RESEARCH ARTICLES

The Hypolipidemic Activity of Benzenetricarboxylic Acids in Rodents

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Abstract: A series of benzenetricarboxylic acids was shown to be potent hypolipidemic agents in rodents. Terephthalic acid proved to be one of the more potent agents, lowering serum cholesterol 42% and serum triglyceride 33% at 20 mg/kg/day for 16 days. The ability to lower serum lipids by this agent appeared to be due to multiple modes of action: (*1*) terephthalic acid suppressed the activities both *in vivo* and *in vitro* of a number of regulatory enzymes involved in cholesterol, fatty acid, and triglyceride syntheses; (*2*) the drug inhibited cholesterol absorption from the GI tract by 43%; and (*3*) the drug accelerated lipid excretion in the feces leading to a reduction of cholesterol in the tissue. Terephthalic acid was effective in lowering lipids in normal and hyperlipidemic animals and possessed a safe therapeutic index.

Benzene-1,2,3-tricarboxylic acid has previously been observed to be a potent inhibitor of citrate transport by rat liver mitochondria (1). Subsequently, studies demonstrated that a series of substituted benzene, pyrimidine and miscellaneous aromatic acids were inhibitors of the citrate transport (2). Limiting the available citrate in the cytoplasm for the conversion to acetyl coenzyme A would limit lipid synthesis by the cell. Acetyl coenzyme A is a required precursor intermediate for cholesterol and triglyceride synthesis. We have demonstrated that a series of cyclic imides inhibit mitochondrial citrate exchange in rodents and are potent hypolipidemic agents (3–6). Consequently, a study was undertaken to examine a series of benzenetricarboxylic acids for their lipid lowering effects in rodents. Those results are now reported.

Experimental

The following compounds were commercially purchased: phthalic acid (Aldrich), isophthalic acid (Matheson, Coleman and Bell), terephthalic acid (Eastman), 1,2,3-benzenetricarboxylic acid, 1,2,4-benzenetricarboxylic acid and 1,3,5-benzenetricarboxylic acid (all Aldrich).

Antihyerlipidemic Screens in Normal Rodents

Benzenetricarboxylic acids were suspended in 1% carboxymethylcellulose-water and administered to CF_1 male mice (~25 g) intraperitoneally for 16 days or to Holtzman male rats (~350 g) orally by an intubation needle for 14 days. On days 9 and 14 or 16, blood was obtained by tail vein bleeding and the serum separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann-Burchard reaction (7). Serum was also collected on day 14 or 16 and the triglyceride content was determined by a commercial kit (Fisher, Hycel Triglyceride Test Kit).

Testing in Hyperlipidemic Mice

CF_1 male mice (~25 g) were placed on a commercial diet (U. S. Biochemical Corporation Basal Atherogenic Test Diet) which contained butterfat (400 g), celufil (cellulose) (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil (Wesson) (40 g), sodium cholate (20 g), sucrose (223 g), vitamin free casein (200 g) and total vitamin supplement for 10 days. After the cholesterol and trigylceride levels were assayed and found to be elevated, the mice were administered test drugs at 20 mg/kg/day, intraperitoneally for an additional 14 day period. Serum cholesterol and trigylceride levels were measured after 14 days of administration of the drugs.

Animal Weights and Food Intake

Animal weights were obtained throughout the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 14 days with test drugs, selected organs were excised, trimmed of fat and weighed.

Toxicity Studies

The acute toxicity (LD_{50} values) (8) was determined in CF_1 male mice by administering test drugs intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths were recorded over 7 day period for each group.

Enzymatic Studies

In vitro enzymatic studies were performed using 10% homogenates of CF_1 male mouse liver containing 2.5–10 μmoles of test drugs. *In vivo* enzymatic studies were performed using 10% homogenates of liver from CF_1 male mice obtained after administering the agents for 15 days at a dose ranging from 10–60 mg/kg/day intraperitoneally. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 mM (ethylenedinitrilo)tetraacetic acid. Acetyl coenzyme A synthetase (9) and adenosine triphosphate dependent citrate lyase (10) activities were determined spectrophotometrically at 540 nm as the hydroxyamate of acetyl coenzyme A (formed after 30 min at 37°C). Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (11, 12) using ^{14}C sodium bicarbonate (41 mCi/mmol) incorporated into mitochondrial ^{14}C-citrate after isolating rat mitochondria (9000 g × 10 min) from the homogenates. The exchanges of the ^{14}C-citrate were determined after incubating the mitochondrial fraction which was loaded with labelled citrate and test drugs for 10 min. Then the radioactivity was measured in the mitochondrial and supernatant fractions in scintillation fluid (Fisher Scintiverse in a Packard Scintillation Counter) and expressed as a percentage. Cholesterol side chain oxidation

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was determined by the method of Kritchevsky and Tepper (13) using 26-^{14}C-cholesterol (50 mCi/mmol) and mitochondria isolated from rat liver homogenates. After 18 hr incubation at 37° C with test drugs, the generated $^{14}CO_2$ was trapped in the center well in {2-[2-(p-1,1,3,3-tetramethylbutylcresoxy)-ethoxy]ethyl}dimethybenzylammonium hydroxide (Hyamine hydroxide, New England Nuclear) and assayed by liquid scintillation counting. Cholesterol synthesis was measured using 1-^{14}C-acetate (56 mCi/mmol) and a post-mitochondrial supernatant (9000 g × 20 min) incubated for 60 min at 37°C (14). The digitonide derivative of cholesterol was isolated and assayed for ^{14}C content (15). Acetyl coenzyme A carboxylase activity was measured by the method of Greenspan and Lowenstein (16). Initially, the enzyme had to be polymerized for 30 min at 37° C, and then the assay mixture containing sodium ^{14}C-bicarbonate (41.0 mCi/mmol) was added and incubated for 30 min at 37° C with test drugs. Fatty acid synthetase activity was determined by the method of Brady *et al.* (17) using 2-^{14}C-malonyl-coenzyme A (37.5 mCi/mmol) which was incorporated into newly synthesized fatty acids that were extracted with ether and assayed for ^{14}C content. *Sn*-Glycerol-3-phosphate acyl transferase activity was determined with glycerol-3-phosphate [L-2-^{3}H(N)] (7.1 Ci/mmol) and the microsomal fraction of the liver homogenates (18). The reaction was terminated after 10 min, and the lipids were extracted with chloroform : methanol (1:2) containing 1% conc. HCl and assayed for ^{3}H content. Phosphatidate phosphohydrolase activity was measured as the inorganic phosphate released after 30 min by the method of Mavis *et al.* (19). The released inorganic phosphate after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

Liver, Small Intestine and Fecal Lipid Extraction

In CF_1 male mice that had been administered test drugs for 16 days, the liver, small intestine and fecal materials (24 hr collection) were removed, and a 10% homogenate in 0.25 M sucrose + 0.001 M (ethylenedinitrilo)tetraacetic acid was prepared. An aliquot (2 ml) of the homogenate was extracted by the methods of Folch *et al.* (20) and Bligh and Dyer (21) and the number of mg of lipid weighed. The lipid was taken up in methylene chloride and the cholesterol level (7), triglyceride levels (Bio-Dynamics/bmc Triglyceride Kit), neutral lipid content (22) and phospholipid content (23) were determined.

^{3}H-Cholesterol Distribution in Rats

Holtzman male rats (~ 350 g) were administered test agents for 14 days orally. On day 13, 10 μCi of ^{3}H-cholesterol was administered orally in rats, and feces were collected after 24 hrs. Twenty-four hours after cholesterol administration, the major organs were excised, and samples of blood, chyme and urine were obtained. Homogenates (10%) were prepared of the tissues which were combusted (Packard Tissue Oxidizer) and assayed for ^{3}H content. Some tissue samples were plated on filter paper (Whatman #1), dried and digested for 24 hr in base (Hyamine hydroxide) at 40° C and assayed. Results were expressed as dpm per total organ.

Cholesterol Absorption Study

Holtzman male rats (~ 400 g) were administered test drugs intraperitoneally for 14 days at 20 mg/kg/day. On day 13, 10 μCi of 1,2-^{3}H(N)-cholesterol (40.7 Ci/mmole) was administered to the rat orally. Twenty-four hours later, the blood was collected and the serum separated by centrifugation (24). Both the serum and the precipitate were assayed for ^{3}H content.

Bile Cannulation Study

Holtzman male rats (~ 400 g) were treated with test drugs at 20 mg/kg/day orally for 14 days. The rats were anesthetized with 25 mg/kg chlorpromazine (Thorazine, Chlorpromazine Hydrochloride. Smith, Kline & French Labs.), followed in 30 min by 22 mg/kg pentobarbital (Nembutal, Sodium Pentobarbital, Abbott Labs.) intraperitoneally. The duodenum section of the small intestine was isolated, ligatures were placed around the pyloric sphincter and distally to a site approximately one third of the way down the duodenum. Sterile isotonic saline was injected into the sectioned off duodenum segment. The saline expanded the duodenum and the common bile duct. Once the bile duct was identified, a loose ligature was placed around it, an incision made and plastic tubing (PE-10 Intramedia Polyethylene) introduced into the duct. Once past the ligature, the tubing was tied in place, and the ligatures around the duodenum were removed. Once bile was freely moving down the cannulated tube, 1,2-^{3}H(N)-cholesterol (40.7 mCi/mmol) was injected intravenously into the rats. The bile was collected over the next 6 hrs and measured (ml). Aliquots were assayed for ^{3}H content as well as analyzed for cholesterol content (3).

Plasma Lipoprotein Fractions

Holtzman male rats (~ 400 g) were administered test drugs at 20 mg/kg/day for 14 days. On day 14, blood was collected from the abdominal aorta. Serum was separated from whole blood by centrifugation at 3500 rpm. Aliquots (3 ml) were separated by density gradient ultracentrifugation according to the method of Havel *et al.* (25) as modified for rat by Mookerjea *et al.* (26) into the chylomicrons, very low density lipoproteins, high density lipoproteins and low density lipoproteins. Each of the fractions was analyzed for cholesterol (4), triglyceride (Biodynamics/bmc Triglyceride Kit), neutral lipids (22), phospholipids (23) and protein levels (27).

Data are expressed in the Tables I–VI as percent of control ± the standard deviation. The probable significant level (P) between each test group and the control group was determined by the Student's "t" test.

Table I. The Effects of Benzenetricarboxylic Acids on Serum Cholesterol and Triglycerides of Normal CF_1 Mice at 20 mg/kg/day i. p

	% Control		
	Serum Cholestrol		Serum Triglyceride
Compound (N = 6)	**9th Day** X ± SD	**16th Day** X ± SD	**4th Day** X ± SD
Phthalic Acid	91 ± 7	78 ± 7[a]	86 ± 4[b]
Isophthalic Acid	75 ± 4[a]	69 ± 4[a]	84 ± 5[a]
Terephthalic Acid	65 ± 4[a]	58 ± 5[a]	47 ± 5[a]
1-2-3-Benzene-tricarboxylic Acid	89 ± 8	81 ± 6[a]	54 ± 7[a]
1-2-4-Benzene-tricarboxylic Acid	79 ± 8[a]	76 ± 3[a]	42 ± 4[a]
1-3-5-Benzene-tricarboxylic Acid	72 ± 7[a]	67 ± 2[a]	59 ± 5[a]
1% Carboxy-methylcellulose	100 ± 5[c]	100 ± 6[d]	100 ± 6[e]

[a]$p \leq 0.001$; [b]$p \leq 0.010$; [c]116 mg%; [d]122 mg%; [e]137 mg%

Table II. The Effects of Terephthalic Acid on Serum Cholesterol, and Triglyceride Levels in Normal CF_1 Male Mice and Sprague Dawley Male Rats.

	% Control					
	CF_1 Male Mice			Sprague Dawley Rats		
Therephthalic Acid (N = 6)	Serum Cholesterol 9th Day X ± S.D.	16th Day X ± S.D.	Serum Triglyceride 16th Day X ± S.D.	Serum Cholesterol 9th Day X ± S.D.	14th Day X ± S.D.	Serum Triglyceride 14th Day X ± S.D.
10 mg/kg	66 ± 5[a]	64 ± 5[a]	62 ± 5[a]	–	–	–
20 mg/kg	65 ± 5[a]	58 ± 5[a]	47 ± 4[a]	84 ± 7	56 ± 6[a]	62 ± 6[a]
40 mg/kg	74 ± 6[a]	67 ± 4[a]	55 ± 5[a]	–	–	–
60 mg/kg	70 ± 4[a]	69 ± 7[a]	66 ± 6[a]	–	–	–
1 % Carboxymethylcellulose	100 ± 5[b]	100 ± 6[c]	100 ± 6[d]	100 ± 9[e]	100 ± 7[f]	100 ± 8[g]

[a]$P \leq 0.001$; [b]118 mg %; [c]122 mg %; [d]137 mg %; [e]73 mg %; [f]78 mg %; [g]110 mg %

Table III. The *in vitro* Effects of Terephthalic Acid and 1,3,5-Benzenetricarboxylic Acid on Liver Enzyme Activities.

	Per cent Control						
	Terephthalic Acid				1,3,5-Benezenetricarboxylic Acid		
Enzyme Assays (N = 6)	Control X ± S.D.	50 μM X ± S.D.	100 μM X ± S.D.	200 μM X ± S.D.	50 μM X ± S.D.	100 μM X ± S.D.	200 μM X ± S.D.
Mitochondrial Citrate Exchange[b]	100 ± 10	63 ± 5[a]	40 ± 5[a]	40 ± 4[a]	65 ± 6[a]	42 ± 4[a]	58 ± 4[a]
Citrate Lyase[c]	100 ± 9	81 ± 6	72 ± 7[a]	72 ± 6[a]	79 ± 7	79 ± 6	71 ± 7[a]
Acetyl CoA Synthetase[d]	100 ± 8	91 ± 5	91 ± 9	71 ± 8[a]	79 ± 8	78 ± 9	33 ± 4[a]
Colesterol Synthesis[e]	100 ± 7	86 ± 6	84 ± 7	79 ± 8	95 ± 6	111 ± –	149 ± 7[a]
Cholesterol Side Chain Oxidation[f]	100 ± 8	–	73 ±5[a]	–	–	119 ± 4_a	–
Acetyl CoA Carboxylase[g]	100 ± 6	99 ± 6	94 ± 5	86 ± 6	95 ± 6	92 ± 7	88 ± 7
Fatty Acid Synthetase[h]	100 ± 7	99 ± 6	83 ± 6	79 ± 7[a]	86 ± 5	52 ± 8[a]	65 ± 6_a
sn-Glycerol-3-Phosphate Acyl Transferase[i]	100 ± 8	66 ± 6[a]	67 ± 7[a]	66 ± 6[a]	115 ± 7	90 ± 7	89 ± 6
Phosphatidate Phosphohydrolase[j]	100 ± 7	64 ± 7[a]	58 ± 6[a]	55 ± 5[a]	12 ± 3[a]	11 ± 2[a]	8 ± 2[a]

[a]$P \leq 0.001$ [b–j] Standard values are given in Table IV.

Table IV. The *in vivo* Effects of Terephthalic Acid on Liver Enzymes Activities of CF_1 Mice Treated at 20 mg/kg/day for 15 Days.

	% Control		
Enzyme Assays (N = 6)	Citrate Lyase X ± S.D.	Acethyl CoA Synthetase X ± S.D.	Cholesterol Synthesis X ± S.D.
1 % Carboxymethylcellulose	100 ± 5[c]	100 ± 8[d]	100 ± 6[e]
Terephthalic Acid			
10 mg/kg	99 ± 9	67 ± 6[a]	127 ± 7[a]
20 mg/kg	86 ± 5[a′]	73 ± 7[a]	163 ± 8[a]
40 mg/kg	77 ± 5[a]	73 ± 7[a]	164 ± 8[a]
60 mg/kg	98 ± 6	79 ± 7[a′]	151 ± 7[a]

	Acetyl CoA Carboxylase X ± S.D.	Fatty Acid Synthetase X ± S.D.	*sn*-Glycerol 3-Phosphate Acyl Transferase X ± S.D.	Phosphatidate Phosphohydrolase X ± S.D.
1 % Carboxymethylcellulose	100 ± 5[g]	100 ± 6[h]	100 ± 8[i]	100 ± 8[j]
Terephthalic Acid				
10 mg/kg	88 ± 4[a′]	116 ± 7[a′]	80 ± 6[a′]	55 ± 5[a]
20 mg/kg	82 ± 5[a]	115 ± 6[a′]	76 ± 7[a]	63 ± 6[a]
40 mg/kg	73 ± 6[a]	112 ± 5[a′]	66 ± 6[a]	64 ± 6[a]
60 mg/kg	71 ± 5[a]	111 ± 6	57 ± 5[a]	61 ± 7[a]

[a]$P \leq 0.001$, [a′]$p \leq 0.010$; [b]30.8 ± 3.1 mg % exchange of mitochondrial citrate; [c]30.5 ± 2.74 mg of citrate hydrolyzed/gm wet tissue/30 min; [d]28.5 ± 3.14 mg of acetyl coenzyme A formed/gm wet tissue/30 min; [e]384,900 ± 26943 dpm cholesterol formed/gm wet tissue/60 min; [g]32.010 ± 1921 dpm/gm wet tissue/30 min; [h]37,656 ± 2635 dpm/gm wet tissue/30 min; [i]16.70 ± 1.16 μg P_i/gm wet tissue/15 min; [j]537800 ± 43024 dpm triglyceride formed/gm wet tissue/10 min;

Table V. The Effect of Terephthalic Acid on Rat Lipid Levels of Liver, Small Intestine, Feces and Lipoprotein Fractions After 14 Day Dosing at 20 mg/kg/Day Orally.

		Percent Control				
	Lipid	**Cholesterol**	**Triglycerides**	**Neutral Lipids**	**Phospholipids**	**Protein**
Liver (N = 6)	X ± S.D.	X ± S.D.	X ± S.D.	X ± S.D.	X ± S.D.	X ± S.D.
Control	100 ± 7	100 ± 7[c]	100 ± 5[d]	100 ± 4[e]	100 ± 8[f]	100 ± 7[g]
Treated	81 ± 6[a]	56 ± 5[a]	75 ± 6[a]	40 ± 3[a]	176 ± 9	93 ± 6
Small Intestine						
Control	100 ± 7	100 ± 7[h]	100 ± 6[i]	100 ± 5[j]	100 ± 8[k]	100 ± 8[l]
Treated	43 ± 4[a]	72 ± 6[a]	17 ± 3[a]	45 ± 3	104 ± 7	79 ± 7
Feces						
Control	100 ± 8	100 ± 8[m]	100 ± 7[n]	100 ± 6[o]	100 ± 6[p]	100 ± 6[q]
Treated	115 ± 7	113 ± 8	235 ± 9[a]	151 ± 8[a]	109 ±	96 ± 5
Bile						
Control		100 ± 8[r]	100 ± 8[s]	100 ± 8[t]	100 ± 6[u]	–
Treated		187 ± 9[a]	100 ± 7	206 ± a	131 ± 7[a]	–
Lipoprotein						
Chylomicrons						
Control		100 ± 9[v]	100 ± 6[w]	100 ± 8[x]	100 ± 10[y]	100 ± 7[z]
Treated		78 ± 8	73 ± 5[a]	93 ± 7	188 ± 12[a]	98 ± 8
VLDL						
Control		100 ± 8[aa]	100 ± 7[bb]	100 ± 9[cc]	100 ± 7[dd]	100 ± 8[ee]
Treated		66 ± 6[a]	65 ± 5[a]	38 ± 3[a]	105 ± 6	50 ± 6[a]
HDL						
Control		100 ± 8[ff]	100 ± 4[gg]	100 ± 9[hh]	100 ± 6[ii]	100 ± 8[jj]
Treated		76 ± 7[a]	100 ± 7	74 ± 8[a]	93 ± 4	96 ± 5
LDL						
Control		100 ± 9[kk]	100 ± 8[ll]	100 ± 7[mm]	100 ± 7[nn]	100 ± 8[oo]
Treated		58 ± 6[a]	76 ± 4[a]	53 ± 5[a]	67 ± 6[a]	107 ± 7

[a]$P < 0.001$; [b]$P < 0.010$; [c]24.03 mg cholesterol/gm tissue; [d]44.11 mg neutral lipid/gm tissue; [e]6.37 mg triglyceride/gm tissue; [f]7.19 mg phospholipid/gm tissue; [g]4.5 mg protein/gm tissue; [h]7.82 mg/gm; [i]6.98 mg/gm; [j]1.12 mg/gm; [k]2.06 mg/gm; [l]42 mg/g; [m]28.47 mg/gm; [n]33.94 mg/gm; [o]1.86 mg/gm; [p]1.39 kg/gm; [q]6.99 mg/gm; [r]118 mg %; [s]5 mg/ml; [t]170 mg/ml; [u]1.75 mg/ml; [v]337 µg/ml; [w]167 µg/ml; [x]420 µg/ml; [y]149 µg/ml; [z]184 µg/ml; [aa]190 µg/ml; [bb]98 µg/ml; [cc]22 µg/ml; [dd]26 µg/ml; [ee]50 µg/ml; [ff]544 µg/ml; [gg]620 µg/ml; [hh]27 µg/ml; [ii]153 µg/ml; [jj]657 µg/ml; [kk]210 µg/ml; [ll]10 µg/ml; [mm]45 µg/ml; [nn]41 µg/ml; [oo]122 µg/ml.

Table VI. The Effects of Terephthalic Acid on Rat Organ Weights and ^{3}H-Cholesterol Distribution 24 HR After Administration.

Organ (N = 6)	**Organ Weight (gm)**		**DPM/Total Organ**	
	Control	**Treated**	**Control**	**Treated**
Liver	14.47	13.00	47429	43354
Brain	1.97	1.70	5877	4213
Lung	2.10	1.60	7069	6045
Heart	1.33	1.48	4004	4742
Spleen	0.67	0.87	2315	4578
Kidney	3.37	3.05	8170	8408
Stomach	2.99	2.90	11375	8479
Small Intestine	10.17	8.65	41656	50343
Large Intestine	4.78	5.45	14104	26269
Adrenals	.050	.049	–	–
Chyme	3.53	7.58	9226	22479
Feces	4.30	5.03	8170	25060
			DPM/mL	
Serum			648	368
Bile			726	879
Urine			281	273

Results

All of the benzenetricarboxylic acids demonstrated significant activity in lowering serum cholesterol and triglyceride levels in mice at 20 mg/kg/day (Table I). Terephthalic acid was the most potent of the compounds tested in lowering serum cholesterol by 42%. 1,2,4-Benzenetricarboxylic acid proved to be the most effective in reducing serum triglyceride levels by 58%. Since terephthalic acid appeared to be a better overall agent in both screens, further pharmacological and biochemical studies were conducted with this agent. In addition, terephthalic acid's LD_{50} value in mice was $\geq$ 1000 mg/kg. A dose response study in mice from 10–60 mg/kg demonstrated that 20 mg/kg/day was the most effective dose in lowering both cholesterol and triglyceride levels. Similar values were obtained when male rats were treated with terephthalic acid; however, the triglyceride levels were not as effectively reduced in rats as they were in mice at 20 mg/kg/day (Table II). In hyperlipidemic induced mice, serum cholesterol levels were elevated 183% (354 mg%) above the normal values (125 mg%) after being on the diet. The cholesterol levels were reduced 57% by drug treatment to 152 mg%. In hyperlipidemic mice, the serum triglyceride levels were elevated 168% (367 mg/dl) above the control values (137 mg/dl) which was lowered 37% by drug administration for 14 days to 231 mg/dl.

Examination of enzyme activities involved in liver cholesterol and triglyceride synthesis demonstrated that mouse mitochondrial citrate exchange was inhibited significantly by terephthalic acid and 1,3,5-benzenetricarboxylic acid, with moderate (20–30 %) inhibition of ATP dependent citrate lyase and acetyl coenzyme A synthetase activity. Terephthalic acid moderately suppressed cholesterol side chain oxidation; however, 1,3,5-benzenetricarboxylic acid had no effect. The agents had essentially no effect on acetyl coenzyme A carboxylase and fatty acid synthetase activities; however, the two enzymes involved in triglyceride synthesis, *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase, were markedly inhibited by terephthalic acid (Table III).

The *in vivo* studies showed that at 40 mg/kg, terephthalic acid reduced citrate lyase activity 23 %, acetyl coenzyme A synthetase activity greater than 25 % from 10–40 mg/kg and actually elevated cholesterol synthesis. Acetyl coenzyme A carboxylase activity was reduced in a dose related manner with 60 mg/kg causing the maximum reduction of 29 %. Similarly *sn*-glycerol-3-phosphate acyl transferase activity was maximally inhibited at 60 mg/kg by 43 %. On the other hand, phosphatidate phosphohydrolase activity was suppressed maximally at 10 mg/kg by 45 %, with 20–60 mg/kg causing at least 35 % reduction (Table IV).

Lipid extraction of the liver and small intestine demonstrated reductions of total lipids, including cholesterol, triglycerides and neutral lipids. Cholesterol was reduced 44 % in the liver, but phospholipid content was elevated 76 %. Triglyceride levels were reduced 83 % in the small intestine, with the phospholipid level essentially unchanged (Table V).

An increase in the cholesterol content of the bile was evident. Likewise there were increases in the cholesterol, triglyceride and neutral lipids of the feces, but not the phospholipids. In the lipoprotein fractions of rat serum, cholesterol content was lower in all four fractions. Triglycerides were lower in chylomicrons, very low and low density lipoproteins. Neutral lipids were reduced in very low, low and high density lipoproteins. Phospholipid content was reduced in the low density fraction but elevated in the chylomicron fraction (Table V).

The cholesterol distribution study showed that there was no major accumulation of ^{3}H-cholesterol or its metabolic products in heart, liver, kidney, lung, brain or stomach. ^{3}H-cholesterol content in the spleen was approximately doubled after drug treatment for 2 weeks. Large increases in the ^{3}H-cholesterol content were observed in the chyme (144 %) and fecal (207 %) collections for 24 hr, as well as a moderate increase in the large intestinal tissues (86 %) and small intestine (21 %) (Table VI). Bile excretion of cholesterol was elevated 21 %, but urine excretion of cholesterol was unaffected by drug treatment. The 24 hr absorption study after oral administration of ^{3}H-cholesterol showed a marked reduction of 43 % of the cholesterol in the blood.

Discussion

The benzenetricarboxylic acid derivatives proved to be effective hypolipidemic agents when tested *in vivo* in rodents. Both intraperitoneal and oral administration routes of terephthalic acid were effective in lowering serum lipids. The agent was more effective in lowering cholesterol levels than triglyceride levels in hyperlipidemic induced mice. Terephthalic acid was more potent than the widely used clinical drug, clofibrate, which is inactive at 20 mg/kg/day. Clofibrate at 100–200 mg/kg lowers cholesterol levels by 15–20 % (28) and triglyceride levels by 25 %. The reduction in serum lipids by terephthalic acid in all probability is due in part to the agent's ability to reduce the activity of liver enzymes in the biosynthetic pathways leading to cholesterol and triglyceride, e. g., citrate lyase ($p < 0.001$ at 40 mg/kg/day), acetyl CoA synthetase ($p < 0.001$ from 10 to 40 mg/kg/day). Reduction of these enzyme activities would theoretically lower the availability of acetyl CoA, an intermediate precursor for both cholesterol and triglyceride synthesis. Reduction of acetyl CoA carboxylase ($p < 0.001$ from 20–60 mg/kg) would lead to reduction of fatty acid synthesis. Terephthalic acid, rather than inhibiting cholesterol synthesis, actually increased it. However, clofibrate does inhibit the activity of HMG CoA reductase activity and cholesterol synthesis. Triglyceride synthesis was further blocked by terephthalic acid at regulatory sites late in the triglyceride pathway; e. g., *sn*-glycerol-3-phosphate acyl transferase ($p < 0.001$) and phosphatidate phosphohydrolase ($p < 0.001$ at 20 mg/kg/day). Other potent hypolipidemic agents reduce the activity of these two enzymes; e. g., cyclic imides (29), 1,3-bis(substituted phenoxy)-2-propane, and clofibrate (30). Lamb *et al.* (30) correlated the inhibition of these two enzyme activities in the liver and small intestine with the reduction of serum triglycerides in the blood.

One of the reasons the serum cholesterol levels are reduced in treated animals is the accelerated excretion of cholesterol and neutral lipids via the bile. Drug treatment resulted in increased excretion of lipids by the fecal route observed not only as an increase in radioactive cholesterol content of chyme and feces but also as quantitative elevations in each lipid class. Clofibrate also increases cholesterol excretion via the fecal route (31). Those lipids removed from the blood were not extensively deposited in the major organs with the exception of the spleen. This may have been facilitated by reduced cholesterol absorption from the intestine, which may also reflect an inhibition of the absorption of cholesterol normally obtained through extrahepatic circulation. The lipid content of the lipoprotein fractions was significantly reduced by terephthalic acid administration. The reduction of the cholesterol content of the low density lipoprotein fraction may be of significance, since the low density fraction supposedly acts as a carrier of the lipids to the atherosclerotic plaques, shedding cholesterol into the foam cell (32–34). The ratio of the cholesterol content of the high to low density lipoprotein fractions probably increased slightly after drug treatment. HDL plays an important role in the interconversion of lipid components to various lipoprotein fractions and the return from peripheral tissue to the liver. Both LDL and HDL are actively taken up by the liver LDL receptors (35, 36). HDL may participate in the transport of cholesterol out of the atherosclerotic plaque, thus slowing the atherogenic process (37). A particular subfraction of the HDL class (HDL_2) has been inversely related to the incidence of coronary heart disease (Troms study) (38–39). Other studies in humans dispute this observation (40–42) and two other indicators have been suggested as predictors of coronary heart disease: (1) the ratio of LDL to HDL; and (2) the ratio of cholesterol content in HDL to the total cholesterol content. Treatment with clofibrate does not significantly alter HDL but there is a slight lowering of the cholesterol content of HDL (39). Probucol reduces the HDL fraction and lowers the ratio of cholesterol of HDL to total cholesterol content. The fact that HDL is lowered does not increase the risk of coronary heart disease in patients treated with probucol; however, in

patients treated with clofibrate, the drug does not protect against coronary heart disease (39). Thus, the effect of a drug on lipoprotein lipids is unclear in its relationship to predict accurately the reduction of cardiovascular related diseases. Whereas it is difficult to extrapolate data from lipoprotein studies in rats to man, these studies do demonstrate modulation of the lipid content of the lipoprotein fractions by terephthalic acid is possible. These preliminary studies suggest that benzenetricarboxylic acids have potential hypolipidemic activity in man and warrant further investigation.

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