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Subchronic Toxicity of Atorvastatin, a Hydroxymethylglutaryl–Coenzyme A Reductase Inhibitor, in Beagle Dogs*

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ABSTRACT

The toxicity of atorvastatin (AT), an inhibitor of hydroxymethylglutaryl–coenzyme A reductase (HMG), was evaluated in beagle dogs. In 4 studies [2-wk rising dose (daily increasing doses for 1 wk; maintenance for 1 wk), 12-wk rising dose (daily dosing with weekly increases in dose), 2-wk toxicity (daily dosing for 2 wk; 3 dose levels), 13-wk toxicity (daily dosing for 13 wk; 3 dose levels)], dogs received up to 400 mg/kg orally. Doses of 180 mg/kg induced moribundity, necessitating euthanasia. Weight losses up to 26% were seen at doses ≥150 mg/kg. Decreases in cholesterol levels were dose-related. Alanine and/or aspartate aminotransferase were increased at doses ≥80 mg/kg; alkaline phosphatase was increased at doses ≥150 mg/kg. Histopathologic findings were seen at ≥150 mg/kg and included hepatocellular eosinophilia related to increased smooth endoplasmic reticulum and cholangiohepatitis and cholecystitis at 150 mg/kg in the 2-wk toxicity study; hepatocellular degeneration, centrilobular bridging, cholecystitis, hemorrhage in gallbladder and brain, demyelination of optic nerve, and skeletal muscle necrosis at ≥280 mg/kg in the 12-wk rising dose study; and erosion and hemorrhage in large intestine, hepatocellular degeneration and necrosis, and inflammation and necrosis of gallbladder epithelium at 320 mg/kg in the 2-wk rising dose study. Doses up to 80 mg/kg for 13 wk did not induce histopathologic lesions in examined organs. AT effectively lowered serum cholesterol in normal lipidemic dogs. Toxicity of AT in dogs was similar to that with other inhibitors of HMG except that lenticular changes were not seen, significant hepatic, testicular, or neurological toxicity was associated only with high doses of AT, and skeletal muscle changes similar to those described in rats and rabbits were identified.

Keywords. Lipid regulation; liver; skeletal muscle; brain; cholesterol

INTRODUCTION

High levels of serum cholesterol have long been considered a significant risk factor in atherosclerosis and coronary artery disease. Endogenous biosynthesis in liver and intestine is the major source of serum cholesterol, and conversion of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) to L-mevalonic acid, catalyzed by HMG-CoA reductase, is the rate-limiting step in the cholesterol pathway. Atorvastatin (AT), a highly substituted pyrrole, is an inhibitor of HMG-CoA reductase (HMGRI) that has decreased low-density lipoprotein (LDL) cholesterol in humans by 60% at doses of 80 mg/day (13). In addition to decreasing overall serum cholesterol levels in dogs and rats, HMGRIs prevent the buildup of toxic sterol intermediates seen with inhibitors that act later in the synthetic cascade (21). AT has been shown to upregulate receptors for LDLs with a subsequent decrease in serum LDLs (3).

HMGRIs have been reported to cause lenticular opacities, sporadic increases in alanine and/or aspartate aminotransferase, degeneration of vascular endothelium in brain, and hyperplasia of gallbladder epithelium in dogs (2, 6). We report here the findings from 4 subchronic studies in dogs with AT.

MATERIALS AND METHODS

Animals. Four studies, 2 oral rising dose studies [2-wk (ORD) and 12-wk (12W)] and 2 toxicity studies [2-wk (2W) and 13-wk (13W)], have been completed with AT in dogs. All studies complied with U.S. Food and Drug Administration Good Laboratory Practice regulations and with National Institutes of Health and Animal Welfare

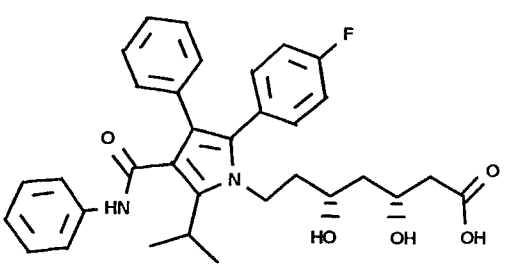

FIG. 1.—Chemical structure of AT.

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TABLE I.—Experimental design.

Study	Number/sex		Dose (mg/kg)										
ORD	1	Day:	1	2	3	4	5	6	7–13	14			
		Dose:	10	20	40	80	150	200	300	400			
12W	2	Week:	1–2	3	4	5	6	7	8	9	10	11	12
		Dose:	80	100	120	140	160	180	200	220	240	280	320
2 W	2/dose		0, 20, 80, 150 (daily for 2 wk)										
13W	3/dose		0, 10, 40, 80 (160 for first 2 wk) (daily for 13 wk)										

Act guidelines for animal welfare. All studies used beagle dogs from Marshall Farms USA (North Rose, NY). Dogs were housed separately in stainless-steel cages, provided water *ad libitum,* and fed 250–300 g of Purina Certified Canine Diet 5007® daily. Dogs were fasted overnight prior to collection of blood samples.

Compound and Dosing. Chemically, AT is designated as 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid (Fig. 1). Compound was administered daily as bulk drug in gelatin capsules. Table I summarizes the experimental dose design of the studies. In ORD, 2 dogs (1/sex) were dosed at 10 mg/kg on day 1; doses increased daily to 300 mg/kg on day 7 and were maintained at 300 mg/kg until day 14, when they were increased to 400 mg/kg. In 12W, 4 dogs (2/sex) were dosed at 80 mg/kg/day for weeks 1 and 2. Doses were increased in weekly increments of 20 mg/kg to reach 240 mg/kg/day in week 10; in week 11, dogs were given 280 mg/kg/day, and in week 12 320 mg/kg/day. Four dogs (2/sex) were untreated and served as controls. In 2W, 2 dogs/sex/dose were given 0, 20, 80, or 150 mg/kg/day. In 13W, 3 dogs/sex/dose were given 0, 10, 40, or 160 mg/kg/day for 2 wk and, because of clinical intolerance at the high dose, dogs were given 0, 10, 40, or 80 mg/kg/day for the remaining 11 wk.

Clinical Observations. Animals were observed daily for signs of toxicity or systemic effects. Food consumption was assessed daily. Dogs were weighed daily (ORD) or weekly (12W, 2W, and 13W). Clinical and ophthalmic examinations, blood pressure measurements, and electrocardiograms were done at protocol-specified intervals (Table II).

Clinical Laboratory Analyses. Blood was collected from each dog pretest and at protocol-specified periods (Table II). Clinical pathology parameters evaluated from blood or serum included red blood cell counts and indices (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin), platelet count, white blood cell (WBC) count (total and differential), prothrombin and activated partial thromboplastin times, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, calcium, phosphorus, alanine and aspartate aminotransferase (ALT and AST), bilirubin, alkaline phosphatase (AP), glucose, cholesterol, high-density lipoproteins (HDLs), LDLs, triglycerides, total protein, albumin, globulin, α-hydroxybutyrate dehydrogenase (HBD), lactate dehydrogenase, creatine phosphokinase (CPK), ornithine carbamyltransferase, gamma-glutamyl transpeptidase, and α-amylase. Urinalysis included specific gravity, pH, protein, bilirubin, glucose, occult blood, ketones, and microscopic evaluation. Bone marrow was taken from all dogs at termination and evaluated by light microscopy or flow cytometry.

Lenses from the right eyes of dogs in 2W and 13W were analyzed for protein, glucose, potassium, sodium, reduced and oxidized glutathione, phosphofructokinase, glucose-6-phosphodehydrogenase (G6PD), AMP, ADP, and ATP. Liver samples from dogs in 12W were analyzed for protein and cytochrome P-450 content and activity of aniline hydroxylase, aminopyrine *N*-demethylase, UDP–glucuronyl transferase, cytochrome *c*-reductase, catalase, carnitine acetyltransferase, and peroxisomal β-oxidation.

Pathology. Dogs were euthanized with iv overdose of short-acting barbiturate and exsanguination. At necropsy, brain, pituitary, heart, liver, spleen, kidneys, adrenals, prostate, and gonads were weighed; in some studies, thyroids, mandibular salivary glands, thymus, lung, uterus, and epididymides were also weighed. Organ-to-body and organ-to-brain weight ratios were calculated. Complete necropsies were done on all dogs. Samples of the following were fixed in 10% buffered formalin (except as noted), embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and examined microscopically: brain, spinal cord, sciatic nerve, pituitary, thyroid, parathyroid, adrenal, pancreas, liver, gallbladder, tongue, parotid and mandibular salivary glands, esophagus, stomach, small and large intestines, trachea, lung, heart, aorta, lymph nodes (mesenteric, tracheobronchial, and prescapular), tonsil, spleen, thymus, kidney, urinary bladder, gonads, uterus, vagina, testis and epididymis (the latter 2 were fixed in Bouin's or Zenker's fixative), prostate, bone, bone marrow, skin, mammary gland, eye

TABLE II.—Protocol-specified intervals for clinical parameters.

	Oral rising dose	12-Wk rising dose	2-Wk toxicity	13-Wk toxicity
Clinical exam	Pretest, day 14	Pretest, week 12	Pretest, day 14	Pretest, monthly
Ophthalmic exam	Pretest, day 14	Pretest, weekly	Pretest, day 14	Pretest, monthly
Electrocardiography/blood pressure	Pretest, days 7, 14	Not done	Pretest, weekly	Pretest, weeks 1, 4, 8, 13
Clinical chemistry/hematology	Pretest, days 7, 14	Week 2, weekly thereafter	Pretest, days 6, 14	Pretest, weeks 1, 4, 8, 13

TABLE III.—Sampling times for pharmacokinetics.

Study	Hours postdosing	Day or week of study
ORD	0, 3	Days 1, 7, 14
12-Wk ORD	0, 1, 2, 4, 6, 8, 24	Weeks 1, 2, 4–12
2-Wk	0, 0.5, 1, 2, 3, 4, 6, 10, 24	Days 1, 14
13-Wk	0, 2	Weeks 1, 4, 8, 13

(fixed in 6% glutaraldehyde or Zenker's fixative), skeletal muscle, and gross lesions. At terminal sacrifice, dogs in 12W were perfused with 4% formaldehyde/1% glutaraldehyde in phosphate buffer to optimize microscopic evaluation of brain and optic nerve. All tissues were examined by light microscopy; selected samples of liver, brain, and optic nerve were examined by electron microscopy.

Plasma AT Determinations. Plasma samples were evaluated for AT concentrations at protocol-specified times (Table III). Plasma concentrations of AT were measured using a validated radioimmunoassay procedure with a lower quantitation limit of 0.350 ng eq/ml dog plasma.

Statistical Analyses. When applicable, group differences were tested in pairwise comparison to controls by the 2-tailed Student's *t*-test. A probability level of 0.99 was used for the criterion of significance. Bone marrow data were tested by analysis of variance (ANOVA) in a sequential monotonic trend test. Results of lens analyses were compared in 1- and 2-factor ANOVAs with a 1% significance level and a test of trend reversal applied at the 2-tailed 0.2% level. All operations were performed on a digital computer.

RESULTS

The following includes only drug-related results; if not reported, results were not different from concurrent or historical controls or were not considered drug-related. Changes were dose-related in severity and/or frequency.

Clinical Signs

Abnormal stools (diarrhea, soft feces, mucoid feces) were seen at ≥40 mg/kg in 13W, at ≥80 mg/kg in 2W, at ≥100 mg/kg in 12W, and at 300 and 400 mg/kg in ORD; melena was generally seen at higher doses (300 mg/kg in ORD, 150 mg/kg in 2W, 160 mg/kg in 13W, and 100–320 mg/kg in 12W). White material identified as compound was noted sporadically in feces of animals given ≥100 mg/kg in 12W and 160 mg/kg in 13W. Weight loss up to 26% of pretest weight occurred at doses of 150 mg/kg and above. Because of a 16% weight loss and bloody diarrhea in dogs given 160 mg/kg during the first 2 wk of 13W, the high dose was decreased to 80 mg/kg/day in that study. Emesis was sporadic at ≥120 mg/kg in 12W, at 160 mg/kg in 13W, and at 300 and 400 mg/kg in ORD. At 320 mg/kg in 12W, dogs had pain upon opening their mouths.

TABLE V.—Selected lenticular parameters from dogs given AT for 13 wk—sexes combined.

Dose (mg/kg)	0	10	40	80
Protein (mg/g wet weight)	512	487	449*	460*
K (μM/g wet weight)	73	71	65*	63*
Glucose (μM/g wet weight)	4.2	3.8	3.5*	3.5*
G6PD (U/g wet weight)	0.62	0.75	0.74	0.58

* Statistically significant difference from control ($p < 0.01$).

Moribund Sacrifice

In 12W, 1 male was sacrificed in week 8 (dosed up to 180 mg/kg) and 1 female in week 11 (280 mg/kg) because animals were hypoactive, dehydrated, prostrate, and hypothermic. Remaining animals in that study were sacrificed in week 12 due to moribundity. There were no early deaths in the other 3 studies.

Hematology

Increases in peripheral leukocyte counts in 1 animal at 80 mg/kg in 13W (transient increase at week 8), in 1 male with cholecystitis at 150 mg/kg in 2W, in 1 male and both females at 280 mg/kg in 12W, and in 1 female with cholecystitis and hepatitis at 300 mg/kg in ORD ranged from 15,000 to 28,000 WBC/μl due to neutrophilia. Increased terminal marrow M:E ratios (2.5:1 to 6:1) in 4 treated animals in 12W and 1 male with cholecystitis in 2W were associated with moderate decreases in erythroid maturation series and minimal increases in myeloid series. Peripheral red blood cells, hemoglobin, and hematocrit were increased ~25 to 45% from pretest in treated dogs in ORD and 12W probably due to dehydration.

Clinical Pathology

Table IV contains results of selected clinical chemistry parameters. As pharmacologically expected, decreases in

TABLE IV.—Serum levels (±SD) of selected parameters in dogs from 13-wk toxicity study with AT.

Dose	HDL (mg/dl)		LDL (mg/dl)		ALT (IU/L)		AST (IU/L)		AP (IU/L)	
(mg/kg)	Pretest	Week 13	Pretest	Week 13	Pretest	Week 13	Pretest	Week 13	Pretest	Week 13
Males (n = 3/dose)										
0	131 ± 5	116 ± 3	21 ± 3	20 ± 1	24 ± 2	22 ± 2	23 ± 3	17 ± 0	37 ± 9	26 ± 5
10	122 ± 17	86 ± 11	16 ± 2	14 ± 3	25 ± 3	24 ± 2	28 ± 3	20 ± 1	54 ± 2	39 ± 2
40	146 ± 6	83 ± 6*	17 ± 1	10 ± 3*	21 ± 2	21 ± 2	19 ± 3	18 ± 1	42 ± 8	39 ± 4
80	131 ± 13	75 ± 6*	15 ± 2	9 ± 1*	22 ± 2	20 ± 1	20 ± 1	16 ± 1	32 ± 3	32 ± 4
Females (n = 3/dose)										
0	120 ± 4	122 ± 6	24 ± 2	24 ± 1	20 ± 2	25 ± 4	19 ± 2	18 ± 3	43 ± 11	39 ± 14
10	168 ± 23	135 ± 17	23 ± 2	17 ± 3	14 ± 6	16 ± 7	15 ± 2	17 ± 1	44 ± 10	53 ± 14
40	162 ± 33	95 ± 18	24 ± 2	11 ± 1*	18 ± 4	23 ± 3	19 ± 3	20 ± 1	47 ± 7	45 ± 10
80	143 ± 28	52 ± 2*	25 ± 6	10 ± 2*	17 ± 2	27 ± 3	20 ± 1	19 ± 1	51 ± 18	72 ± 18

* Statistically significant difference from control ($p < 0.01$).

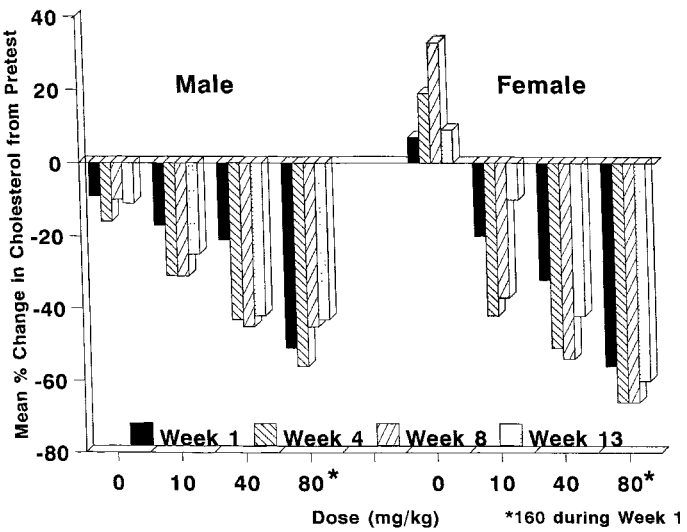

FIG. 2.—Mean percentage of change from pretest values in cholesterol in dogs treated for 13 wk with AT. N = 3/sex/dose.

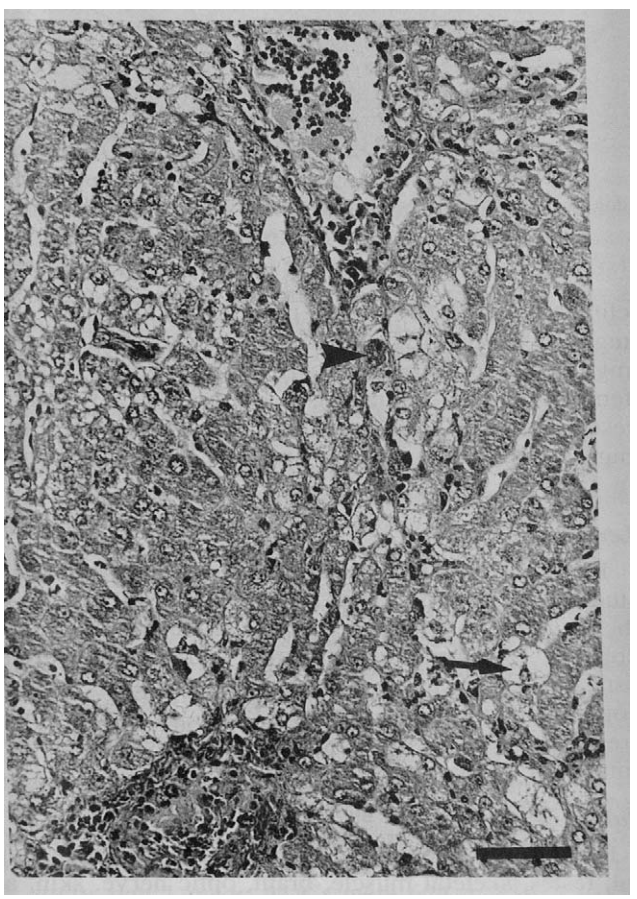

FIG. 3.—Photomicrograph of liver from a female given up to 280 mg/kg AT. There are hepatocellular degeneration (arrowhead) and vacuolation (arrow), inflammatory cell infiltrates, and centrilobular bridging. H&E. Bar = 50 μm.

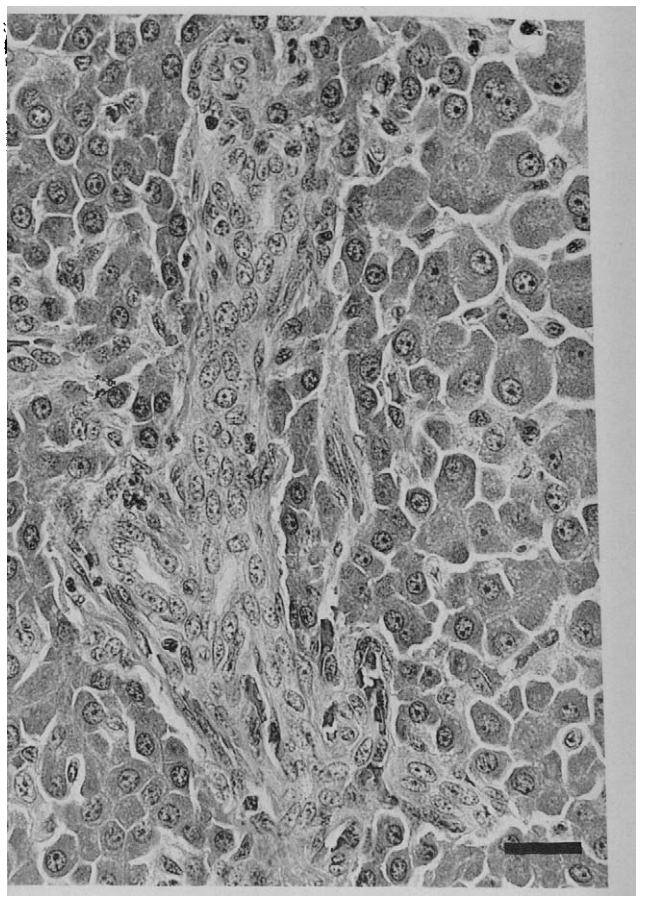

FIG. 4.—Photomicrograph of bile duct hyperplasia in male dog given up to 320 mg/kg AT. Bar = 25 μm.

cholesterol were dose-dependent. Cholesterol progressively decreased to less than 33% of pretest in ORD and to less than 16% of pretest levels at doses up to 220 mg/kg of AT in 12W (334 mg/dl pretest to 47 mg/dl at 220 mg/kg). In 13W, cholesterol levels decreased with increasing dose and length of treatment until week 4 or 8 when they slightly reversed by week 13 (Fig. 2). By week 13 in 13W, cholesterol levels were approximately 25, 40, and 43% less than pretest values in males and 10, 40, and 60% less than pretest in females at 10, 40, and 80 mg/kg, respectively. HDL and LDL decreases were also dose-dependent (Table IV). Triglycerides were decreased from pretest levels in dogs in ORD (64 mg/dl pretest to 26 mg/dl at week 2) and in males (pretest: 38, 48, 50, and 39 mg/dl; week 13: 37, 35, 19, and 26 mg/dl; at 0, 20, 80, and 150 mg/kg, respectively) but not in females in 13W; decreases were significant only at 40 mg/kg in 13W.

ALT and/or AST were increased in all studies. In ORD, increase in ALT was 2–3 times pretest (28 and 56 IU/L) by termination on day 14 (56 and 92 IU/L). In 12W, ALT increased 4–6 times pretest values from week 2 (80 mg/kg) to termination, with a 60-fold increase in 1 female (28 IU/L pretest to 1,784 IU/L at termination) prior to termination in week 11 (280 mg/kg). In 2W, ALT was increased 5-fold over pretest (24 IU/L) in males giv

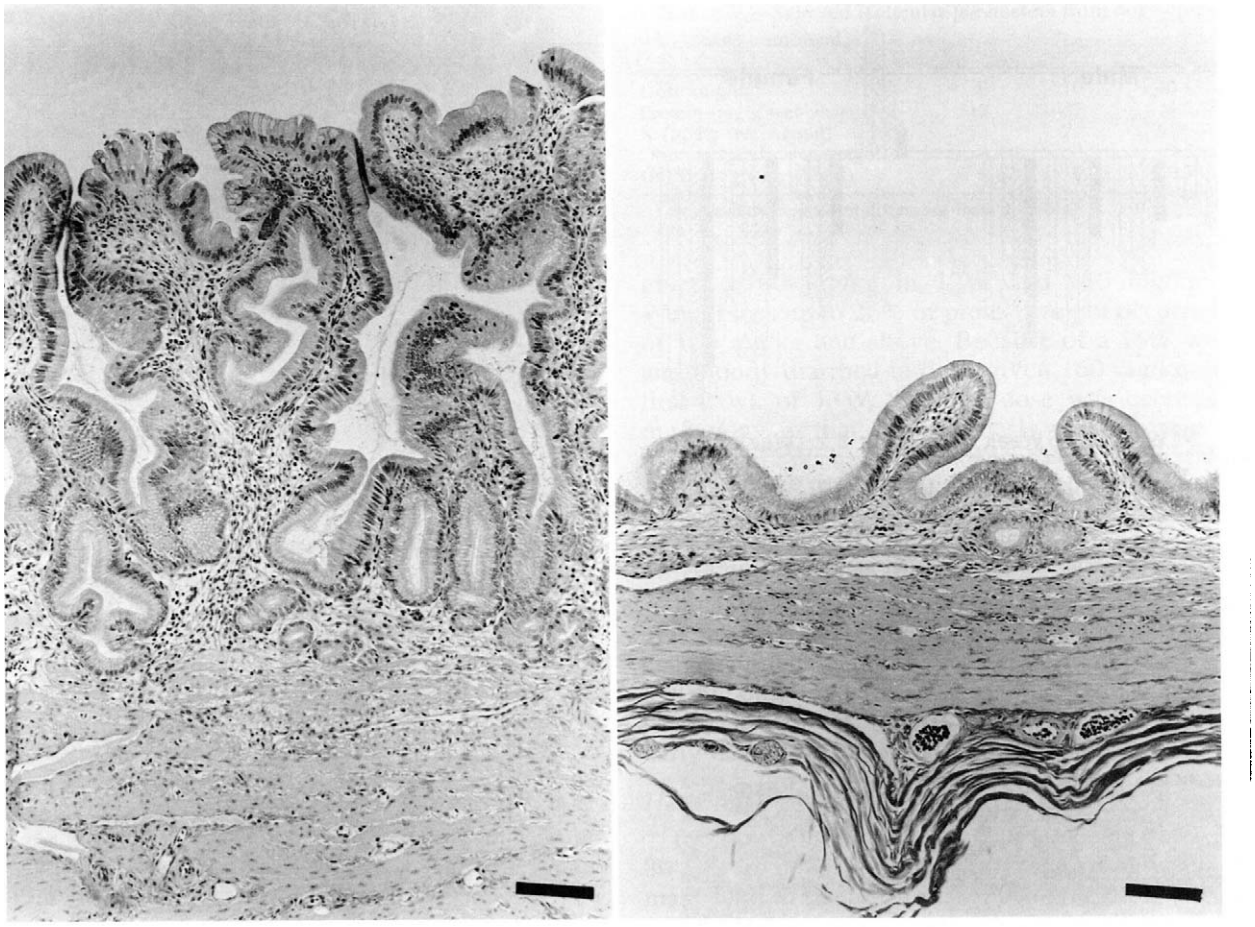

FIG. 5.—A) Photomicrograph of hyperplastic mucosa of gallbladder from dog treated with AT. B) Photomicrograph of normal gallbladder muc from control dog. H&E. Bar = 112 μm.

en 150 mg/kg on days 6 (124 IU/L) and 14 (127 IU/L); ALT did not increase in females. In 13W, 2–4-fold increases in ALT in dogs given 80 mg/kg were transient, occurring during week 1 (89 IU/L vs 17 IU/L pretest) or 4 (45 IU/L vs 21 IU/L pretest): by study termination at 13 wk, ALT was not significantly different from controls (Table IV). In 12W, AST was increased up to 10 times pretest at doses ≥240 mg/kg (up to 325 IU/L vs 31 IU/L pretest); in animals sacrificed moribund at 280 mg/kg, AST reached 76-fold higher than pretest values (34 IU/L pretest to 2,596 IU/L). In 2W, AST was increased 3-fold over pretest values (24 IU/L) in males given 150 mg/kg (80 IU/L) and, in 13W, a 2.5-fold increase was seen only in week 1 in 160-mg/kg females (20 IU/L pretest to 49 IU/L at week 1).

Two- to 4-fold increases in AP were seen in 1 male (170 IU/L pretest to 716 IU/L) and 1 female (223 IU/L pretest to 1,035 IU/L) in 12W at 280 mg/kg and 1 male (34 IU/L pretest to 83 IU/L) at 150 mg/kg in 2W; in the 150-mg/kg male, this increase correlated with cholangiohepatitis and cholecystitis seen histopathologically. Decreases in albumin, globulin, total protein (up to 74% of pretest values), glucose, sodium, and/or chloride were seen at 150 mg/kg in 2W and/or 300 mg/kg in ORD. In 2W, these changes were related to emesis and diarrhea clinically. CPK (274 IU/L pretest to 4112 IU/L at 3 mg/kg AT) and HBD (25 IU/L pretest to 168 IU/L at 2 mg/kg AT) increased up to 15-fold in 3 dogs (1 male females) at 240–320 mg/kg in 12W. BUN (5.3 mg/dl p test to 38 mg/dl) and creatinine (0.8 mg/dl pretest to mg/dl) were increased (2–7 times) in 1 male at 180 mg/ in 12W.

Lens Biochemistry

Results of evaluation of lenses from 2W and 1 studies will be reported in full elsewhere. Briefly, in 2 there were no differences between treated and cont dogs. In 13W, protein, potassium, and glucose were s tistically significantly decreased in 40- and 80-mg/ dogs compared to the control (Table V). G6PD was creased at 10 and 40 mg/kg and decreased at 80 mg/ compared to the control.

Pathology

Lesions identified in liver, gallbladder, intestine, ad nal, testes, skeletal muscle, brain, optic nerve, skin, a pancreas were related to dose. Most severe and extens changes were seen in 12W. In 13W, with doses up to mg/kg, there were no drug-related histopathological fi ings.

In the 2W study, lesions related to drug were identified in dogs given 150 mg/kg only. Increased eosinophilia of periportal hepatocytes was seen in all 4 dogs and cholangiohepatitis and cholecystitis with epithelial hyperplasia were present in 1 dog. Hepatocellular eosinophilia was related to increased smooth endoplasmic reticulum (SER) and decreased glycogen identified by electron microscopic evaluation in livers from 80- and 150-mg/kg dogs.

In ORD, in which doses reached 400 mg/kg, minimal multifocal mucosal erosions in large intestine were accompanied by congestion, hemorrhage, and neutrophilic infiltrates. Centrilobular hepatocytes were degenerate and/or necrotic. Gallbladder changes included submucosal edema and mixed inflammatory cell infiltrate, necrosis of epithelium, and arteriolar medial necrosis.

In 12W, liver lesions correlated with elevations in hepatic enzymes in dogs given ≥280 mg/kg and included hepatocellular degeneration (Fig. 3) and/or atrophy, bridging of centrilobular areas by hepatocellular degeneration and vacuolation (Fig. 3), and bile duct hyperplasia (Fig. 4). Gallbladder changes included hemorrhage, serosal edema, epithelial hyperplasia (Fig. 5), and mixed inflammatory infiltrate in the mucosa. In 2 dogs, multifocal necrosis and regeneration of skeletal (tongue) muscle (Fig. 6) correlated with increases in AST and CPK and clinical signs of pain. In a female euthanatized at 280 mg/kg, hemorrhage, necrosis, and neutrophil infiltrates in the neuropil of cerebral cortex, amygdala, caudate nuclei, putamen, and accumbens nuclei were associated with vascular fibrinoid necrosis and perivascular hemorrhage (Fig. 7). Optic nerves in this dog had multiple vacuoles as a result of focal demyelination (Fig. 8). Bone marrow was hypocellular in 3 of 4 dogs and populated mainly with mature leukocytes; this correlated with decreased erythroid series and increased myeloid series in bone marrow. Decreased fine vacuolation (lipid) of adrenal zona fasciculata cells and multifocal severe vacuolation of zona reticularis cells were seen in all dogs. Erythrophagocytosis was common in medullary sinuses of lymph nodes. Degeneration of seminiferous tubules, atrophy of dermal sebaceous glands and pancreatic acinar cells, congestion and hyalinization of enteric mucosa with or without epithelial erosion, and fibrin thrombosis of small vessels in gallbladder, lymph node, or intestine were seen sporadically in some dogs.

Plasma Drug Concentrations

There was extensive inter- and intraanimal variability in all studies. In ORD, plasma samples taken 3 hr postdosing on days 1, 7, and 14 (corresponding to doses of 10, 300, and 400 mg/kg) had drug concentrations up to 5,860, 29,620, and 5,067 ng eq/ml, respectively. Mean AUC levels in 12W were not proportional to dose and varied from 3,510 ng eq·hr/ml (range 1,266–6,373 ng eq·hr/ml) at 80 mg/kg, to 6,428 ng eq·hr/ml (range 5,277–7,577 ng eq·hr/ml) at 160 mg/kg, to 25,549 ng eq·hr/ml (range 18,950–34,919 ng eq·hr/ml) at 240 mg/kg. In the 2W study, day 1 mean AUCs were 624 ± 353, 7,157 ± 6,440, and 29,956 ± 22,581 ng eq·hr/ml and day 14 mean AUCs were 510 ± 333, 1,500 ± 544, and 9,790 ± 2,529 ng eq·hr/ml for 20, 80, and 150 mg/kg, respectively. In the 13W study, mean plasma drug levels 2 hr postdosing in week 4 were 61 ± 46, 93 ± 61, and 436 ± 243 ng eq/ml and in week 13 were 18 ± 14, 79 ± 36, and 111 ± 80 ng eq/ml at 10, 40, and 80 mg/kg.

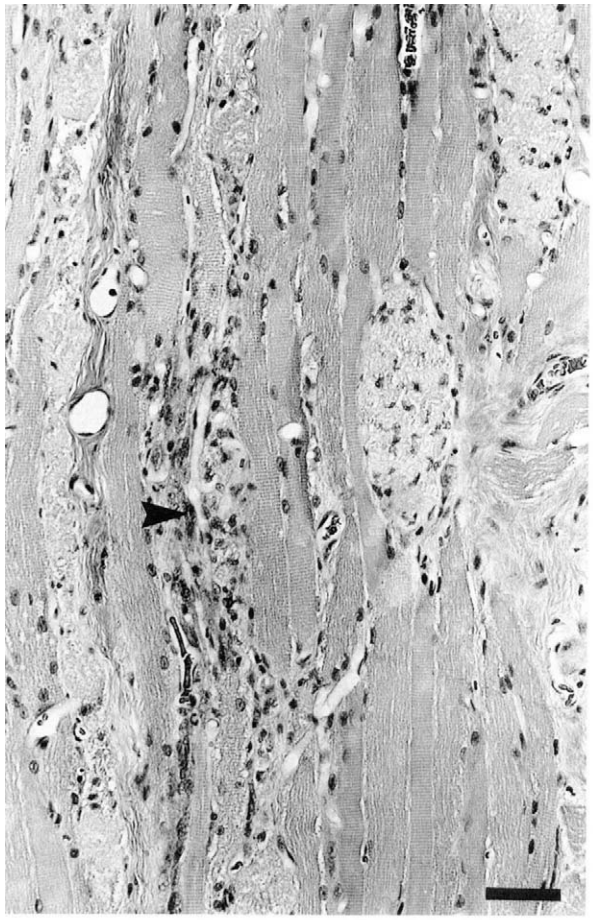

FIG. 6.—Photomicrograph of glossal skeletal muscle from dog given up to 280 mg/kg AT. Prominent myonecrosis and regeneration (arrowhead) are accompanied by infiltration of mixed inflammatory cells. H&E. Bar = 56 μm.

DISCUSSION

In dogs, AT effectively lowered serum cholesterol at all doses in a dose-related manner. Decreases in HDL and LDL fractions were dose-related; the effect on triglycerides was not consistent.

In dogs in these studies, the occurrence, severity, and distribution of toxic signs were dose-related. Dose-limiting toxicity was seen at doses of 120 mg/kg and above at which decreases in cholesterol and lipoproteins were marked. Clinical signs necessitating sacrifice or decreases in dose were seen at 160 mg/kg and above. Although there were clinical signs of gastrointestinal injury, only occasionally were these confirmed on gross or histopathologic examination. Transient increases in ALT suggestive of hepatic insult were seen at doses of 80 mg/kg and above; histological hepatic changes were seen at 150 mg/kg and above.

The primary target organ of AT was the liver. This was

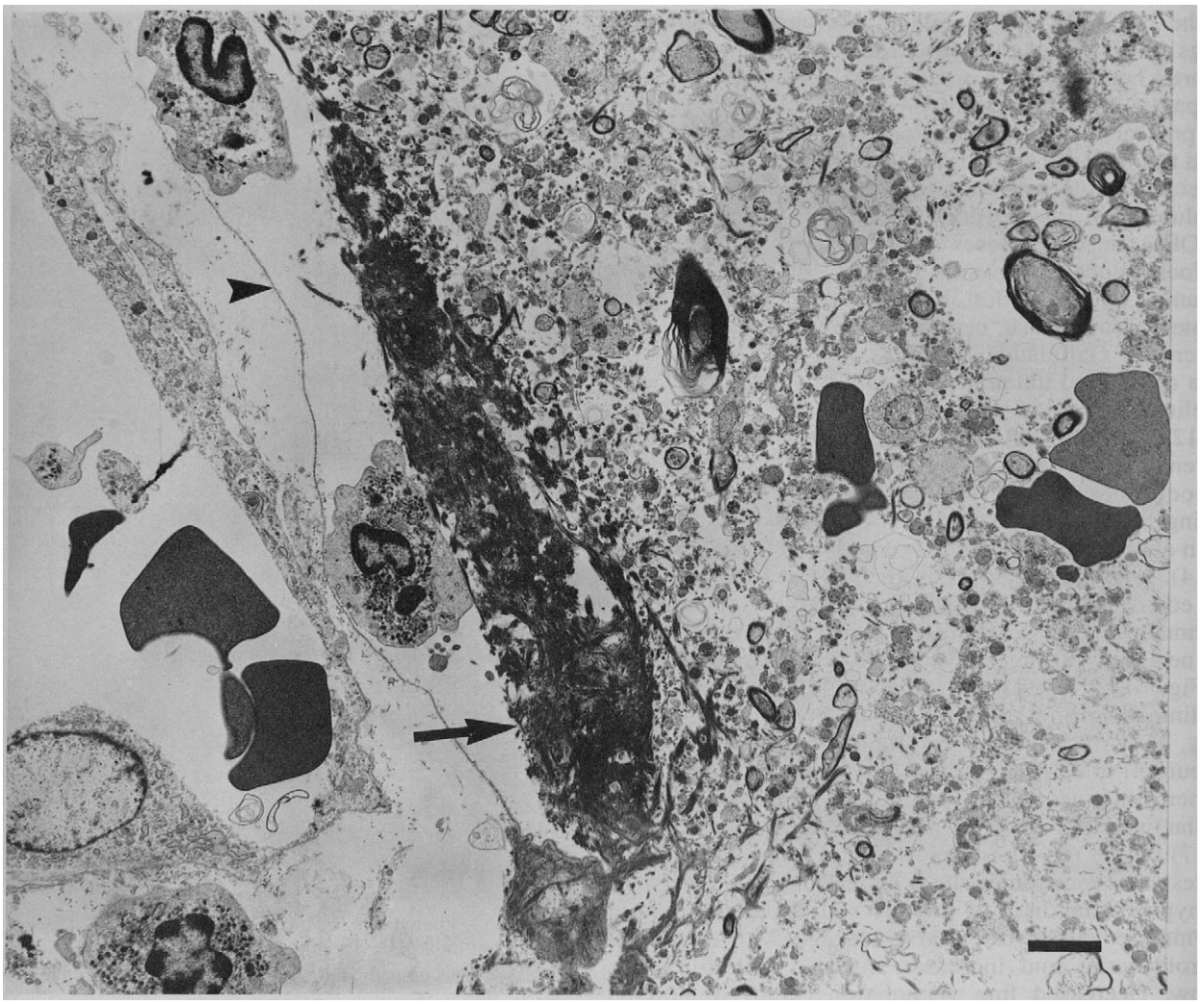

FIG. 7.—Electron micrograph of brain with marked vascular and perivascular changes from dog given up to 280 mg/kg AT. There is separation of endothelial cells from basal lamina (arrowhead) with fibrin deposition (arrow) and neutrophilic infiltration. Note cellular debris and erythrocytes in neuropil. Bar = 2.8 μm.

not unexpected, as the liver is the primary site of cholesterol synthesis and the primary site of drug delivery for HMGRI (1) including AT (3, 17). Although transient increases in ALT and AST were described for lovastatin in dogs (up to 180 mg/kg/day) (11) and monkeys (800 mg/kg for 7 days) (12) and for simvastatin (8), histopathologic hepatic lesions were not reported. Histopathologic hepatic lesions were not seen in dogs given up to 25 mg/kg pravastatin for 104 wk (20). In our studies, increases in hepatic transaminases were seen at doses ≥80 mg/kg but increases were reversible in spite of continued treatment. In rising dose studies (2 wk and 12 wk), hepatocellular degeneration was concomitant with ALT elevations and, in a 2-wk study in which the ALT increase at 80 mg/kg was transient, increased SER was the only hepatocellular change present at terminal sacrifice. Transient elevations in hepatic transaminase in dogs and hepatocellular atypia with proliferation of SER in rats treated with HMGRI were blocked by coadministration of mevalonate (1, 11).

Increases in AP were sporadic, correlated with cholangiohepatitis or cholecystitis, and were induced by high doses (≥150 mg/kg) of AT. These changes could be due to relatively increased concentrations of AT in bile and gallbladder because AT is excreted primarily via bile. It is also possible that low cholesterol levels affected bile composition because cholesterol is the immediate precursor to bile acids (14, 17). Hyperplasia and/or inflammation of gallbladder have been described in dogs given other HMGRIs (2, 6).

Neurological lesions seen in 1 dog given high doses (280 mg/kg) of AT were multifocal, affected mainly basal ganglia, and were similar to those described in the brain (hemorrhage, fibrinoid necrosis of vessels, edema) and optic nerve (axonal degeneration) of dogs given other HMGRIs (2, 6). HMGRI-induced neurological lesions in marmosets were much less severe, consisting mainly of perivascular cuffing and multifocal gliosis (15). Supplementation with α-tocopherol did not prevent the neurologic lesions (2). Time of onset of neurologic signs in

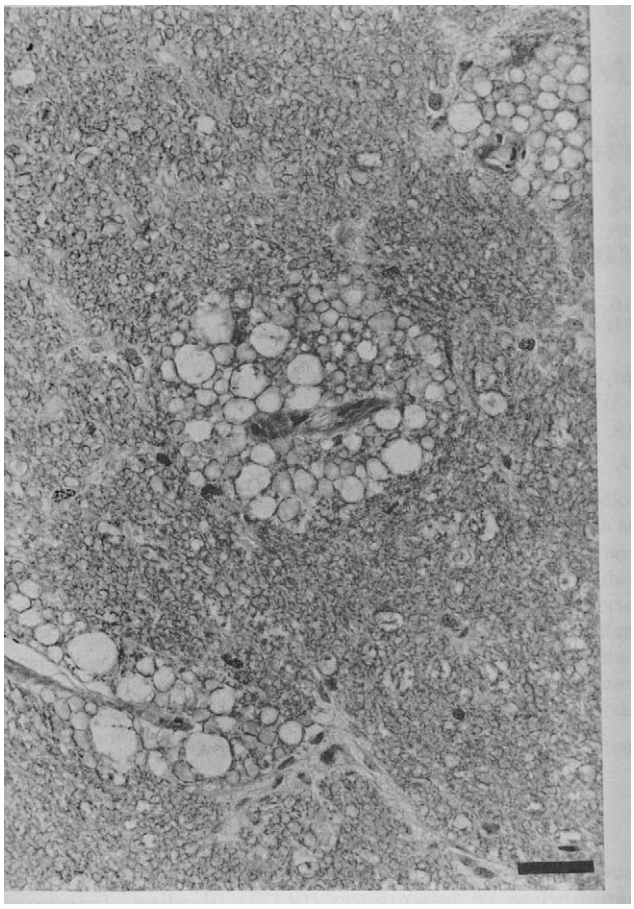

FIG. 8.—Photomicrograph of optic nerve from dog given AT demonstrates multifocal swelling and vacuolation of myelin. Luxol fast blue. Bar = 28 μm.

dogs given AT was after 10 wk of weekly dose escalation, later than reported with lovastatin (11–91 days), when high doses were reached.

Skeletal muscle degeneration has not been previously reported with HMGRI in dogs although it has been described in rats (8, 11, 16), rabbits (5), and marmosets (15) but not in cynomolgus monkeys (12). In our studies, skeletal muscle degeneration was seen in tongue of 2 dogs that had pain upon opening the mouth. These animals had increased CPK and AST and had been dosed with up to 280 mg/kg of AT before pain was elicited. In rabbits, simvastatin and pravastatin induced skeletal muscle hyperexcitability (decreased threshold current and repetitive firing) and rhabdomyolysis by reducing chloride conductance via chloride channel blocking (19). Skeletal muscle degeneration did not correlate with ubiquinone content of muscle in rabbits (5). Increased skeletal muscle degeneration in rats treated with combination HMGRI and cyclosporine A suggested that myopathy may be due to altered clearance with resulting increased tissue exposure (18). Skeletal muscle degeneration in dogs in our studies did not correlate with plasma AT levels. The 2 affected animals had lower plasma concentrations than did other dogs in which degeneration was not found. However, inter- and intraanimal variability in drug concentration, particularly at the high doses, was extensive and it may be that these 2 animals had transient, extremely high plasma concentrations that were not reflected in sampling. In addition, AT radioequivalents could not be detected in muscle (3).

AT induced changes in lenticular glucose, protein, K, and reduced glutathione. Lenticular opacities associated with HMGRI (6, 8, 9) have been related to plasma levels of drug and not to magnitude of cholesterol decrease nor to drug concentration in affected lenses (7, 11). However, HMG-CoA reductase activity was not demonstrated in lenses of multiple species (9, 10), and it has been suggested that cataractogenesis may be due to decreased cholesterol levels in lens and aqueous humor secondary to decreased serum cholesterol (10). However, although AT induced extensive decreases in serum cholesterol and in spite of the changes in lenticular concentrations of glucose, K, reduced glutathione, and protein, there was no clinical or morphological evidence of cataract in dogs in these studies.

Testicular degeneration in dogs has been seen with some HMGRIs (4, 8, 11) but was an inconsistent finding seen only at very high near-lethal doses with AT treatment in dogs in these studies. AT has been shown to inhibit sterol synthesis in rat liver, spleen, and adrenal but not in testis, kidney, muscle, or brain (3).

An explanation for the variability of plasma AT levels between animals and within a single animal is unknown. Similar variability has occurred with other HMGRIs in dogs (2) and marmosets (15). In spite of this variability, pharmacological effects, such as decreases in cholesterol, suggest a dose–response; this dose–response was considered to apply to toxicity with the understanding that some changes may have been idiosyncratic in occurrence or severity. Most toxic effects were related to high doses of AT.

AT was very effective in lowering serum cholesterol in dogs. Toxicity of AT in dogs was similar to other HMGRIs except that lenticular changes were not seen with AT, significant hepatic, testicular, and neurological toxicity was associated only with high doses of AT, and skeletal muscle degeneration, similar to that in rat and rabbit, has not been seen in dogs treated with other HMGRIs.

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