The Hypolipidemic Effects of 1-Acetyl-4-phenyl-1,2,4-triazolidine-3,5-dione in Rodents

Iris H. Hall,[1] Rupenra Simlot,[1] Patricia Day,[1] O. T. Wong,[1] Hamby ElSourady,[1] and Robert A. Izydore[2]

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1-Acetyl-4-phenyl-1,2,4-triazolidine,5-dione (APTD), a potent hypolipidemic agent, lowered both serum cholesterol and triglyceride levels in normo- and hyperlipidemic rats at 10 or 20 mg/kg/day. The agent effectively lowered VLDL-cholesterol (VLDL-C) and LDL-C content and raised HDL-C content in normal and hyperlipidemic rats treated from 4 to 8 weeks. Similar effects on the incorporation of cholesterol into the lipoprotein fractions were observed after drug treatment. Tissue lipids, e.g. cholesterol, were lowered, whereas fecal cholesterol levels were increased. APTD's primary targets were acyl CoA cholesterol acyl transferase (ACAT) for cholesterol ester synthesis and *sn*-glycerol-3-phosphate acyl transferase (GPAT) and phosphatidylate phosphohydrolase (PPH) for triglyceride synthesis.

KEY WORDS: triazolidine-3,5-dione; hyperlipidemic; high-density lipoprotein C (HDL-C); atherosclerosis.

INTRODUCTION

1,2,4-triazolidine-3,5-diones have been observed to be potent hypolipidemic agents in rodents at 20 mg/kg/day (1). In a 2-week study, the compounds lowered both serum cholesterol (SC) and serum triglyceride (ST) levels as well as VLDL-C and LDL-C levels, while elevating HDL-C levels. *In vitro* mouse hepatic enzyme activities of HMG CoA reductase, acetyl CoA synthetase, ACAT, acetyl CoA carboxylase, GPAT, PPH, and lipoprotein lipase activities were inhibited between 25 and 100 μM (1). *In vitro* studies of human liver and fibroblasts, rat small intestinal mucosal and aorta foam cells, and mouse macrophages showed that APTD reduced LDL receptor binding, internalization, and degradation while accelerating HDL binding, internalization, and degradation (2). ACAT and GPAT activities in these cultured cells were also reduced. The purposes of these studies are to establish the long-term (8-week) effects of APTD on tissue and serum lipoprotein (SLp) lipid content and on important enzyme inhibitions in normolipidemic rats and to determine effects of APTD administration on lipid metabolism in diet-induced hyperlipidemic and fasted rats.

METHODS AND PROCEDURES

General Procedure

Radioisotopes (New England Nuclear), biochemical reagents, cofactors (Sigma Chemical Co.), and Sprague Dawley male rats (~230 g; Charles River Laboratory) were obtained commercially. Rats were maintained under 12-hr light:dark cycles at 22°C. Food and water were given ad libitum.

Source of Compounds

APTD synthesis (Fig. 1) was reported previously (1). The chemical and physical characteristics were identical to those originally reported: 216.5–218.5°C (mp) white solid, 54% yield. IR C=O cm^{-1} 1726, 1710, 1688; NMR 7.90 (S, 5H) 2.55 (S, 3H) $C_{10}H_9O_3N_3$. Calc: C 57.08, H 3.24, N 13.45. Found: C 57.00 H 3.20 N 13.31 (1).

Pharmacological Methods

Normolipidemic Study

Rats were administered APTD, orally, at 10 or 20 mg/kg/day for 2, 4, 6, and 8 weeks, with six animals in control and treatment groups. Weekly blood samples were obtained by tail vein bleeding between 7:30 and 8:30 AM using nonheparinized microhematocrit tubes (4). The following were determined: SC (3) and ST levels (Bio-Dynamic/bmc Triglyceride Kit), BUN, glucose, LDH, CP-kinase, bilirubin (direct and indirect), albumin, total protein, SGPT, creatinine (Sigma Clinical Chemistry Kit), uric acid, cholic acid, hematocrit, differential blood count, and platelet estimates.

Fasted normolipidemic rats were treated with drug for 2 weeks at 20 mg/kg/day, orally. Food was removed 18 hr prior to blood collection and water was available ad libitum.

Hyperlipidemic Study

Rats were maintained on a commercial diet (U.S. Biochemical Corporation Basal Atherogenic Diet) containing butterfat (400 g), celufil (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), Wesson oil (40 g), sodium cholate (20 g), sucrose (223 g), vitamin-free cassein (200 g), and total vitamin supplement. After SC and ST levels were elevated, i.e., after 28 days on the diet, rats were administered APTD at 10 mg/kg/day, orally. APTD-treated and diet-control animals were maintained on the atherogenic diet for 4 weeks.

Enzymatic Studies and Tissue Lipids

In vivo enzymatic studies were performed using 10% homogenates prepared in 0.25 *M* sucrose + 0.001 *M* EDTA (pH 7.2) of liver or small intestinal mucosa obtained from control rats and those treated rats dosed with APTD for 8 weeks at 10 mg/kg/day, orally ($N = 6$). Enzyme reactions were conducted as outlined previously (4,5). Homogenates (10%) of liver, small intestinal mucosa, aorta, and a 24-hr fecal sample were extracted and analyzed for lipid content (4,5).

Animal Weight, Organ Weight, and Food Consumption

Weekly body weights from control and treated normolipidemic rats ($N = 6$) were obtained and are expressed as a percentage of the initial body weight (week 0). Food consumption (g/day/rat) was noted for weeks 6, 7, and 8 for control and treated rats. After the 8-week drug regimen, rats were sacrificed and individual organ weights were obtained (1).

[1] Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina—Chapel Hill, Chapel Hill, North Carolina 27599.

[2] Department of Chemistry, North Carolina Central University, Durham, North Carolina 27707.

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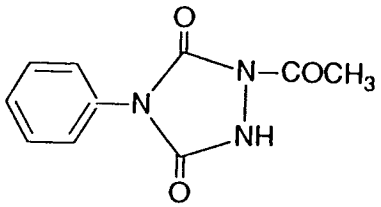

Fig. 1. 1-Acetyl-4-phenyl-1,2,4-triazolidine-3,5-dione.

Serum Lipoprotein (SLp) Fractions

Normolipidemic control rats and rats treated for 2, 4, 6, and 8 weeks with APTD at 10 or 20 mg/kg/day orally, and hyperlipidemic control and rats treated for 4 weeks at 10 mg/kg/day ($N = 6$) were anesthesized (ether). Blood (~10 mL) was collected from the abdominal vein (no anticoagulant). Serum was centrifuged at 3500 rpm × 10 min and then separated by density-gradient techniques into chylomicron (Chy), VLDL, HDL, and LDL fractions specified for rats (4,5).

Rate of Lipoprotein Synthesis in Normolipidemic Male Rats

Normolipidemic control and treated rats were administered APTD at 20 mg/kg/day for 2 weeks ($N = 6$). On day 13, 20 μCi of L-[4,5-^{3}H(N)] leucine (58.5 Ci/mmol), 1,2-^{3}H-cholesterol (40.7 mCi/mmol), ^{14}C-palmitic acid (57 mCi/mmol), or ^{32}P (H_2PO_3) buffered (2 μCi) was injected into the tail vein suspended in isotonic PBS, pH 7.4 (22–24). After 16 hr, blood was collected and SLp fractions were separated (5).

RESULTS

APTD lowered both SC and ST levels at 10 and 20 mg/kg/day orally (Table I). Only after 6 to 8 weeks of treatment was the 20 mg/kg/day dose more effective than the 10 mg/kg/day dose in lowering both SC and ST levels greater than 50% of control values. After discontinuation of APTD, SC and ST levels returned to normal levels by week 12. Daily food intake after APTD treatment (10 mg/kg/day) was not reduced compared to control levels (18.79 g/day), with no increase in total body weight. However, dosing at 20 mg/kg/day caused a 17% reduction of daily food intake, with a 30% reduction in total body weight after 8 weeks.

After drug treatment for 8 weeks, cytoplasmic acetyl CoA synthetase, ACAT, GPAT, and PPH activities were reduced significantly in the liver and SI mucosa. After 14 days of APTD treatment at 20 mg/kg/day to normolipidemic rats, a purified liver and SI microsomal preparation of ACAT showed suppression of activity by 58 and 72%, respectively. Liver supernatant ACAT activity (9000g × 20 min) was reduced by 36%; SI mucosal activity was reduced by 21.5%. Acid phosphatase activity was reduced in the liver; cathepsin activity was reduced in the SI mucosa (Table II).

Total liver and aorta lipids were reduced at 8 weeks for both doses (Table III). Total SI mucosal lipids were only reduced at the 20 mg/kg/day dose for 8 weeks. Tissue cholesterol (C), triglycerides (T), and neutral lipids (NL) levels were generally reduced; phospholipids (PH) were elevated. Fecal lipids, particularly C, were elevated at both doses at 8 weeks. PH was not elevated at 8 weeks. Bile C and T levels were elevated after 2 weeks of treatment. However, the bile flow rate was reduced after 2 weeks of treatment at 20 mg/kg/day (data not shown).

Table I. (A) Effects of APTD on Serum Cholesterol and Serum Triglyceride Levels of Normolipidemic Sprague Dawley Rats Over 8 Weeks at 10 and 20 mg/kg/day, Orally; (B) Effects of APTD at 10 mg/kg/day Orally on Serum Cholesterol and Serum Triglyceride Levels in Sprague Dawley Rats

A ($N = 6$)

	Serum cholesterol (mg/dL; $\bar{X}$ + SD)			Serum triglycerides (mg/dL; $\bar{X}$ + SD)		
Weeks	Control	10 mg/kg/day	20 mg/kg/day	Control	10 mg/kg/day	20 mg/kg/day
0	75 ± 5	75 ± 5	75 ± 4	120 ± 8	120 ± 8	120 ± 8
1	75 ± 4	52 ± 5*	67 ± 4	120 ± 7	74 ± 7*	73 ± 6*
2	76 ± 5	56 ± 4*	59 ± 4*	119 ± 8	54 ± 7*	58 ± 5*
4	75 ± 4	47 ± 5*	58 ± 4*	121 ± 7	64 ± 5*	76 ± 7*
6	76 ± 5	51 ± 6*	31 ± 3*	120 ± 6	71 ± 6*	67 ± 6*
8	75 ± 5	49 ± 5*	32 ± 4*	119 ± 7	66 ± 4*	49 ± 6*

B ($N = 6$)

Treatment	Serum cholesterol (mg/dL; $\bar{X}$ + SD)	Serum triglycerides (mg/dL; $\bar{X}$ + SD)
Fasted rats		
Nonfasted controls	78 ± 5	121 ± 7
Control fasted (18 hr)	39 ± 3	40 ± 5
APTD fasted (18 hr) (20 mg/kg/day, 2 wk)	31 ± 4	32 ± 3
Hyperlipidemic rats		
Diet control, 4 wk	344 ± 10*	506 ± 11*
Diet-induced + APTD (10 mg/kg/day, 2 wk)	220 ± 8*	386 ± 12*
Diet-induced + APTD (10 mg/kg/day, 4 wk)	107 ± 7	151 ± 6

* $P \leq 0.001$ Student's t test.

VLDL-C reductions were maximum after 4 weeks of treatment at 10 mg/kg/day (Table IV) and 6 weeks at 20 mg/kg/day. Elevated HDL-C levels were maximal after 2 weeks, although they remained significantly higher than control levels throughout the 8 weeks of APTD administration. Chy-C levels were reduced; maximal reductions were observed during week 4 at 20 mg/kg/day. VLDL-T and HDL-T were lowest during week 2 for the 10 and 20 mg/kg/day doses; VLDL-T values were 31 and 36%, respectively, and HDL-T values were 22 and 28%, respectively. Chy-T and LDL-T levels for the 10 and 20 mg/kg day doses were lowest during week 6; Chy-T values were 12 and 29%, respectively, and LDL-T values were 22 and 41%, respectively. After administration of the 10 and 20 mg/kg/day doses, VLDL-NL were lowest on week 4 (49 and 63%, respectively), LDL-NL were lowest on week 8 (28 and 36%, respectively), and Chy-NL were lowest on week 8 (43 and 22%, respectively). HDL-NL were actually elevated (22–117%) on weeks 6 and 8. PH were reduced in the VLDL fraction (45 and 170%) on week 2 for the 10 and 20 mg/kg day doses, respectively. Over time, VLDL-PH returned to control values and then, on week 8, were elevated (130%) at the 10 mg/kg/day dose. Chy-PH were elevated (86% for both doses) at 8 weeks. LDL-PH peaked in week 2 by 88 and 102% at the 10 and 20 mg/kg/day doses, respectively; they then declined, with the 20/mg/kg/day dose remaining elevated (56%) longer than the 10 mg/kg/day dose. HDL-PH at 20 mg/kg/day were reduced at weeks 2 and 4 but recovered during weeks 6 and 8. The 10 mg/kg/day dose caused a steady increase in HDL-PH to 135% over the 8-week dosing period. Protein was reduced in the VLDL and LDL fractions during weeks 2 and 4 by 19 and 41%, respectively, but returned to normal levels on weeks 6 and 8.

Incorporation of radiolabeled precursors into SLps was measured after 14 days at 20 mg/kg/day orally in normolipidemic rats. ^{3}H-Cholesterol incorporation in the Chy (65%), VLDL (28%), and LDL (47%) fractions was significantly reduced but was increased in the HDL fraction (97%). ^{14}C-Palmitic acid incorporation was reduced significantly in all fractions (Chy, 76%; VLDL, 79%; LDL, 80%; HDL, 38%). ^{32}P incorporation into PH was elevated only in the Chy fraction (21%). ^{3}H-Leucine incorporation was elevated in the Chy (43%), VLDL (91%), and LDL (50%) fractions.

^{3}H-Cholesterol uptake into tissue in normolipidemic rats was decreased in the heart (33%), spleen (24%), kidney (41%), reproductive organs (76%), stomach (40%), and large intestine (51%). Liver (40%), thymus (60%), and chyme (28%) were significantly elevated in ^{3}H-cholesterol content. ^{14}C-Palmitic acid incorporation was reduced significantly in brain (77%), lung (32%), heart (74%), liver (23%), spleen (69%), stomach (85%), small intestine (91%), large intestine (68%), chyme (90%), and feces (56%). ^{32}P-Incorporation into PH was elevated in the lung (24%), chyme (27%), spleen (19%), and reproductive organs (19%), while being reduced in the heart (37%) and large intestine (38%). Remaining tissues were within normal limits. Organ weights of normolipidemic rats treated with APTD for 2 weeks did not differ significantly from control values.

Hyperlipidemic rats required 4 weeks on the diet to induce high levels of serum lipid (Table I). Drug treatments were commenced at this time and continued for the next 4 weeks. After the first 14 days of drug treatment, SC levels

Table II. *In Vivo* Effects of APTD on Activity of Enzymes Involved in Lipid Metabolism of Normolipidemic Sprague Dawley Male Rat Liver and Small Intestine After 8 Weeks of Oral Administration at 10 mg/kg/day

	Percentage of control ($\bar{X}$ ± SD)			
	Liver		Small intestine	
Enzyme assay (N = 6)	Control	Treated	Control	Treated
ATP-dependent citrate lyase	100 ± 8[a]	119 ± 8	100 ± 8	101 ± 7
Acetyl CoA synthetase	100 ± 7[b]	81 ± 7	100 ± 7	44 ± 5*
HMG CoA reductase	100 ± 9[c]	108 ± 8	100 ± 6	98 ± 4
Cholesterol-7α-hydroxylase	100 ± 7[d]	84 ± 5	100 ± 7	104 ± 7
Acyl CoA cholesterol acyl transferase	100 ± 7[e]	64 ± 5	100 ± 7	53 ± 5*
Cholesterol ester hydrolase (neutral)	100 ± 8[f]	106 ± 5	100 ± 7	72 ± 6*
Acetyl CoA carboxylase	100 ± 6[g]	107 ± 6	100 ± 8	130 ± 5*
sn-Glycerol-3-phosphase acyl transferase	100 ± 5[h]	30 ± 3*	100 ± 5	47 ± 4*
Phosphatidylate phosphohydroxylase	100 ± 6[i]	25 ± 5*	100 ± 7	48 ± 4*
Cathepsin proteolytic	100 ± 6[j]	76 ± 6*	100 ± 6	94 ± 6
Acid phosphatase	100 ± 5	86 ± 5	100 ± 6	78 ± 7*

[a] 30.5 mg citrate hydrolyzed/g wet tissue/20 min.
[b] 28.5 mg acetyl CoA formed/g wet tissue/20 min.
[c] 384,900 dpm cholesterol formed/g wet tissue/60 min.
[d] 4808 dpm/mg microsomal protein/20 min.
[e] 224,000 dpm/mg microsomal protein/30 min.
[f] 56,436 dpm/g wet tissue/hr.
[g] 32,010 dpm/g wt tissue/30 min.
[h] 537,800 dpm/g wet tissue/20 min.
[i] 16.7 μg/iP/g wet tissue/15 min.
[j] 278,583 dpm/g wet tissue/hr.
* $P \leq 0.001$, Student's t test.

Table III. Effects on Tissue Lipid Levels of Sprague Dawley Rats After Oral Administration of APTD at 10 or 20 mg/kg/day for 8 Weeks

	Percentage of control ($\bar{X}$ ± SD)					
Tissue (N = 6)	Total lipid (mg)	Cholesterol	Triglyceride	Neutral lipids	Phospholipids	Proteins
Liver						
APTD (10 mg/kg)	71 ± 2	92 ± 7	70 ± 9	89 ± 5	94 ± 8	102 ± 5
APTD (20 mg/kg)	98 ± 6	77 ± 5*	104 ± 7	110 ± 6	166 ± 9*	100 ± 7
Control (1% CMC)	100 ± 6[a]	100 ± 5[b]	100 ± 7[c]	100 ± 6[d]	100 ± 6[e]	100 ± 5[f]
Small intestine						
APTD (10 mg/kg)	158 ± 5	122 ± 7	91 ± 9	72 ± 8*	106 ± 8	65 ± 8*
APTD (20 mg/kg)	97 ± 3	96 ± 7	72 ± 5*	44 ± 3*	163 ± 10*	104 ± 6
Control (1% CMC)	100 ± 7[g]	100 ± 6[h]	100 ± 7[i]	100 ± 6[j]	100 ± 8[k]	100 ± 6[l]
Aorta						
APTD (10 mg/kg)	48 ± 5*	69 ± 4*	95 ± 6	50 ± 6*	104 ± 7	95 ± 2
APTD (20 mg/kg)	67 ± 5*	67 ± 4*	31 ± 3*	38 ± 4*	251 ± 7*	112 ± 7
Control (1% CMC)	100 ± 6[m]	100 ± 5[n]	100 ± 6[o]	100 ± 5[p]	100 ± 6[q]	100 ± 4[r]
Feces						
APTD (10 mg/kg)	118 ± 6	93 ± 3	113 ± 5	103 ± 6	97 ± 5	96 ± 8
APTD (20 mg/kg)	173 ± 7*	137 ± 8*	10 ± 4*	87 ± 6	82 ± 6	127 ± 6*
Control (1% CMC)	100 ± 7[s]	100 ± 6[t]	100 ± 6[u]	100 ± 5[v]	100 ± 7[w]	100 ± 7[x]

[a] 50.0 mg lipid/g wet tissue.
[b] 9.18 mg cholesterol/g wet tissue.
[c] 6.37 mg triglyceride/g wet tissue.
[d] 15.70 mg neutral lipid/g wet tissue.
[e] 27.19 mg phospholipid/g wet tissue.
[f] 4.5 mg protein/g wet tissue.
[g] 68.20 mg lipid/g wet tissue.
[h] 12.02 mg cholesterol/g wet tissue.
[i] 11.20 mg triglyceride/g wet tissue.
[j] 16.98 mg neutral lipid/g wet tissue.
[k] 20.06 mg phospholipid/g wet tissue.
[l] 42.0 mg protein/g wet tissue.
[m] 67.5 mg lipid/g wet tissue.
[n] 5.77 mg cholesterol/g wet tissue.
[o] 9.85 mg triglyceride/g wet tissue.
[p] 15.28 mg neutral lipid/g wet tissue.
[q] 28.8 mg phospholipid/g wet tissue.
[r] 11.71 mg protein/g wet tissue.
[s] 11.58 mg lipid/g wet tissue.
[t] 2.84 cholesterol/g wet tissue.
[u] 1.85 mg triglyceride/g wet tissue.
[v] 3.39 mg neutral lipid/g wet tissue.
[w] 5.70 mg phospholipid/g wet tissue.
[x] 6.99 mg protein/g wet tissue.
* $P \leq 0.001$, Student's t test.

were lowered 36% (Table IV), and after 28 days it was reduced 69%, which approached normolipidemic control values. ST levels were reduced 25% by 14-day drug treatment and 70% by 4-week drug treatment. Examination of SLp levels in these hyperlipidemic rats after 4 weeks of drug treatment revealed that reductions in the Chy-C, VLDL-C, and LDL-C levels; HDL-C levels were elevated threefold. Chy-T and LDL-T levels were reduced. VLDL-NL levels were reduced. VLDL-PH and LDL-PH levels were elevated. Protein was reduced in the VLDL and the LDL fractions. The agent was equally effective in lowering SC and ST in normal and hyperlipidemic mice at 20 mg/kg/day, ip.

Clinical chemistry evaluations were made in normolipidemic rats after 8 weeks at 10 and 20 mg/kg day orally and in rats fasted for 18 hr treated after treatment for 2 weeks at 20 mg/kg/day. All tested values were within normal limits except SC and ST levels. The hematocrit, platelet estimates, and differential white blood cell counts were all within normal limits. Organ weights after 2- and 8-week drug administration, orally, were all within normal limits.

DISCUSSION

APTD is a potent hypolipidemic agent in rats over an 8-week period, lowering effectively both SC and ST levels when administered orally at both 10 and 20 mg/kg/day. APTD had the same ability to lower SC and ST levels in hyperlipidemic rats treated for 2 weeks. These lipid levels were even lower after 4 weeks of administration, approaching normolipidemic control values. The decremental effects of APTD on SC and ST levels of fasted rats were present but were not as potent, causing approximately a 20% reduction in ST but only a 9% reduction in SC levels. This suggested that the drug's suppression of appetite is a major reason for

Table IV. Effects of 1-Acetyl-4-phenyl-1,2,4-triazolidine-3,5-dione on Lipid Content of Sprague Dawley Male Rat Serum Lipoproteins After Oral Administration

	Percentage of control ($\bar{X}$ ± SD)				
	Cholesterol	Triglyceride	Neutral lipids	Phospholipids	Protein
Two weeks					
Chylomicrons					
Control	100 ± 7	100 ± 6	100 ± 7	100 ± 7	100 ± 5
Treated, 10 mg/kg/day	111 ± 7*	111 ± 5	112 ± 7	72 ± 7*	97 ± 6
Treated, 20 mg/kg/day	39 ± 3*	105 ± 6*	111 ± 7	96 ± 9	91 ± 4
VLDL					
Control	100 ± 6	100 ± 5	100 ± 7	100 ± 6	100 ± 6
Treated, 10 mg/kg/day	20 ± 7*	64 ± 5*	101 ± 6	55 ± 5*	73 ± 4*
Treated, 20 mg/kg/day	44 ± 5*	69 ± 4*	72 ± 3*	24 ± 6*	81 ± 2*
LDL					
Control	100 ± 6	100 ± 7	100 ± 6	100 ± 8	100 ± 6
Treated, 10 mg/kg/day	68 ± 3*	138 ± 5*	140 ± 5*	188 ± 9*	40 ± 5*
Treated, 20 mg/kg/day	74 ± 6*	94 ± 5	120 ± 5*	202 ± 5*	93 ± 4
HDL					
Control	100 ± 5	100 ± 6	100 ± 7	100 ± 7	100 ± 7
Treated, 10 mg/kg/day	417 ± 16*	78 ± 5*	106 ± 8	132 ± 9*	120 ± 6
Treated, 20 mg/kg/day	441 ± 6*	72 ± 5*	89 ± 6	44 ± 3*	117 ± 7
Four weeks					
Chylomicrons					
Control	100 ± 7	100 ± 6	100 ± 7	100 ± 8	100 ± 6
Treated, 10 mg//kg/day	81 ± 4	126 ± 7*	91 ± 5	157 ± 8*	75 ± 5*
Treated, 20 mg/kg/day	33 ± 3*	105 ± 6	94 ± 4	89 ± 6	76 ± 6*
VLDL					
Control	100 ± 6	100 ± 5	100 ± 8	100 ± 7	100 ± 5
Treated, 10 mg/kg/day	13 ± 4*	98 ± 6	51 ± 3*	91 ± 5	75 ± 6*
Treated, 20 mg/kg/day	40 ± 5*	88 ± 7	37 ± 4*	71 ± 5*	61 ± 5*
LDL					
Control	100 ± 6	100 ± 7	100 ± 6	100 ± 7	100 ± 5
Treated, 10 mg/kg/day	59 ± 5*	85 ± 7	117 ± 6	89 ± 9	65 ± 6*
Treated, 20 mg/kg/day	51 ± 6*	82 ± 6*	107 ± 6	176 ± 9*	45 ± 4*
HDL					
Control	100 ± 5	100 ± 6	100 ± 7	100 ± 6	100 ± 5
Treated, 10 mg/kg/day	163 ± 5*	79 ± 6*	122 ± 9	146 ± 8*	75 ± 8*
Treated, 20 mg/kg/day	254 ± 6*	58 ± 5*	115 ± 6	60 ± 5*	87 ± 8
Six weeks					
Chylomicrons					
Control	100 ± 7	100 ± 6	100 ± 7	100 ± 8	100 ± 6
Treated, 10 mg/kg/day	79 ± 6*	88 ± 7	80 ± 7	176 ± 6*	96 ± 4
Treated, 20 mg/kg/day	64 ± 5*	71 ± 5	78 ± 4*	132 ± 5*	89 ± 5
VLDL					
Control	100 ± 6	100 ± 5	100 ± 5	100 ± 7	100 ± 5
Treated, 10 mg/kg/day	55 ± 4*	83 ± 6	72 ± 6*	134 ± 7*	97 ± 7
Treated, 20 mg/kg/day	24 ± 3*	86 ± 5	73 ± 7*	93 ± 5	86 ± 4
LDL					
Control	100 ± 6	100 ± 7	100 ± 6	100 ± 8	100 ± 7
Treated, 10 mg/kg/day	78 ± 5*	64 ± 5*	101 ± 6	84 ± 8	91 ± 7
Treated, 20 mg/kg/day	59 ± 5*	77 ± 5*	73 ± 7*	156 ± 9*	89 ± 8
HDL					
Control	100 ± 5	100 ± 7	100 ± 8	100 ± 7	100 ± 6
Treated, 10 mg/kg/day	134 ± 5*	88 ± 5	150 ± 7*	153 ± 6*	112 ± 6
Treated, 20 mg/kg/day	231 ± 11*	75 ± 7*	217 ± 8*	91 ± 5	104 ± 5
Eight weeks					
Chylomicrons					
Control	100 ± 7	100 ± 7	100 ± 8	100 ± 7	100 ± 6
Treated, 10 mg/kg/day	84 ± 6	111 ± 5	57 ± 7*	186 ± 8*	101 ± 7
Treated, 20 mg/kg/day	55 ± 5*	85 ± 6*	78 ± 6*	186 ± 8*	101 ± 6
VLDL					
Control	100 ± 7	100 ± 7	100 ± 6	100 ± 8	100 ± 5
Treated, 10 mg/kg/day	106 ± 4	105 ± 5	125 ± 2*	149 ± 6*	110 ± 6
Treated, 20 mg/kg/day	82 ± 9	92 ± 5	114 ± 4	196 ± 8*	131 ± 8*

Table IV. Continued

	Percentage of control ($\bar{X}$ ± SD)				
	Cholesterol	Triglyceride	Neutral lipids	Phospholipids	Protein
LDL					
Control	100 ± 6	100 ± 7	100 ± 5	100 ± 8	100 ± 7
Treated, 10 mg/kg/day	86 ± 3	115 ± 5	72 ± 6*	80 ± 4	98 ± 7
Treated, 20 mg/kg/day	87 ± 6	85 ± 6	64 ± 4*	122 ± 6	101 ± 6*
HDL					
Control	100 ± 5	100 ± 6	100 ± 5	100 ± 7	100 ± 7
Treated, 10 mg/kg/day	124 ± 5*	102 ± 8	122 ± 6	235 ± 9*	104 ± 8
Treated, 20 mg/kg/day	138 ± 7*	75 ± 5*	137 ± 5*	113 ± 6	93 ± 6

reduction of serum lipids. APTD lowered tissue lipids probably because its major effects were on cytoplasmic acetyl CoA synthetase, which regulated the early synthesis of fatty acids and cholesterol. Lowering of GPAT and PPH activities by APTD would explain reduction in tissue triglycerides. ACAT activity was significantly inhibited in liver and small intestine mucosa after 8 weeks of APTD treatment. When this enzyme was examined after 2 weeks of treatment, activity was significantly inhibited *in vivo*. This enzyme regulates cholesterol ester synthesis which is particularly important in aorta foam cells. Previously, APTD was shown to inhibit ACAT activity in culture cells, e.g., human hepatocytes and fibroblasts, rat small intestine mucosa cells and aorta foam cells, and mouse macrophages (2). It is important to note that total cholesterol in the aorta was reduced after drug treatment *in vivo*. HDL-C elevation after APTD treatment may indicate that free cholesterol from peripheral cells (including plaque cells) is taken up by HDL returning to the liver for excretion via the bile. Bile-C levels and fecal lipids were indeed elevated after 2 weeks of treatment, suggesting that the drug accelerated HDL-C reverse transport to the liver and elimination from the body (1).

Previous studies on HDL receptor binding, internalization, and degradation in human hepatocytes also support the premise that APTD accelerates the reverse-cholesterol transport to the liver (2). HDL apoproteins (apo) of treated rats showed increased apo E and apo AI, both of which are recognition moieties for the liver HDL receptor (2). *In vivo*, APTD at 20 mg/kg/day accelerated ^{3}H-HDL clearance from plasma of rats; also, hepatic ^{3}H-HDL levels were high in treated animals (2).

APTD modulated lipid content of rat SLps in a clinically useful manner. While HDL-C levels were elevated, VLDL-C and LDL-C levels were lowered. This pattern of cholesterol levels of SLps supposedly protects against myocardial infarction in man (6). HDL-C levels are inversely linked with growth of aorta plaques cells in man. Surprisingly, APTD's maximum effects on HDL-C levels occurred after 2 weeks. The effects, although present, were attenuated over 8 weeks, again suggesting drug treatment accelerated clearance of cholesterol from the serum and tissues. Probably the reverse transport of cholesterol to the liver peaks and then levels out over time after drug treatment. The effects on VLDL-C and LDL-C content peaked between week 4 and week 6; these effects decreased by week 8. Reductions in efficacy may be due to feedback regulation on the synthetic processes of these biochemical components of SLps. Similar reactions were also observed with other lipid contents of the SLps from week 2 to week 8. In rats, APTD appeared to alter lipoprotein lipids by affecting lipid incorporation, e.g., cholesterol incorporation was reduced at 2 weeks into the Chy, VLDL, and LDL fractions, whereas HDL incorporation of cholesterol was accelerated. APTD blocked lipid uptake into tissue in both the isotope and the chemical analysis studies. In hyperlipidemic rats, the effects of APTD on modulating SLp were still present, although elevations in HDL-C were only approximately threefold. Hematopoietic parameters, organ weights, and clinical chemistry values suggested that APTD had no deleterious effects at 8 weeks, findings supported by previous mouse studies (7). APTD caused a lowering of total body weight at 20 but not at 10 mg/kg/day. This may have resulted from reduced daily food intake, increased lipid excretion into the feces, or reduced lipid content of organ and tissues of the body.

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