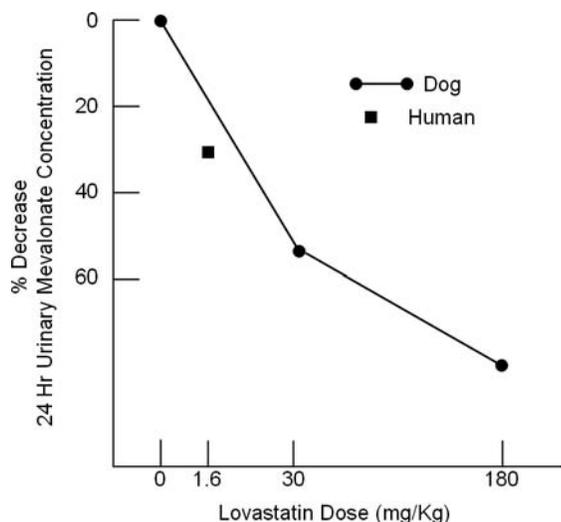


FIGURE 13.—Effect of lovastatin on urinary mevalonate concentration in dogs and humans. Urinary mevalonate levels measured from 24-hour urine samples normalized to urinary creatinine concentrations. Data are expressed as percent decrease from pretreatment levels. Dogs were treated with the indicated doses for 1 month, p.o. (n = 8/group). Human data derived from hypercholesterolemic patients treated for 6 weeks at 80 mg (approx. 4 times the usual clinical dose), n = 15. (From MacDonald et al., 1988.)

specificity thought to be related to the relative lipophilicity of the various statins, and suggested to explain the differential ocular (Mosley et al., 1989; de Vries et al., 1993) or skeletal muscle (Masters et al., 1995) toxicity seen preclinically. As data have become available with more drugs in this class, it appears that the predominant predictor of toxicity is degree of systemic exposure, cellular penetration and potency against the target enzyme.

With the understanding that much of the toxicity of these agents is mechanism-based, it is possible to develop a more rigorous method of assessment of potential human risk by looking at a surrogate marker of the relative biochemical effect in the target species (Beil et al., 1990). The result of this type of evaluation is shown in Figure 13 (MacDonald, 1988; Gerson, 1990). Using 24-hour urinary mevalonate excretion as a measure of the magnitude of the biochemical effect of these drugs, it can be seen that the degree of inhibition of mevalonate synthesis at exposures associated with significant toxicity in dogs exceeds that seen in humans even at the highest therapeutic dose. This approach provides a greater degree of assurance that doses associated with effective therapy in humans will not be associated with the kind of adverse effects seen in animals at higher exposures.

An appropriate question to ask is “what was the predictive value of the non-clinical studies performed with these agents?” The animal studies very clearly identified potential target organs and helped understand the relationship of these effects for example in the liver to the biochemical effect of these drugs providing some perspective to assess potential hepatic effects in people. In addition, the potential to induce myopathy was identified in animals, although it must be acknowledged that this potential was only definitively identified in retrospective studies after the first observation of this adverse event in the clinic. The available data show that the

TABLE 9.—Metabolic properties of HMG-CoA reductase inhibitors.

Drug	Predominant metabolic enzyme	Number of active metabolites	t 1/2
Lovastatin	CYP 3A4	3	2
Pravastatin	Hydroxylase	2	2
Simvastatin	CYP3A4	3	3
Fluvastatin	CYP2C9	No	3
Atorvastatin	CYP3A4	2	15
Cerivastatin	CYP3A4, CYP2C8	2	3
Rosuvastatin	CYP 2C9	1	18

CYP: Cytochrome P450; t 1/2 expressed in hours in human subjects.

(Data from Williams et al., 2002; rosuvastatin data from rosuvastatin FDA Summary Basis of Approval).

potential to induce myopathy by this class of agents is rare in monotherapy and increases greatly when these drugs are given with other pharmaceutical agents. It is understood that the basis for this enhanced toxicity in combination in many cases can be ascribed to an increase in systemic exposure to the statin resulting from metabolic interactions. As indicated in Table 9, most of these statins are metabolized by various members of the cytochrome P450 family of enzymes. Inhibition of metabolic clearance of the statin due to simple competitive action by other P450 substrates leads to significantly elevated plasma levels of the respective statin and associated adverse events. While these direct interactions can account for most of the reported AE's attributed to drug-drug interactions, other metabolic interactions may also play a role (effects on transport proteins, potential effects on Phase II drug metabolizing enzymes) (Bays et al., 1998; Williams, 2002). Data on routes of metabolism of these agents permits a better understanding of potential drug-drug interactions when administered in combination with other compounds.

The available data suggest that the additional target organs identified in nonclinical studies do not predict human risk. This is either because the toxicity was only expressed at exposure levels significantly greater than those achievable under therapeutic conditions (even in the presence of drug-drug interactions) or these adverse effects are expressed in target organs that are not relevant for human risk assessment (e.g., forestomach). Perhaps the best example of the former case where exposures required to produce toxicity are not achievable under therapeutic conditions is the case of cataracts. This finding is consistent across all statins and can be observed in multiple species. A careful evaluation of the available data, however, illustrates why the adverse effects in the lens of humans have not been observed in extensive human experience with this class of agents.

The experience with this class of agents demonstrates that the tools that have been developed in toxicologic pathology are effective in contributing to the understanding of the pathogenesis of lesions observed at high exposures in animals and the relative importance of these findings for potential human risk. These tools in essence, then, facilitated the safe exploration of efficacy of these agents in humans suffering from hypercholesterolemia. While these studies demonstrate the value of these tools in assessing potential human risk, it is also clear that there are opportunities to improve the power of this process. The value of new molecular approaches such as genomics, proteomics, and metabonomics (discussed elsewhere in this symposium) are important to further develop

and pursue so as to enhance the ability of toxicologic pathology to predict human risk with increased efficiency and facilitate the safe exploration of efficacy of potentially important human medicines.

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