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The Hypolipidemic Activity of Furoic Acid and Furylacrylic Acid Derivatives in Rodents

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Abstract: 2-Furoic acid, 3-furoic acid, 3,4-furan dicarboxylic acid and furyl-acrylic acid were evaluated for hypolipidemic activity in mice and rats. 2-Furoic acid was the most potent agent of the four tested, lowering serum cholesterol levels 41 % and serum triglyceride levels 56 % at 20 mg/kg/day in mice and serum cholesterol 50 % and serum triglyceride levels 42 % in rats. 2-Furoic acid effectively suppressed liver mitochondrial citrate exchange, ATP dependent citrate lyase, acetyl CoA synthetase, acyl CoA cholesterol acyl transferase, *sn*-glycerol-3-phosphate acyl transferase, phosphatidate phosphohydrolase and hepatic lipoprotein lipase enzymatic activities. Lipid levels after 16 days in mice were reduced in the liver. In the rat cholesterol content of the HDL fraction was elevated and lowered in the chylomicron fraction. 2-Furoic acid administration for 14 days resulted in a large portion of ^{3}H-cholesterol being excreted by the biliary route. The furoic acid derivatives appear to have promise as hypolipidemic agents and further studies on their ability to lower lipids are warranted.

Recently we have shown that a number of five-member cyclic ring structures possess potent hypolipidemic activity in rodents, e. g. succinimide (1), pyrrolidine (2) and 2-pyrrolidinones (3) have proven to lower both serum cholesterol and triglycerides in mice and rats at the low dose level of 20 mg/kg/day. These agents as well as the cyclic imides, that contain also one aromatic cyclic ring, were more potent than most commercially available agents (4), e. g. colfibrate, tiadenol, cholestyramine, nicotinic acid. All of these agents lowered serum cholesterol and triglycerides levels in CF_1 male mice approximately 40 %. Since furoic acid derivatives are available commercially, we decided to examine their capability to lower serum cholesterol and triglyceride levels in rodents. Those results are reported herein.

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Materials and Methods

Source of Compounds

2-Furoic acid, 3-furoic acid, 3,4-furandicarboxylic acid and furyl-acrylic acid were purchased from Aldrich Chemical Company, Inc. Radioisotopes were purchased from New England Nuclear. Substrates and cofactors for the enzyme reaction medium were obtained from Sigma Chemical Company.

Antihyperlipidemic Screens in Normal Rodents

Test compounds were suspended in an aqueous 1 % carboxymethylcellulose solution, homogenized, and administered to CF_1 male mice (~ 25 g) intraperitoneally for 16 days or Sprague Dawley 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 (5). 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) that produced a "hyperlipidemic" state (4). After the serum cholesterol and triglyceride levels were observed to be elevated, the mice were administered test drugs at 20 mg/kg/day, intraperitoneally for an additional 14-day period. Serum cholesterol and triglyceride levels were measured at that time.

Animal Weights and Food Intake

Periodic animal weights were obtained during 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. Food consumption was determined daily.

Toxicity Studies

The acute toxicity (LD_{50} values) (6) was determined in CF_1 male mice (~ 25 g) by administering test drugs intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths was recorded over a 7-day period for each group.

Enzymatic Studies

In vitro enzymatic studies were determined using 10 % homogenates of CF_1 male mouse liver with 50–200 μM of test drugs. *In vivo* enzymatic studies were determined using 10 % liver homogenates [prepared in 0.25 M sucrose + 0.001 M (ethylenedinitrilo)-tetraacetic acid, pH 7.2] from CF_1 male mice

obtained after administering the agents for 16 days at a dose ranging from 10–60 mg/kg/day, intraperitoneally. The enzyme activities were determined by following literature procedures (2): acetyl coenzyme A synthetase (7); adenosine triphosphate dependent citrate lyase (8); mitochondrial citrate exchange (9, 10); cholesterol 7α-hydroxylase (11); 3-hydroxy-3-methylglutaryl coenzyme A (12, 13); acetyl coenzyme A carboxylase activity (14); fatty acid synthetase activity (15); *sn*-glycerol-3-phosphate acyl transferase activity (16), phosphatidate phosphohydrolase activity (17), acyl-CoA-cholesterol acyl transferase (18) and heparin activated hepatic lipoproteine/-lipase (19). Protein was determined for all enzyme assays by the technique of Lowry et al.

Liver, Small Intestine and Fecal Lipid Extraction

In CF_1 male mice that had been administered 2-furoic acid for 16 days, the liver, small intestine and fecal materials (24 h collection) were removed, extracted (21, 22) and analyzed for cholesterol levels (5), triglyceride levels (Bio-Dynamics/bmc Triglyceride Kit), neutral lipid content (23) and phospholipid content (24).

^{3}H-Cholesterol Distribution in Rats

Sprague Dawley rats (~300 g) were administered 2-furoic acid for 14 days orally. On day 13, 10 μCi of ^{3}H-cholesterol was administered, orally by intubation needle to male rats, and according to the procedures described previously (4); some tissue samples were combusted in a Packard Tissue Oxidizer or plated on filter paper, dried and digested for 24 h in Hyamine Hydroxide (New England Nuclear) at 40° C and counted (Fisher Scintiverse in a Packard Scintillation Counter). Results were expressed as disintegration/min (dpm) per total organ.

Cholesterol Absorption Study

Sprague Dawley rats (~300 g) were administered 2-furoic acid intraperitoneally for 14 days at 20 mg/kg/day. On day 13, 10 μCi of 1,2-^{3}H-cholesterol (40.7 mCi/mmole) was administered to the rat, orally. Twenty-four hours later, the blood was collected and the serum separated by centrifugation (25).

Bile Cannulation Study

Sprague Dawley male rats (~300 g) were treated with test drugs at 20 mg/kg/day orally for 14 days. After anesthetizing the animal, the bile duct was cannulated as previously described (4). 1,2-^{3}H-Cholesterol (40.7 mCi/mmole) (10 μCi) was administered orally 18 h prior to commencing the surgery. The bile was collected over the next 6 h and the volume (ml) measured. Aliquots were counted (Fisher Scintiverse in a Packard Scintillation Counter) as well as analyzed for ^{3}H-cholesterol content (5).

Plasma Lipoprotein Fractions

Sprague Dawley male rats (~300 g) were administered test drugs at 20 mg/kg/day orally. Blood was collected from the abdominal aorta and lipoprotein fractions were obtained by the method of Hatch and Lees (26) and Havel et al. (27). Each of the fractions was analyzed for cholesterol (5), triglyceride (Bio-Dynamics/bmc Triglyceride Kit), neutral lipids (23) and protein levels (20).

Results

All four compounds demonstrated hypolipidemic activity in CF_1 male mice (Table I). 2-Furoic acid afforded the best hypocholesterolemic activity with 42 % reduction at 20 mg/kg/day on day 16. Serum triglyceride levels were reduced 56 % at this dose on day 16 by 2-furoic acid. 3-Furoic acid reduced serum cholesterol levels 46 % at 10 mg/kg/day;

Table I. The Hypolipidemic Activity of Furoic Acid and Furylacrylic Acid Derivatives in Rodents.

	Per cent of control						
	CF_1 Mice			Sprague Dawley rats			
(N = 6) 1 % CMC	Serum cholesterol 9th day (X ± S.D.)	Serum cholesterol 16th day (X ± S.D.)	Serum triglyceride 16th day (X ± S.D.)	Serum cholesterol 9th day (X ± S.D.)	Serum cholesterol 16th day (X ± S.D.)	Serum triglyceride 9th day (X ± S.D.)	Serum triglyceride 16th day (X ± S.D.)
2-Furoic acid	100 ± 6[a]	100 ± 7[b]	100 ± 7	100 ± 7[d]	100 ± 7[e]	100 ± 6[f]	100 g
10 mg/kg/day	80 ± 7	62 ± 5*	89 ± 8	—	—	—	—
20 mg/kg/day	68 ± 6*	59 ± 5*	44 ± 5*	77 ± 6*	50 ± 5*	84 ± 7	58 ± 6*
40 mg/kg/day	90 ± 8	84 ± 6	62 ± 6*	—	—	—	—
60 mg/kg/day	81 ± 8	91 ± 7	76 ± 8*	—	—	—	—
3-Furoic acid							
10 mg/kg/day	71 ± 8*	54 ± 5*	93 ± 7				
20 mg/kg/day	72 ± 7*	68 ± 6*	95 ± 6				
40 mg/kg/day	82 ± 7	81 ± 6	63 ± 6*				
60 mg/kg/day	88 ± 5	86 ± 8	61 ± 7*				
Furylacrylic acid							
10 mg/kg/day	70 ± 7*	57 ± 5*	62 ± 8*				
20 mg/kg/day	72 ± 8*	72 ± 5*	95 ± 7				
40 mg/kg/day	86 ± 7	73 ± 6*	94 ± 10				
60 mg/kg/day	80 ± 9	74 ± 6*	63 ± 6*				
3,4-Furandicarboxylic acid							
10 mg/kg/day	92 ± 8	68 ± 6*	87 ± 7				
20 mg/kg/day	90 ± 8	79 ± 8	79 ± 6*				
40 mg/kg/day	87 ± 7	80 ± 7	93 ± 8				
60 mg/kg/day	70 ± 5	65 ± 6*	83 ± 7				

[a]118 mg%; [b]122 mg%; [c]137 mg%; [d]73 mg%; [e]78 mg%; [f]110 mg%; [h]112 mg%
*$p < 0.001$

however, the highest reduction of serum triglyceride levels was at 60 mg/kg/day with 39 % reduction. 3,4-Furandicarboxylic acid reduced serum cholesterol 44 % at 10 mg/kg/day but required a dose of 60 mg/kg to reduce serum triglyceride levels 37 %. Furylacrylic acid demonstrated 32 % and 35 % reductions in serum cholesterol at 10 and 60 mg/kg/day, respectively. Serum triglyceride levels were lowered 25 % at 20 mg/kg/day with furylacrylic acid. From these results it would appear that 2-furoic acid was the drug of choice of the four compounds tested, and thus, it was used to further evaluate the hypolipidemic activity of this group. In diet-induced hyperlipidemic CF_1 mice where serum cholesterol levels had been elevated 183 % to 365 mg % and serum triglycerides were elevated 168 % to 367 mg/dl, 2-furoic acid at 20 mg/kg/day for 14 days lowered the serum cholesterol to 173.5 mg %, which was 39 % above control value (125 mg %), and lowered serum triglyceride levels to 93 mg/dl, which was 68 % of control values (138 mg/dl). In Sprague Dawley rats 2-furoic acid at 20 mg/kg/day after 14 days dosing afforded a 50 % reduction in serum cholesterol values and 42 % reduction in serum triglyceride levels. For these rats the food consumption for the control group was 19.39 g/day, whereas for the treated group it was 16.35 g/day, a 15.8 % reduction in food consumption/day by 2-furoic acid.

The *in vitro* enzymatic studies on CF_1 male mouse liver (Table II) demonstrated that 2-furoic acid reduced the level of mitochondrial citrate exchange 36 % at 200 μM. Moreover, ATP dependent citrate lyase was reduced significantly by all four compounds, but inhibition was observed at different concentrations, e. g. 2-furoic acid resulted in 37 % inhibition at 200 μM; 3-furoic acid resulted in 63 % inhibition at 50 μM, furylacrylic acid caused 46 % inhibition at 50 μM, and 3,4-furandicarboxylic acid caused 63 % at 50 μM. Acetyl CoA synthetase activity was inhibited 40 % by 2-furoic acid and 33 % by 3-furoic acid at 200 μM. HMG CoA reductase and cholesterol 7α-hydroxylase activities of mouse liver were unaffected by the furoic acid derivatives. Acyl CoA cholesterol acyl transferase activity was inhibited 40 % at 50 μM, 53 % at 100 μM, and 62 % at 200 μM 2-furoic acid. The fatty acid synthetase complex of enzymes was not inhibited by the furoic acid derivatives. *sn*-Glycerol-3-phosphate acyl transferase activity was reduced 30 % by 2-furoic acid, 34 % by 3-furoic acid, 25 % by furylacrylic acid and 35 % by 3,4-furandicarboxylic acid. Phosphatidate phosphohydrolase activity was also reduced 30 % by 2-furoic, 36 % by 3-furoic, 18 % by furylacrylic acid and 21 % by 3,4-furandicarboxylic acid. Hepatic lipoprotein lipase was inhibited 27 % by 2-furoic acid at 100 μM and 200 μM.

Examination of some of the same enzymes *in vivo* using 2-furoic acid from 10–60 mg/kg/day demonstrated that ATP dependent citrate lyase activity was suppressed 53 % at 10 mg/kg and 51 % at 20 mg/kg/day; acetyl CoA synthetase was inhibited 62 % at 20 mg/kg and 77 % at 40 mg/kg (Table III). HMG CoA reductase activity was suppressed 38–44 % by all four doses employed. Acetyl CoA carboxylase activity was reduced, maximally, 34 % at 10 mg/kg/day. *sn*-Glycerol-3-phosphate acyl transferase was inhibited 74–70 % between 10–40 mg/kg/day and phosphatidate phosphohydrolase activity was suppressed maximally at 20 mg/kg by 86 %. The lipid content of the livers (Table IV) after *in vivo* treatment showed that the drug lowered cholesterol content 16 % at 20 mg/kg, triglyceride content in a dose dependent man-

Table II. *In Vitro* Effect of Furoic Acid and Furylacrylic Acid Derivatives on CF_1 Mouse Liver Enzymes Activities.

		Per cent of control (X ± S.D.)											
		2-Furoic acid			3-Furoic acid			Furylacrylic acid			3,4-Furandicarboxylic acid		
Enzyme (N = 6)	Control	50 μm	100 μm	200 μm	50 μm	100 μm	200 μm	50 μm	100 μm	200 μm	50 μm	100 μm	200 μm
Mitochondrial Citrate exchange	100 ± 7[a]	92 ± 6	92 ± 6	64 ± 5*	( )	( )	( )	( )	( )	( )	( )	( )	( )
ATP dependent Citrate Lyase	100 ± 6[b]	134 ± 7	94 ± 5	63 ± 4*	37 ± 4*	76 ± 6*	63 ± 5*	54 ± 5*	96 ± 8	89 ± 7	27 ± 5*	88 ± 6	85 ± 7
Acetyl CoA Synthetase	100 ± 5[c]	96 ± 6	91 ± 7	60 ± 5*	76 ± 7*	75 ± 6*	67 ± 6*	93 ± 11	107 ± 5	119 ± 8	106 ± 8	110 ± 9	89 ± 7
HMG CoA Reductase	100 ± 9[d]	83 ± 10	98 ± 8	106 ± 9	98 ± 9	119 ± 10	119 ± 9	105 ± 10	134 ± 11	142 ± 10*	103 ± 10	110 ± 11	114 ± 7
Cholesterol 7α Hydroxylase	100 ± 5[e]	99 ± 8	102 ± 6	141 ± 7*	( )	( )	( )	( )	( )	( )	( )	( )	( )
Acyl CoA cholesterol Acyl transferase	100 ± 8[f]	60 ± 5*	47 ± 5*	38 ± 6*	( )	( )	( )	( )	( )	( )	( )	( )	( )
Acetyl CoA Carboxylase	100 ± 6[g]	105 ± 7	100 ± 7	99 ± 6	108 ± 8	88 ± 8	78 ± 7*	109 ± 7	99 ± 6	105 ± 8	99 ± 8	97 ± 7	94 ± 5
Fatty acid Synthetase	100 ± 8[h]	103 ± 8	101 ± 7	99 ± 7	110 ± 9	96 ± 8	96 ± 8	97 ± 10	99 ± 8	101 ± 7	98 ± 7	100 ± 6	104 ± 6
sn-Glycerol 3 phosphate Acyl transferase	100 ± 7[i]	82 ± 6	71 ± 5*	70 ± 7*	98 ± 8	75 ± 6*	66 ± 5*	85 ± 6	78 ± 5*	75 ± 6*	80 ± 5*	66 ± 7*	65 ± 6*
Phosphatidate Phosphohydrolase	100 ± 7[j]	95 ± 8	91 ± 6	70 ± 4*	116 ± 8	82 ± 4	64 ± 6*	86 ± 8	82 ± 5	82 ± 7	106 ± 7	82 ± 5	79 ± 6*
Hepatic Lipoprotein lipase	100 ± 6[k]	68 ± 5	73 ± 5*	73 ± 7*	( )	( )	( )	( )	( )	( )	( )	( )	( )

[a]30.8 % exchange of mitochondrial citrate; [b]30.5 mg citrate hydrolyzed/g wet tissue/20 min; [c]28.5 mg acetyl CoA formed/g wet tissue/20 min; [d]384,900 dpm cholesterol formed/g wet tissue/60 min; [e]224,000 dpm/μg microsomal protein; [f]4,808 dpm/mg microsomal protein/20 min; [g]32,010 dpm/g wet tissue/30 min; [h]37,656 dpm/g wet tissue/20 min; [i]537,800 dpm/g wet tissue/20 min; [j]16.7 μP_i/g wet tissue/15 min; [k]278,583 dpm/g wet tissue/h.

*$p \leq 0.001$

Table III. The *In Vivo* Effects of 2-Furoic Acid given I.P. in CF_1 Mice After 16 Days Dosing.

	Percent of control (X ± S.D.)					
	ATP dependent Citrate Lyase	Acetyl CoA synthesis	HMG CoA reductase	Acetyl CoA carboxylase	*sn*-Glycerol 3 P acyl transferase	Phosphatidate Phosphohydrolase
Control (N=6)	100 ± 8a	100 ± 7b	100 ± 6e	100 ± 5d	100 ± 7e	100 ± 6f
Treated						
10 mg/kg/day	47 ± 5*	65 ± 6*	60 ± 7*	66 ± 4*	30 ± 2*	60 ± 7*
20 mg/kg/day	49 ± 6*	38 ± 5*	62 ± 6*	73 ± 5*	26 ± 3*	14 ± 2*
40 mg/kg/day	80 ± 5	23 ± 3*	62 ± 7*	81 ± 3*	27 ± 3*	77 ± 5*
60 mg/kg/day	105 ± 7	50 ± 4*	56 ± 8*	124 ± 7*	92 ± 5	98 ± 4

See footnotes from Table II for control enzymatic activity.

Table IV. The Effect of 2-Furoic Acid on the Lipid Content of CF[1] and Rat Liver, Rat Small Intestine Feces, and Lipoprotein Fractions after *In Vivo* Administration at 20 mg/kg/day.

	Per cent of control					
CF_1 liver (I.P.) (N = 6)	Wt Lipids (mg) X ± S.D.	Cholesterol X ± S.D.	Triglyceride X ± S.D.	Neutral lipids X ± S.D.	Phospholipids X ± S.D.	Protein X ± S.D.
Control						
1 % CMC	100 ± 7	100 ± 6[a]	100 ± 5[b]	100 ± 7[c]	100 ± 8[d]	100 ± 7[e]
Treated						
10 mg/kg/day	114 ± 8	136 ± 8	87 ± 5*	130 ± 8	120 ± 9*	122 ± 8
20 mg/kg/day	87 ± 6	86 ± 7	68 ± 4*	89 ± 7	67 ± 7*	112 ± 8
30 mg/kg/day	86 ± 6	98 ± 8	45 ± 5*	78 ± 7*	67 ± 5*	118 ± 7
40 mg/kg/day	87 ± 5	92 ± 6	41 ± 4*	65 ± 6*	47 ± 8	93 ± 8
Sprague Dawley (orally) liver						
Control	100 ± 8	100 ± 7[f]	100 ± 6[g]	100 ± 7[h]	80 ± 9[i]	100 ± 8[j]
Treated	108 ± 9	112 ± 10	90 ± 7	101 ± 7	100 ± 6	108 ± 9
Small intestine						
Control	100 ± 7	100 ± 6[k]	100 ± 7[l]	100 ± 8[m]	100 ± 8[n]	100 ± 7[o]
Treated	79 ± 6	108 ± 5	96 ± 4	72 ± 6*	86 ± 9	85 ± 8
Feces						
Control	100 ± 8	100 ± 7[p]	100 ± 6[q]	100 ± 8[r]	100 ± 8[s]	100 ± 8[t]
Treated	97 ± 10	107 ± 11	97 ± 5	97 ± 6	91 ± 7	96 ± 5
Lipoprotein fraction chylomicrons						
Control		100 ± 7[u]	100 ± 5[v]	100 ± 7[w]	100 ± 7[x]	100 ± 6[y]
Treated		68 ± 6*	77 ± 6*	102 ± 8	19 ± 5*	106 ± 7
VLDL						
Control		100 ± 7[z]	100 ± 6[aa]	100 ± 7[bb]	100 ± 8[cc]	100 ± 5[dd]
Treated		112 ± 7	93 ± 7	102 ± 8	12 ± 8	101 ± 6
LDL						
Control		100 ± 6[ee]	100 ± 7[ff]	100 ± 8[gg]	100 ± 7[ghh]	100 ± 7[ii]
Treated		100 ± 7	84 ± 6	99 ±	22 ± 5*	102 ± 6
HDL						
Control		100 ± 6[jj]	100 ± 7[kk]	100 ± 6[ll]	100 ± 7[mm]	100 ± 7[nn]
Treated		134 ± 7*	73 ± 6*	93 ±	112 ± 8	116 ± 8

[a] 12.24 mg Cholesterol/gm tissue; [b] 4.77 mg triglyceride/g tissue; [c] 28.35 mg neutral lipid/g tissue; [d] 4.39 mg phospholipid (P)/g tissue; [e] 4.5 mg of protein/g tissue; [f] 24.03 mg cholesterol/g tissue; [g] 44.11 mg neutral lipid/g tissue; [h] 6.37 mg triglyceride/g tissue; [i] 7.19 mg phospholipid(P)/g tissue; [j] 4.5 mg protein/g wet tissue; [k] 7.82 mg/g; [l] 6.98 mg/g; [m] 1.12 mg/g; [n] 2.06 mg/g; [o] 42 mg/g; [p] 28.47 mg/g; [q] 33.94 mg/g; [r] 1.86 mg/g; [s] 1.239 kg/g; [t] 6.99 mg/g; [u] 337 μg/ml; [v] 67 μg/ml; [w] 420 μg/ml; [x] 149 μg/ml; [y] 184 μg/ml; [z] 190 μg/ml; [aa] 98 μg/ml; [bb] 22 μg/ml; [cc] 26 μg/ml; [dd] 50 μg/ml; [ee] 210 μg/ml; [ff] 10 μg/ml; [gg] 45 μg/ml; [hh] 41 μg/ml; [ii] 122 μg/ml; [jj] 544 μg/ml; [kk] 620 μg/ml; [ll] 27 μg/ml; [mm] 153 μg/ml; [nn] 657 μg/ml

ner with 60 mg/kg resulting in a 59 % reduction. Neutral lipids followed the same pattern, but lagged behind the triglyceride reduction affording only 35 % reduction at 60 mg/kg. The phospholipid content was reduced 33 % at 20 mg/kg and 53 % at 40 mg/kg. Protein content was not reduced by drug treatment in mice.

When rats were treated with 2-furoic acid at 20 mg/kg/day, the changes in lipid content of the liver, small intestine and fecal excretion were not significantly altered with the exception that in liver phospholipid content was reduced 50 %; in the small intestine neutral lipids were reduced 28 % and phospholipids 14 % (Table IV). The fecal material

demonstrated no increase in the excretion of any of the lipid classes. Rat lipoproteins did demonstrate changes in the lipid content. Cholesterol content was reduced 32 % in the chylomicron and elevated 34 % in the HDL; triglycerides were lowered 23 % in the chylomicron and HDL fractions and 16 % in the LDL fraction. Phospholipid content was reduced 81 % in the chylomicron, 89 % in VLDL, and 78 % in the LDL fractions. ^{14}C-Cholesterol distribution studies in rats treated with 2-furoic acid at 20 mg/kg/day (Table V) showed that cholesterol was not accumulating in any of the major organs of the body. Rather the chyme collection from the intestine demonstrated a 10.74 % increase in cholesterol content after drug treatment. The cholesterol absorption from the intestine into the blood was reduced 14 % after 14 days dosing with 2-furoic acid. Bile excretion of cholesterol into the intestine was also elevated 14 % in the rats.

The LD_{50} for 2-furoic acid in CF_1 male mice I.P. was 100 mg/kg.

Discussion

The furoic acid and furylacrylic acid derivatives proved to be active hypolipidemic agents. These agents demonstrated activity superior to the commercially available agent clofibrate, which at 150 mg/kg lowers serum cholesterol levels 13 % and triglyceride levels 25 %. Clofibrate is essentially nonactive at 20 mg/kg in mice (28). 2-Furoic acid showed improved activity over clofibrate. 2-Furoic acid suppressed significantly both serum cholesterol and serum triglyceride levels in rodents unlike some commercially available agents that lower only cholesterol levels, e. g., *d*-thyroxine, probucol, cholestyramine, neomycin, sitosterols (29). 2-Furoic acid was active in hyperlipidemic induced mice, lowering blood lipids to near normal levels. The agent is active both by oral and intraperitoneal administration. Enzymatic studies suggest that 2-furoic acid suppresses enzyme activities at multiple sites in cholesterol, fatty acid and triglyceride *de novo* synthetic pathways. Cytoplasmic acetyl CoA is a key intermediate for both cholesterol and fatty acid synthesis. 2-Furoic acid suppresses mitochondrial citrate exchange, ATP-dependent citrate lyase and acetyl CoA synthetase activities. All three lead to reduced levels of cytoplasmic acetyl CoA. The regulatory enzyme of cholesterol synthesis, HMG CoA reductase, was inhibited *in vivo* but not *in vitro*. The key regulatory enzymes of triglyceride synthesis, *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase, were suppressed by 2-furoic acid *in vivo* and *in vitro*. The most effective dose for lowering blood lipids was 20 mg/kg/day which also correlated with the maximum suppression of these two enzymes.

Cholesterol 7α-hydroxylase is the regulatory enzyme for the conversion of cholesterol to bile acid; 2-furoic acid *in vitro* resulted in a significant increase in the enzyme activity. Acyl CoA cholesterol acyl transferase activity was also suppressed by the drug. This enzyme converts cholesterol to cholesterol ester for storage in tissue and particularly in the atherogenic plaques. Suppression of enzymatic activities should lead to reduced levels of cholesterol storage. Heparin induced hepatic lipoprotein lipase is the enzyme that releases fatty acids from the serum lipoproteins. 2-Furoic acid reduced the activity of this enzyme, suggesting that the lipoprotein would have difficulty releasing fatty acids to the tissues. The lipids which were lowered in the blood compartment by drug treatment were not being deposited in the major tissues. This can be observed first in the lipid analysis of the liver and small intestine. In fact, phospholipid content was reduced markedly in the livers of both mice and rats. Secondly, the ^{3}H-cholesterol distribution study demonstrated no increase in cholesterol in the major organs; rather there was an increase in the chyme content of ^{3}H-cholesterol indicating that the cholesterol was not being absorbed in the presence of drug. The cholesterol absorption studies demonstrated 14 % reduction in cholesterol absorption from the intestine. There was no increase in adrenal weight which is indicative that the drug did not cause hypertrophy of the adrenal cortex as a compensatory mechanism to increase steroidogenesis. The rat lipoprotein study demonstrated that 2-furoic acid in general caused a decrease in the lipid content of some of the lipoprotein fractions. However, perhaps the most significant finding was that after drug treatment, the cholesterol content of the HDL fraction was significantly elevated. The elevated cholesterol content of the HDL fraction supposedly is linked with the protection against cardiovascular infarctions according to the study of Miettinen et al. (30). The phospholipid content was decreased in the chylomicrons, VLDL and LDL. If the phospholipid is removed from these lipoproteins, the remnant is retarded in its uptake by the tissues.

Other reported hypolipidemic agents that contain the furan ring structure include benzofuran, 2,3-dihydrobenzofuran, 3-(2*H*)-benzofuranone-2-carboxylates (31), tricyclic benzofurans, e. g. ethyl-2-(4-dibenzofuranyloxy)-2-methylpropionate (32), coumarilic acid, benzo[*b*]thiophen- and indole-2-carboxylic acid (33). Parker et al. (34) have shown that 5-(tetradecyloxy)-2-furancarboxylic acid lowers blood lipids and inhibited fatty acid synthesis in rats and monkeys.

Although these 5-substituded 2-furoic acid derivatives required a higher dose to effectively lower serum lipid levels, they appear similar to the compounds discussed in this paper in that they inhibit acetate uptake into fatty acids but did not effect the regulatory enzyme of cholesterol synthesis (34). Both classes of compounds had similar effects on the liver lipid content of rodents.

Table V. The Effect of 2-Furoic Acid at 20 mg/kg/day, Orally on ^{3}H-Cholesterol Distribution in Sprague Dawley Rats.

(N = 6)	Organ Weight (grams) Control	Treated	DPM/Total Organ (% total) Control		Treated	
Brain	1.77	1.70	19,655	(0.216 %)	12,530	(0.133 %)
Heart	0.80	1.03	55,637	(0.610 %)	62,652	(0.665 %)
Lung	1.44	1.43	279,924	(3.073 %)	306,005	(3.248 %)
Kidney	1.93	1.73	137,238	(1.507 %)	127,760	(1.303 %)
Spleen	0.56	0.62	279,993	(3.074 %)	199,167	(2.114 %)
Liver	9.56	9.00	2,260,397	(24.814 %)	2,369,468	(25.150 %)
Stomach	2.10	1.54	87,747	(0.963 %)	80,458	(0.854 %)
Small intestine	7.23	6.1	3,003,579	(32.972 %)	3,288,614	(34.906 %)
Large intestine	3.92	3.14	244,838	(2.688 %)	281,227	(2.985 %)
Chyme	4.46	2.86	706,211	(7.752 %)	1,742,666	(18.497 %)
Feces	4.47	5.30	2,029,921	(22.2836 %)	952,448	(10.110 %)
Adrenals	66.6 mg	66.3 mg				

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