TOXICOLOGIC PATHOLOGY, vol. 27, no. 4, pp. 395–401, 1999

Hepatic Effects in Beagle Dogs Administered Atorvastatin, a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitor, for 2 Years*

KATHLEEN M. WALSH AND CHARLES E. ROTHWELL

Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, Michigan

ABSTRACT

The chronic toxicity of atorvastatin (AT), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, was evaluated in beagle dogs. Dogs were treated with 0, 10, 40, or 120 mg/kg of AT daily. Treatment lengths were 52 wk, 52 wk followed by 12 wk without drug, or 104 wk. Decreases in cholesterol levels were dose related and stable throughout the treatment period. Increases in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were transient and dose related in severity at ≥40 mg/kg. Two dogs administered 120 mg/kg of AT daily were sacrificed moribund during the first 9 wk of treatment. Hepatic lesions were reversible with or without continued treatment and dose related in severity and distribution. Hepatic microgranulomas and hepatocellular degeneration were seen at the 120-mg/kg dose in dogs sacrificed before 53 wk. Before 53 wk, hepatocellular lipofuscin deposits were increased in dogs given ≥40 mg/kg of AT daily but were similar to controls after 12 wk without drug and after 104 wk of continuous treatment. Bile stasis occurred in dogs given ≥40 mg/kg of AT daily at all time points but was less severe after reversal and at week 104 compared with week 52.

Keywords. Lipid regulation, liver, cholesterol, microgranuloma, lipofuscin

INTRODUCTION

Atorvastatin (AT) (CI-981, Lipitor®) is a potent member of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (HMGRIs). Endogenous biosynthesis in liver and intestine is the major source of serum cholesterol, and conversion of HMG-CoA to L-mevalonic acid, catalyzed by HMG-CoA reductase, is the rate-limiting step in the cholesterol pathway. HMGRIs effectively lower overall cholesterol levels, decrease low-density lipoprotein (LDL) levels, and minimally increase high-density lipoprotein (HDL) levels (26) and are considered important hypolipidemic and antiatherosclerotic compounds in humans. AT, a highly substituted pyrrole, has been shown to decrease LDL cholesterol in humans (19) via upregulation of receptors for LDL (3). In addition to decreasing overall serum cholesterol levels in normolipidemic dogs and rats (24, 31), HMGRIs prevent the buildup of toxic sterol intermediates seen with inhibitors that act later in the synthetic cascade (29).

Despite the fact that in the dog the major cholesterol carrier is HDL and not LDL as in humans (32), the dog is a reasonable animal model to study HMGRIs. In dogs, HMGRIs lowered cholesterol levels and induced lenticular opacities, sporadic transient increases in alanine and/or aspartate aminotransferase, degeneration of vascular endothelium in brain, and hyperplasia of gallbladder epithelium (2, 7). In subchronic studies (up to 13 wk), AT induced hepatocellular degeneration, cholecystitis, skeletal muscle necrosis, and optic nerve demyelination at doses ≥150 mg/kg (31). Because of these findings and because treatment in humans is expected to be long-term, a long-term study in dogs was performed. This report presents the findings in livers of dogs treated for up to 2 yr with AT.

MATERIALS AND METHODS

This study complied with U.S. Food and Drug Administration Good Laboratory Practice regulations and with the National Institutes of Health and Animal Welfare Act guidelines for animal welfare (18, 30). Full details of this study will be reported elsewhere. Briefly, beagle dogs were given 0, 10, 40, or 120 mg/kg of AT in gelatin capsules daily (10 dogs/sex/dose). Chemically, AT is designated as 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid. At 52 wk, 3 dogs/sex/dose were sacrificed and necropsied, and 2 dogs/sex/dose were withdrawn from treatment. These latter dogs were sacrificed and necropsied in week 64 to assess reversibility. The remaining dogs were continuously treated daily for 104 wk before termination. In addition to routine hematology, clinical chemistry, and urinalysis, lenses of one eye from each dog were analyzed for protein, glucose, and potassium, and semen and sperm samples were taken at multiple time points for analysis. Lenticular results have been reported previously (23), and procedures and results of semen and sperm analyses will be reported separately (6).

At scheduled terminations, all dogs given 0 and 120 mg/kg of AT daily and 2 males given 10 mg/kg of AT daily were anesthetized intravenously with short-acting barbiturate and perfused with 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer via carotid artery to optimize brain and optic nerve fixation. During perfusion, femoral arteries were severed to ensure exsanguination, and jugular veins were transected to limit

* Address correspondence to: Dr. K. M. Walsh, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

0192-6233/99$3.00+$0.00

TABLE I.—Severity[a] of hepatic findings (different from controls) in dogs given AT in various dosages for up to 104 wk. The week given is the week of sacrifice.

	10 mg/kg/day			40 mg/kg/day			120 mg/kg/day			
	Week 52	Week 64	Week 104	Week 52	Week 64	Week 104	MS[b]	Week 52	Week 64	Week 104
Hepatocellular degeneration/necrosis	–	–	–	–		–	3	1	–	–
Mononuclear cell foci	–	–	–	1	–	–	1	1	–	–
Microgranuloma	–	–	–	1	–	–	1	3	–	1
Increased lipofuscin deposits	–	–	–	–	–	–	4	3	1	–
Cholestasis	–	–	–	1	–	1	2	2	1	1
Bile duct proliferation	–	–	–	1	–	–	3	1	–	–
Cholecystitis/hemorrhage	–	–	–	–	–	–	3	1	–	–
Increased AST/ALT	–	–	–	1	–	1	4	1	–	1
Decreased cholesterol	2	–	2	3	–	3	3	4	–	4

[a] 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[b] MS = moribund sacrifice of 2 dogs (weeks 7 or 9).

amount of fixative reaching viscera. Right eyes, testes, and epididymides were removed from these dogs before perfusion. All other dogs were euthanatized by intravenous overdose of barbiturate and exsanguination.

At termination, selected organs, including liver, were weighed. Organ-to-body and organ-to-brain weight ratios were calculated. Complete necropsies were performed on all dogs. Samples of multiple organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin-eosin. Some liver sections were stained with Schmorl's stain for lipofuscin, periodic acid–Schiff (PAS), Hall's for bilirubin, Ziehl-Neelsen (ZN) for acid fastness, Mallory's for hemofuscin, Masson's trichrome, Stein's for bile, Prussian blue for ferric iron, Turnbull blue for ferrous iron, rhodanine for copper, or mouse antirat ED1 monoclonal antbody (marker for lysosomal membrane, Serotec, Raleigh, NC). Samples of left lateral hepatic lobe from each animal were fixed immediately after exsanguination in 2.5% glutaraldehyde, postfixed in 1.0% osmium tetroxide, and processed and embedded in plastic. Ultrathin liver sections from the dogs receiving 120 mg/kg of AT and selected control animals were examined with a Hitachi H-600 electron microscope. All other tissues were examined by light microscopy.

RESULTS

The following includes only drug-related hepatic results, which are summarized in Table I. Complete results of clinical observations, clinical pathology, and pharmacokinetics will be reported separately. Increased emesis and fecal changes (diarrhea, soft or bloody stool samples) occurred sporadically throughout the study period and mainly at 120 mg/kg. Two males receiving 120 mg/kg of AT were sacrificed moribund in week 7 or 9 due to 25% to 27% body weight loss, bloody diarrhea, and nonresponse to symptomatic treatment. Body weight gain during weeks 1 to 52 was minimally reduced at 120 mg/kg compared with controls but was similar to controls during weeks 53 through 104. AT induced consistent dose-related decreases in cholesterol, LDL, and HDL levels. After 12 wk without AT, cholesterol, HDL, and LDL levels in treated dogs were similar to pretest values and values in control dogs.

Increases in aspartate aminotransferase (AST), alanine aminotransferae (ALT), and alkaline phosphatase (AP) ranged from 2- to 28-fold in the males receiving 120 mg/kg of AT who were sacrificed moribund. Mean increases in ALT, AST, and AP levels were minimal (2- to 4-fold more than pretest) in both sexes at 120 mg/kg in weeks 13 and 26. Mean ALT and AP levels were also increased (2- to 3-fold more than pretest) in weeks 52, 78, and/or 104 in females in the 120-mg/kg group. At 40 mg/kg, mean increases in ALT levels were approximately 2 times pretest and occurred at weeks 13 and 26 in males and week 104 in females.

Diagnostic criteria for liver changes were as follows: (a) microgranuloma: accumulation of mononuclear phagocytes (weakly decorated with antimacrophage antibody); ± pigmentation (positive with Schmorl's, ZN, PAS); ± necrotic cells; ± neutrophils; ± lymphocytes; random distribution (mainly midzonal and centrilobular); (b) foci of mononuclear cells (MN foci): collections of mononuclear cells including macrophages but mainly lymphocytes; often in sinusoids surrounding single or few hepatocytes; ± neutrophils; (c) cholestasis: centrilobular canaliculi distended with linear deposits of bile (positive with Hall's stain); minimal severity equivalent to widely scattered in few lobules; mild equivalent to 1 to 5 filled canaliculi in many lobules; and (d) lipofuscin: in hepatocytes or phagocytes, fine (1–2 μm) golden-brown to brown granules (positive with Schmorl's, PAS, and ZN [weakly] stains); minimal equivalent to few readily visible granules in scattered centrilobular hepatocytes in few or many lobules, moderate equivalent to numerous granules in most centrilobular hepatocytes in many to most lobules.

Moribund and Week 52

The most severe changes were in 1 male dog in the 120-mg/kg group who was sacrificed *in extremis* and included moderate degeneration with multifocal necrosis of centrilobular hepatocytes, multifocal minimal MN foci, multifocal microgranuloma, mild cholestasis, and multifocal mild-to-moderate hyperplasia of bile ducts. Compared with livers from control dogs (Fig. 1), hepatocel-

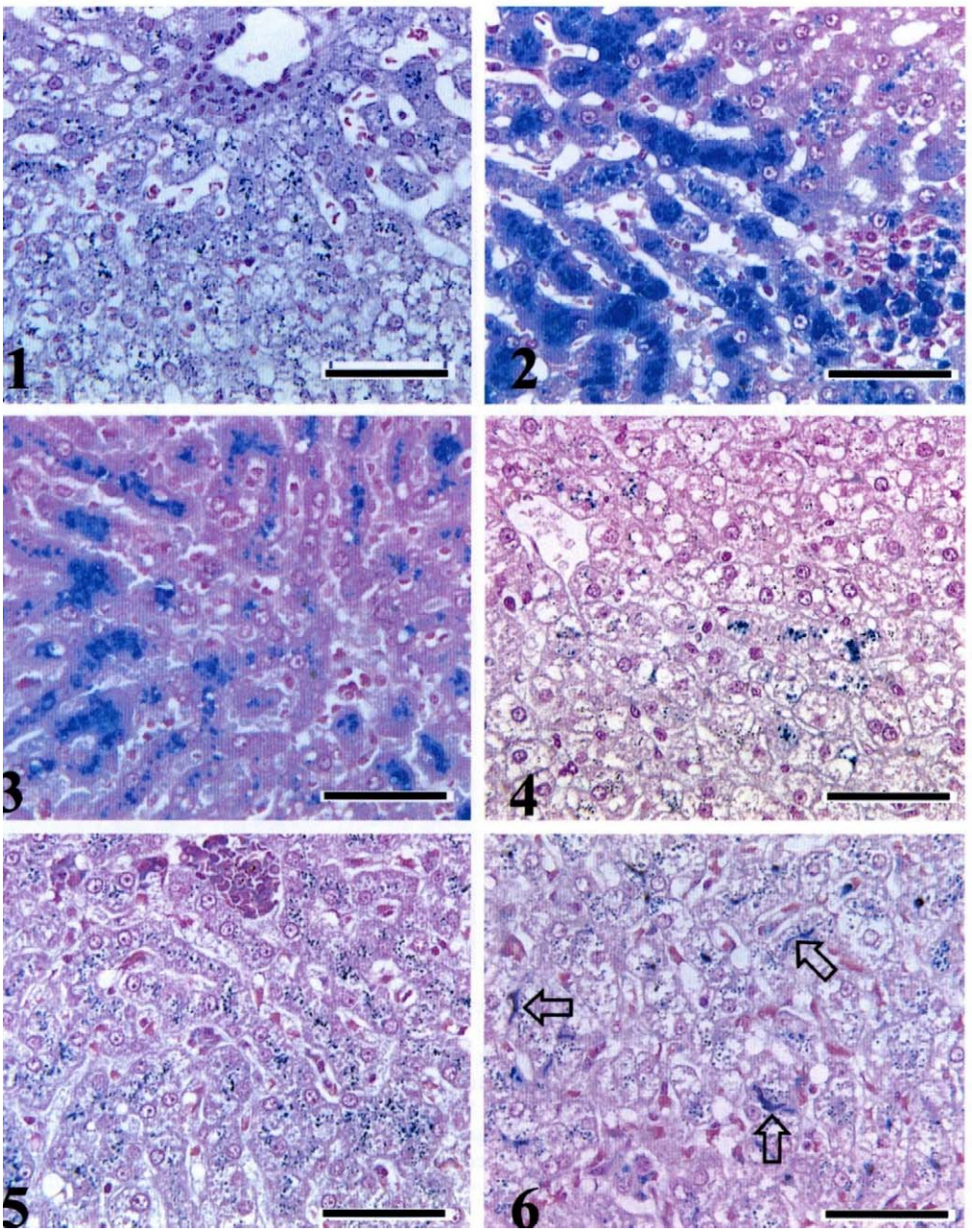

FIG. 1.—Liver from a control dog at 104 wk. Lipofuscin granules (fine blue intracytoplasmic granules) in centrilobular hepatocytes stained with Schmorl's stain. Bar = 100 μm.

FIG. 2.—Liver from a dog given 120 mg/kg of AT for 9 wk before moribund sacrifice. Lipofuscin accumulation markedly increased in centrilobular hepatocytes. Many cells in microgranuloma (lower right) also contain lipofuscin. Schmorl's stain. Bar = 100 μm.

FIG. 3.—Liver from dog given 120 mg/kg of AT for 52 wk. Compared with control, centrilobular hepatocytes contain increased deposits of lipofuscin. Schmorl's stain. Bar = 100 μm.

FIG. 4.—Liver from dog given 120 mg/kg of AT for 52 wk followed by 12 wk without drug treatment. Lipofuscin granules are much less than in dogs at 52 wk and in most hepatocytes similar in quantity to control. Schmorl's stain. Bar = 100 μm.

FIG. 5.—Liver from dog given 120 mg/kg of AT for 104 wk. Despite continuous treatment with AT, hepatocellular lipofuscin is similar to control. Schmorl's stain. Bar = 100 μm.

FIG. 6.—Cholestasis (arrow) in dog given 120 mg/kg of AT for 104 wk. Note lipofuscin deposits are similar to control dog in Fig. 1. Schmorl's stain. Bar = 100 μm.

lular lipofuscin accumulation was moderately to markedly increased (Fig. 2). At 52 wk, liver changes were present with dose-related frequency and/or severity. At 120 mg/kg, most of the dogs had diffuse mild-to-moderate centrilobular lipofuscin deposits (Fig. 3), mild cholestasis, multifocal MN foci (Fig. 7), and minimal-to-moderate multifocal microgranulomas (Figs. 8 and 9). One dog had diffuse mild vacuolation and multifocal

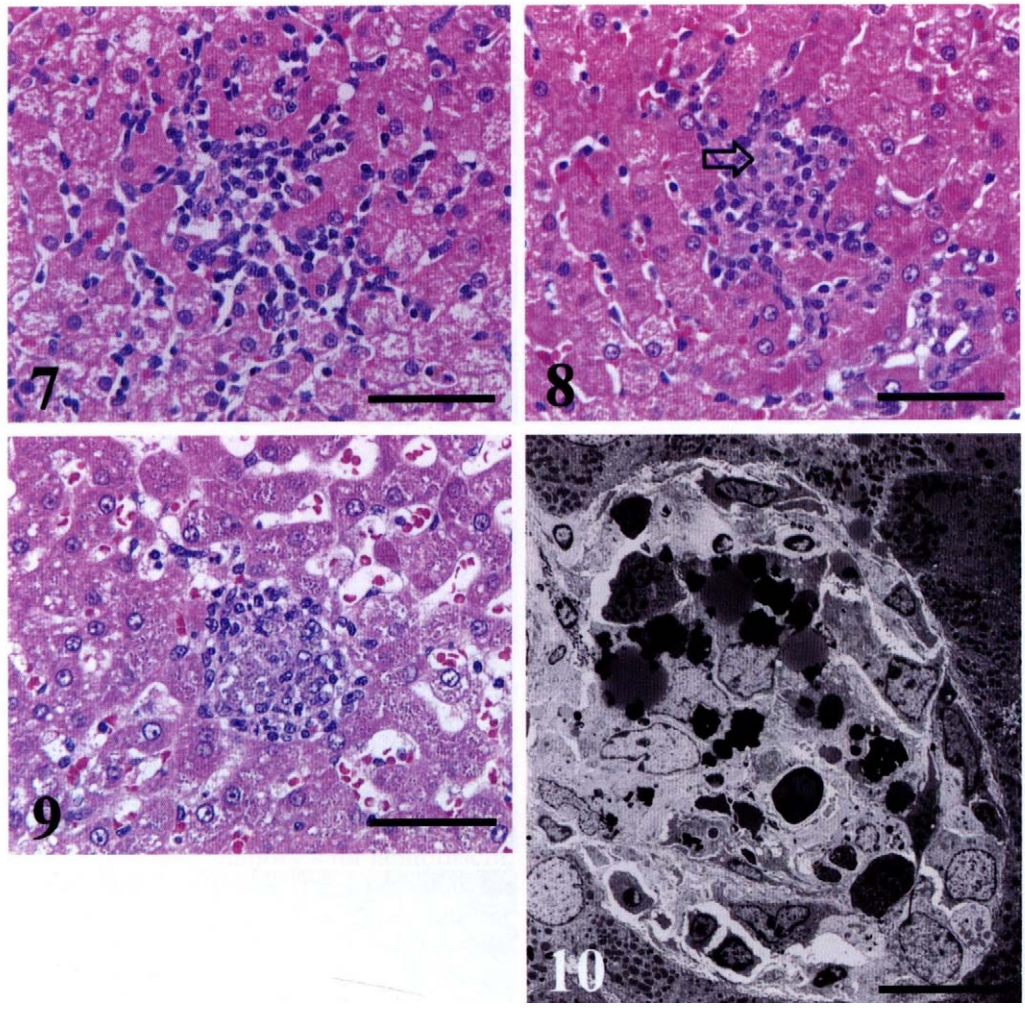

FIG. 7.—MN focus from dog given 120 mg/kg AT for 52 wk; lymphocytes in sinusoids surrounding hepatocytes. This was considered an early step in formation of microgranulomas. H&E. Bar = 100 μm.

FIG. 8.—Hepatic MN focus from dog given 120 mg/kg of AT for 52 wk. This was considered an intermediate but more chronic organized form of MN focus than the MN focus in Fig. 7. Macrophage with brown pigment (arrow) can be identified in middle of lymphocytes. H&E. Bar = 100 μm.

FIG. 9.—Hepatic microgranuloma from dog given 120 mg/kg of AT for 52 wk. Microgranuloma has pigmented mononuclear cells (macrophages) with lymphocytes and neutrophils. Lipofuscin granules can be seen in adjacent hepatocytes and within phagocytes. H&E. Bar = 100 μm.

FIG. 10.—Electron micrograph of hepatic microgranuloma from dog given 120 mg/kg of AT for 52 wk. Mononuclear phagocytes contain numerous secondary lysosomes filled with amorphous electron-dense material. Bar = 12.2 μm.

minimal necrosis of centrilobular hepatocytes. Two dogs had multifocal minimal bile duct hyperplasia. At 40 mg/kg, drug-related changes were minimal and multifocal and included bile duct hyperplasia and bile stasis. A focal microgranuloma was seen in 1 dog. MN foci were similar in appearance in controls and all dose groups but were minimally increased in number in dogs in the 40-mg/kg and 120-mg/kg groups compared with controls, particularly in those animals with microgranulomas. Lipofuscin accumulation was similar in severity in controls and dogs in the 10-mg/kg and 40-mg/kg groups.

Ultrastructurally, hepatocytes in the 120-mg/kg group contained increased membrane-bound, heterogeneous, electron-dense, irregularly shaped material (secondary lysosomes). Centrilobular hepatocytes were most affected, but midzonal and portal hepatocytes were also affected minimally. Microgranulomas consisted of spheroid collections of phagocytes packed with secondary lysosomes similar to those in hepatocytes (Fig. 10). There were also increased numbers of Kupffer cells containing secondary lysosomes.

Two males in the 120-mg/kg group had gallbladder changes; those in 1 dog sacrificed early correlated with gross findings of abnormal color and content and thick walls and were more severe that those in 1 male at 52 wk. Edema and hemorrhage of wall and/or mucosa were seen in both dogs. In addition, the more severely affected dog had transmural neutrophil infiltrate, fibrinoid necrosis and thrombosis of vessels, multifocal minute erosions, and fibrosis in gallbladder; this animal also had inflammation, granulation tissue, and ulcers in the common bile duct.

Week 64 Sacrifice (Recovery)

Of the drug-induced hepatic lesions identified in dogs sacrificed in week 52 or before, some were still present but markedly reduced in severity and distribution. In centrilobular hepatocytes, lipofuscin deposits were minimal in all groups, including controls, except in the 120-mg/kg group in which the severity was mild (Fig. 4). Microgranulomas were not identified in any dog and MN foci were similar in drug-treated and control dogs. Minimal bile stasis and/or bile duct hyperplasia were seen in the 120-mg/kg group.

Week 104 Sacrifice

Hepatic alterations in dogs treated for 104 wk were much less severe and involved less parenchyma than in dogs sacrificed after 52 wk of treatment. A focal microgranuloma similar to those seen in week 52 was seen in 1 dog. MN foci were similar in controls and drug-treated dogs. Lipofuscin deposits were similar in severity in treated and control livers (Fig. 5), but more dogs given 40 and 120 mg/kg of AT (11 of 18) were affected than controls (3 of 10). Eleven of 18 dogs given 40 and 120 mg/kg of AT had minimal cholestasis (Fig. 6). Mixed cell infiltrates around or near central veins were slightly increased in drug-treated females compared with controls. Hepatocellular ultrastructure was similar in control and dogs in the 120-mg/kg group.

Discussion

Since the liver is the primary site of cholesterol synthesis and the primary site of drug delivery (1, 3, 25), as expected the primary organ affected by AT was the liver. Microgranulomas, lipofuscinosis, cholestasis, bile duct proliferation, and hepatocellular degeneration were noted by 52 wk mainly in dogs given 120 mg/kg of AT. These changes were absent or much less pronounced in reversal animals after 12 wk without treatment and were no longer present or were similar to controls except for bile stasis by week 104 despite continuous dosing. Increases in AST, ALT, and AP levels suggestive of hepatic insult were most severe in moribund animals but were sporadic, transient, and minor during the second year of the study. Increases in ALT and AST levels have been described in dogs given HMGRIs (7, 8, 10, 15), but hepatic necrosis and degeneration were noted only with fluvastatin administration (10). Liver lesions were not reported in dogs given pravastatin for up to 104 wk (28). In our previous studies in dogs, AT given at ≥80 mg/kg for up to 13 wk induced transient increases in ALT, but histopathologic evidence of hepatocellular insult was inconsistent (31).

Hepatic changes in this study suggest that the likely chronology of the lesions induced by AT is altered hepatocellular metabolism (e.g., increased membrane turnover) with overloading of removal mechanisms and buildup of degradation products (lipofuscin) intracellularly. As lipofuscin-laden hepatocytes die, resident macrophages and/or recruited mononuclear cells phagocytize cell debris and become overloaded, in turn inducing further phagocyte activity. With time, hepatocytes returned to homeostasis despite continued treatment, and lipofuscin deposits and microgranulomas are resolved.

Granulomas are characteristic of chronic inflammation and consist of accumulations of activated monocytes or macrophages (5). They are often associated with substances that cannot be degraded by macrophage lysosomes. Hepatic granulomas are uncommon in dogs and are most often associated with infectious agents (4). In humans, granulomatous response in liver has been associated with several drugs (14). Microgranulomas were described in monkeys treated with an experimental diuretic (16), in dogs treated with a protease inhibitor (17), and in rats given a sterol compound (13). The microgranulomas in livers of dogs in this study consisted mainly of mononuclear phagocytes, many of which were pigmented, a result of incomplete degradation of senescent, lipofuscin-filled hepatocytes. Similar microgranulomas were reported in dogs given another lipid-lowering agent (16) but were not seen in dogs given 80 mg/kg of AT for up to 13 wk (31). In this study, microgranulomas were seen in dogs given ≥40 mg/kg of AT for up to 52 wk. After 12 wk without treatment and after 104 wk of continuous treatment, microgranulomas were rare. Resolution of microgranulomas is consistent with hepatic accommodation with decreased accumulation of lipofuscin and less of a load on degradation pathways.

The incidence of MN foci was assessed in this study because the number of foci per animal was minimally increased with AT treatment and dogs with microgranulomas had increased numbers of MN foci compared with dogs without microgranulomas. Because these foci were present in control dogs at all periods, they were considered to be part of the normal process of removal of senescent cells. MN foci consisted mostly of lymphocytes, but macrophages could be identified in them and they appeared to be closely associated with degenerated hepatocytes. Because MN foci were minimally increased in dogs with increased lipofuscin and microgranulomas, it is possible that they represent an early stage in microgranuloma development.

Cholestasis appears to be a class effect of HMGRIs (27) and was present at all sacrifice time points in this study, but severity was less at 104 wk than at 52 wk. Bile stasis could be a result of focal cell swelling, membrane alterations (altered membrane flow, change in permeability), or changes in bile salt or bile secretion (21). It could also be an exaggerated pharmacologic action of HMGRIs, since decreases in cholesterol can affect bile salt formation and transport as well as membrane formation and maintenance. Rats with HMGRI-induced myotoxic effects have elevated serum conjugated bile acid levels consistent with cholestasis, and both myotoxicity and serum elevations can be blocked by coadministration of mevalonate (27). Indigestible contents of secondary lysosomes are excreted in bile (11), so decreased bile excretion could result in higher levels of degradation products that would need to be cleared by macrophages and result in microgranulomas.

Although lipofuscin can be identified in centrilobular hepatocytes of control dogs, the distribution and quantity of lipofuscin in secondary lysosomes were moderately increased in dogs given 120 mg/kg of AT for up to 52 wk. Within 12 wk of dosing cessation, lipofuscin was

only minimally increased in treated dogs compared with controls. By 104 wk, lipofuscin deposits in drug-treated dogs were similar to those in controls consistent with accommodation and return to homeostasis despite continuous treatment. Lipofuscin is a lipopigment often seen in highly metabolizing cells (5, 9) and may be a result of increased elimination of abnormal or excess proteins, modulation of synthetic pathways (9), or errors in lysosomal catabolism (12). It is often called a "wear and tear" pigment associated with organelle turnover following sublethal damage (22). It stains positively with Schmorl's iron reduction stain (12) and PAS, may be acid-fast positive, and stains negatively or weakly positively for iron with Prussian blue or Turnbull's stain. Ultrastructurally, deposits are membrane-bound aggregates of electron-dense granules (secondary lysosomes) (5, 9). Hepatic lipofuscin deposition, foci of pigmented macrophages (microgranulomas), and transient ALT increases consistent with mild, reversible cellular injury have been described in rats and dogs treated with a histamine H_1-receptor antagonist (22). Although the pathogenesis of lipofuscin deposition in this study is not known, considering hepatic metabolism, pharmacologic effect of AT on the liver, and the extensive decrease in cholesterol, it is likely that the basal maintenance and turnover of membranes were affected, with a resulting increased accumulation of degradation products.

Unlike L-645,164, another HMGRI, which induced epithelial hyperplasia in gallbladder (7) and AT at ≥280 mg/kg (31), AT at 120 mg/kg induced only cholecystitis in this study, although hepatic bile duct proliferation was seen at ≥40 mg/kg. Increases in AP consistent with bile duct alterations were sporadic, seen only at 120 mg/kg, and were previously seen at doses of >150 mg/kg of AT (31). These changes could be due to relatively increased concentrations of AT in bile and gallbladder, since AT is excreted primarily via bile. It is also possible that low cholesterol levels affected bile composition, since cholesterol is the immediate precursor to bile acids (20, 25). Hyperplasia and/or inflammation of gallbladder have been described in dogs given other HMGRIs (2, 7) and have been suggested to be related to chemical irritation secondary to biliary excretion (10).

AT was very effective in lowering serum cholesterol levels in dogs. In beagle dogs given AT for up to 2 yr, the occurrence, severity, and distribution of hepatic lesions were dose related. Dose-limiting toxic effects were seen at doses of 120 mg/kg at which decreases in cholesterol and lipoproteins were marked. Transient increases in ALT levels suggestive of hepatic insult and histopathologic changes were seen at doses of ≥40 mg/kg. Hepatic toxic effects were dose dependent, sporadic in occurrence (not all animals in a group affected or severity greatly different between members of a group), and reversible with or without continued drug administration. Histopathologic hepatic changes seen after 52 wk of treatment with AT were no longer present or were minimal after 12 wk without dosing and did not progress or completely resolved with another 52 wk of treatment.

REFERENCES

1. Alberts AW, MacDonald JS, Till AE, and Tobert JA (1989). Lovastatin. *Cardiovasc. Drug Rev.* 7: 89–109.
2. Berry PH, MacDonald JS, Alberts AW, Molon-Noblot S, Chen JS, Lo CYL, Greenspan MD, Allen H, Durand-Cavagna G, Jensen R, Bailly Y, Delort P, and Duprat P (1988). Brain and optic system pathology in hypocholesterolemic dogs treated with a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Am. J. Pathol.* 132: 427–443.
3. Bocan TMA, Ferguson E, McNally W, Uhlendorf PD, Mueller SB, Dehart P, Sliskovic DR, Roth BD, Krause BR, and Newton RS (1992). Hepatic and nonhepatic sterol synthesis and tissue distribution following administration of a liver selective HMG-CoA reductase inhibitor, CI-981: Comparison with selected HMG-CoA reductase inhibitors. *Biochim. Biophys. Acta* 1123: 133–144.
4. Chapman BL, Hendrick MJ, and Washabau RJ (1993). Granulomatous hepatitis in dogs: Nine cases (1987–1990). *J. Am. Vet. Med. Assoc.* 203: 680–684.
5. Cheville NF (1983). *Cell Pathology,* 2nd ed. Iowa State University Press, Ames, pp. 155–158, 278.
6. Dostal L, Juneau P, and Rothwell CE. Semen analysis in a 2-year study with HMG-coA reductase inhibitor, atorvastatin, in beagle dogs. Manuscript in preparation.
7. Gerson RJ, Allen HL, Lankas GR, MacDonald JS, Alberts AW, and Bokelman DL (1991). The toxicity of a fluorinated-biphenyl HMG-CoA reductase inhibitor in beagle dogs. *Fundam. Appl. Toxicol.* 16: 320–329.
8. Gerson RJ, MacDonald JS, Alberts AW, Kornbrust DJ, Majka JA, Stubbs RJ, and Bokelman DL. (1989). Animal safety and toxicology of simvastatin and related hydroxymethylglutaryl-coenzyme A reductase inhibitors. *Am. J. Med.* 87: 28S–38S.
9. Harman D (1990). Lipofuscin and ceroid formation: The cellular recycling system. *Adv. Exp. Med. Biol.* 266: 3–15.
10. Hartman HA, Myers LA, Evans M, Robison RL, Engstrom RG, and Tse RLS (1996). The safety evaluation of fluvastatin, an HMG-coA reductase inhibitor, in beagle dogs and rhesus monkeys. *Fundam. Appl. Toxicol.* 29: 48–62.
11. Havel RJ (1992). Role of the liver in hyperlipidemia. *Semin. Liver Dis.* 12: 356–363.
12. Jolly RD and Dalefield RR (1990). Lipopigments in veterinary pathology: Pathogenesis and terminology. *Adv. Exp. Med. Biol.* 266: 157–167.
13. Jones TW, Fisher LF, Meador VP, Perry DN, and Eacho PI (1996). Sterol-induced hepatic microgranulomas. *Fundam. Appl. Toxicol.* 30(1): 102 (abstract 522).
14. Lee WL (1995). Drug-induced hepatotoxicity. *N. Engl. J. Med.* 333: 1118–1127.
15. MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, Alberts AW, and Bokelman DL (1988). Preclinical evaluation of lovastatin. *Am. J. Cardiol.* 62: 16J–27J.
16. Mesfin GM, Higgins MJ, Thornburgh BA, Jones BW, Van de Giessen TL, and Sinha AJ (1992). Drug-induced hepatic microgranulomatosis in cynomolgus monkeys. *Toxicol. Pathol.* 20: 7–17.
17. Nakano S, Yada H, Irimura K, Asanoma H, Hirota T, and Terazawa K (1992). A 52-week oral toxicity study of 6-amidino-2-naphthyl-4-[(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethanesulfonate (FUT-187) in dogs. *J. Toxicol. Sci.* 17: P163-P199.
18. National Institutes of Health (1985). Animal Welfare Act, 1966, amended in 1970, 1976, and 1985, 9 CFR Parts 1, 2, and 3. Publication 86–23.
19. Nawrocki JW, Weiss SR, Davidson MH, Sprecher DL, Schwartz SL, Lupien PJ, Jones PH, Haber HE, and Black DM (1995). Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. *Arterioscler. Thromb. Vasc. Biol.* 15: 678–682.
20. Okolicsanyi L, Lirussi F, Strazzabosco M, Jemmolo RM, Orlando R, Nassuato G, Muraca M, and Grepaldi G (1986). The effect of drugs on bile flow and composition: An overview. *Drugs* 31: 430–438.

21. Philips MJ, Poucell S, and Oda M (1986). Mechanisms of cholestasis. *Lab. Invest.* 54: 593–608.
22. Poole A, Betton GR, Salmon G, Sutton T, and Atterwill CK (1990). Comparative toxicology of temalastine. *Fundam. Appl. Toxicol.* 14: 71–83.
23. Robertson DG, Urda ER, Rothwell CE, and Walsh KM (1997). Atorvastatin is not cataractogenic in beagle dogs. *Curr. Eye Res.* 16: 1229–1235.
24. Sigler RE, Dominick MA, and McGuire EJ (1992). Subacute toxicity of a halogenated pyrrole hydroxymethylglutaryl-coenzyme A reductase inhibitor in Wistar rats. *Toxicol. Pathol.* 20: 595–602.
25. Sliskovic DR, Roth BD, and Bocan TMA (1992). Tissue selectivity of HMG-CoA reductase inhibitors. *Drug News Perspect.* 5: 517–533.
26. Smellie WAS and Lorimer AR (1992). Adverse effects of the lipid-lowering drugs. *Adverse Drug React. Toxicol. Rev.* 11: 71–92.
27. Smith PF, Eydelloth RS, Grossman SJ, Stubbs RJ, Schwartz MS, Germershausen JI, Vyas KP, Kari PH, and MacDonald JS (1991). HMG-CoA reductase inhibitor-induced myopathy in the rat: Cyclosporine A interaction and mechanism studies. *J. Pharmacol. Exp. Ther.* 257: 1225–1235.
28. Tarumi C, Matsunumo N, Miyakoshi N, Yamashita K, and Masuda H (1989). Long term oral administration study of pravastatin sodium to beagles for 104 weeks. *J. Toxicol. Sci.* 14: 85–101.
29. Tobert JA (1987). New developments in lipid-lowering therapy: The role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. *Circulation* 76: 534–538.
30. U.S. Department of Health and Human Services (1985). *Guide for the Care and Use of Laboratory Animals.* Animal Resources Program, Division of Research Resources, National Institutes of Health, Bethesda, Maryland.
31. Walsh KM, Albassam M, and Clarke DE (1996). Subchronic toxicity of atorvastatin, a hydroxymethylglutaryl-Coenzyme A reductase inhibitor, in beagle dogs. *Toxicol. Pathol.* 24: 468–476.
32. Willingham MC (1989). A role for intracellular traffic of HDL in cholesterol metabolism. *Lab. Invest.* 61: 253–254.