The Hypolipidemic Activity of 3-Iminophthalimidine in Rodents

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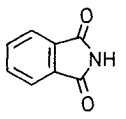
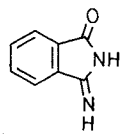
Abstract: 3-Iminophthalimidine (3-imino-2,3-dihydroisoindol-1-one) was examined for its hypolipidemic activity in rodents as a chemical modification of the phthalimide nucleus. Administration of 3-iminophthalimidine for 16 days, at 20 mg/kg/day, demonstrated potent hypolipidemic activity, decreasing serum cholesterol and triglyceride levels by 44 % and 41 %, respectively. No effects on organ or body weight, or food consumption were noted as a result of administration of this agent to rats. However, reductions of liver lipid levels, i.e., triglycerides and neutral lipids, as well as lipid content of serum lipoprotein fractions, i.e., cholesterol, triglyceride and neutral lipids, were observed following administration of this agent. An increase was noted in fecal excretion of cholesterol and/or its metabolites. This effect was not correlated with an increase in bile acid synthesis, but may due in part to increased biliary excretion of cholesterol. Significant inhibitory effects of 3-iminophthalimidine on liver enzyme activities were noted, including acetyl CoA carboxylase and *sn*-glycerol-3-phosphate acyl transferase. Mitochondrial citrate exchange was also greatly reduced at low concentrations of the agent. Inhibition at these sites could account for the reduction in serum triglyceride levels observed after treatment in rodents.

Phthalimide, when administered to rodents at 20 mg/kg/day for 16 days, demonstrated potent hypolipidemic activity, decreasing serum cholesterol and triglyceride levels by 41 % and 56 %, respectively (1,2). Since the initial report of the hypolipidemic activity of phthalimide and a series of *N*-substituted phthalimide derivates, a number of analogs similar in structure to phthalimide have also been found to possess hypolipidemic activity at low doses in rodents (2–4). 3-Imino-2,3-dihydroisoindol-1-one, more commonly known as 3-iminophthalimidine,was examined for its hypolipidemic activity as an extension of structure activity relationship studies of the parent compound, phthalimide. 3-Iminophthalimidine was generally more active than the *N*-substituted derivatives of this compound series. This compound differs in structure from phthalimide in that an imino group is substituted for an imido carbonyl group. Previously, studies have shown that removal of one of the carbonyl groups reduces the hypolipidemic activity. Substitution of a sulfur or nitrogen also leads to less activity. This is the first substitution of a nitrogen outside the ring. The purpose of this study was to examine the hypolipidemic activity of 3-iminophthalimidine in rodents as well as its effects on lipid metabolism. Previously, similar mechanism studies have been conducted with phthalamide (4) and the current data on 3-iminophthalimidine can be compared to those published for phthalamide (4).



Phthalimide



1 3-Iminophthalimidine

Materials and Methods

Source of Compounds

3-Iminophthalimidine was synthesized according to the methods of Byrne et al. (5). Phthalimide (Eastman Organic Chemicals) (10.0 g, 0.07 mol) was suspended in 60 ml of ammonium hydroxide and stirred at room temperature for 24 hours. The white solid product was collected and washed with cold water to afford 10.2 g (86 %) of phthalamide. Phthalamide[1] (2.0 g, 0.01 mol) was suspended in 14 ml of acetic anhydride, quickly heated to boiling, and allowed to boil for one hour until the solution was clear. Upon cooling, the solid which precipitated was collected and washed with cold ethanol to afford 0.6 g (40 %) of crude *o*-cyanobenzamide; m. p. 172°C. *o*-Cyanobenzamide (0.5 g, 0.003 mol) was heated for 15 minutes at 210°C under nitrogen. The product which sublimed on the reaction vessel wall was collected upon cooling and recrystallized from ethanol to afford 170 mg (34 %) of 3-iminophthalimidine; m. p. 198–201° C (sub) (Lit. mp 201°C) (5).

Hypolipidemic Screens in Normogenic Rodents

The test compound was suspended in 1 % carboxymethylcellulose-water and administered intraperitoneally to CF_1 male mice (~ 25 g) or orally to Holtzman male rats (~ 300 g) using an intubation needle, for 16 days. On days 9 and 16, blood was collected from non-fasted rodents 24 h after the last dose of drug in microcapillary tubes by tail vein bleeding and the serum obtained by centrifugation. Serum cholesterol levels were determined by a modification of the Liebermann-Burchard reaction (6). Blood was also collected on day 14 and serum triglyceride levels were determined by a commercial kit (Fisher, Hycel Triglyceride Test (1975), Hycel Inc.).

Testing in Hyperlipidemic Mice

CF_1 male mice (~ 25 g) were administered 50 % W/W ground lab chow (Wayne Blox Rodent Chow) and 50 % W/W commercial diet (US Biochemical Corporation Basal Atherogenic Test Diet) consisting of butterfat (400 g), fine mesh cellulose (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil (Wesson Oil) (40 g), sodium cholate (20 g), sucrose (223 g), vitamin free casein (200 g), and a total vitamin supplement for two weeks. Daily animal weights and food intake per animal were determined throughout the course of

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the experiment. After cholesterol and triglyceride levels were determined and observed to be elevated, the mice were administered the test compound intraperitoneally at 20 mg/kg/day for an additional 16 days. Serum cholesterol (6) and triglyceride (1) were again measured after 14 days of dosing in non-fasted mice 24 h after the last dose, and expressed as percent of control for animals on that diet.

Animal Weight and Food Intake

Animal weights were determined periodically during the course of the experiment and expressed as percentage of the animal's weight on day 0. The average daily food intake per animal (g/day) was also determined after 16 days, and major organs were excised, trimmed of fat, and weighted.

Toxicity Studies

The acute toxicity (LD_{50} values) (7) was determined by the administration of the test compound intraperitoneally, from 100 mg to 500 mg/kg as a single dose, in CF_1 male mice. The number of deaths in the group was recorded.

Enzymatic Studies

All subsequent studies were conducted on non-fasted animals where the tissue sample was excised 24 h after the last dose.

In vitro enzymatic studies were determined using CF_1 male mouse liver homogenates and drug from 2.5 to 10 mM final concentrations. *In vivo* enzymatic studies were determined using 10% homogenates of liver from CF_1 male mice treated for 16 days from 5 to 50 mg/kg/day in 0.25 M sucrose and 0.001 M ethylenediaminetetraacetic acid using literature procedures: acetyl coenzyme A synthetase and citrate lyase (8); 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (cholesterol synthesis) (9, 10); acetyl coenzyme A carboxylase (11); fatty acid synthetase (12); *sn*-glycerol-3-phosphate acyl transferase (13); phosphatidate phosphohydrolase (14); 7-α-hydroxylase activity (15); mitochondrial citrate exchange (16, 17); cholesterol side chain oxidation (18); and, acyl coenzyme A: cholesterol acyl transferase activity (19). Counting was performed in a Packard Scintillation Counter, and the samples were corrected for quench by using an internal standard and a quench curve.

Liver Lipid Extraction

CF_1 male mice were administered the test compound at doses ranging from 5 to 50 mg/kg/day for 16 days. Afterwards, the livers were excised and a 10% homogenate prepared. An aliquot of the homogenate was extracted by the method of Folch et al. (20) and Bligh and Dyer (21) and analyzed for cholesterol (6), triglyceride with the commercial kit (Hycel Inc.), neutral lipid (22) and phospholipid level (23) content.

Determination of ^{14}C-Cholesterol Distribution in Rats

Holtzman male rats (~400 g) were administered the test compound orally at 20 mg/kg/day. On day 13, 10 μCi of 1,2-^{3}H(N)-cholesterol (40.7 Ci/mmol) was administered orally. Twenty-four hours after cholesterol administration, the major organs were exercised and samples of blood, chyme, and feces were collected. Homogenates were prepared and samples were combusted or plated on filter paper, dried and digested for 24 h in Hyamine Hydroxide® and counted. Results are expressed as dpm of total organ and percent of radioactivity recovered.

Bile Cannulation Study

Holtzman male rats (~400 g) were administered the test compound orally at 20 mg/kg/day. On the 14th day, the rats were anesthetized, the bile duct was cannulated as previously described (4). 4-^{14}C-Cholesterol (52.5 mCi/mmol) (10 μCi) was injected intravenously into the base of the tail of the rats. The bile was collected over the next six hours and the total volume, in milliliters, measured. Aliquots (100 μl) were counted and analyzed for cholesterol and phospholipid content.

Plasma Lipoprotein Study

Holtzman male rats (~400 g) were administered the test drug intraperitoneally at 20 mg/kg/day. On the 14th day, the blood was collected from the abdominal aorta and the lipoprotein fractions were separated by the method of Hatch and Lees (24) and Havel et al. (25). The cholesterol (6), triglyceride (Hycel Inc.), neutral lipid (22), phospholipid (23), and protein (26) levels were determined for each lipoprotein fraction.

Statistical Measurement

The mean and the standard deviation were calculated and are designated as $\bar{X} \pm$ S.D. The number of the animals or test samples per group is designated by the letter "N". The probable significant level (P) between test sample and control samples was determined using the Students "t" test (27).

Results

3-Iminophthalimidine was observed to greatly reduce serum lipid levels in rodents after administration at low doses for two weeks (Table I). This agent showed almost equal hypocholesterolemic activity at all four doses tested, with a dose of 50 mg/kg/day affording a 47% reduction in serum cholesterol levels after 16 days intraperitoneal administration to mice. In mice,

Table I. The Effects of 3-Iminophthalimidine Administered Intraperitoneally to CF_1 Male Mice, or Orally to Holtzman Male Rats for 16 Days

Species (N = 6)	Dose (mg/kg/day)	Percent Control, $\bar{X} \pm$ S.D. Serum Cholesterol Day 9	Day 16	Serum Triglyceride Day 14
Mouse	Control (1% CMC)	100 ± 7[b]	100 ± 9[c]	100 ± 8[d]
	3-Iminophthimidine			
	5	68 ± 6[a]	54 ± 5[a]	65 ± 6[a]
	10	76 ± 7[a]	55 ± 4[a]	51 ± 4[a]
	20	64 ± 5[a]	56 ± 4[a]	59 ± 5[a]
	50	71 ± 4[a]	53 ± 6[a]	64 ± 4[a]
	Clofibrate			
	150	88 ± 7	87 ± 5	75 ± 5
Rat	Control 1% CMC	100 ± 9[e]	100 ± 8[f]	100 ± 7[g]
	3-Iminophthalimidine			
	10	87 ± 8[a]	80 ± 7[a]	51 ± 4[a]
	20	69 ± 6[a]	60 ± 5[a]	52 ± 4[a]
	Clofibrate			
	20	98 ± 12	96 ± 15	91 ± 10

[a] p ± 0.001; [b] 118 mg%; [c] 122 mg%; [d] 137 mg%; [e] 73 mg%; [f] 78 mg%; [g] 110 mg%.

maximum hypotriglyceridemic activity was demonstrated after 14 days dosing at 10 mg/kg/day with reductions in serum triglyceride levels of 49%. 3-Iminophthalimidine demonstrated good hypolipidemic activity when administered orally to rats, with a dose of 20 mg/kg/day reducing serum cholesterol levels by 40% and serum triglyceride by 48%. 3-Iminophthalimidine, when administered to diet-induced hyperlipidemic mice for two weeks at 20 mg/kg/day, was able to decrease serum cholesterol and triglyceride levels by 26% and 33%, respectively, from the maximum levels attained after two weeks of feeding the atherogenic diet (Table II).

The administration of 3-iminophthalimidine at low doses, such as 10 or 20 mg/kg/day, to rodents for 16 days had no effect on rodent body weight nor on the appetite of the rats or mice. No significant differences were observed in the weight of the major organs of rats after 2 weeks administration at 20 mg/kg/day.

The *in vivo* effects of 3-iminophthalimidine, after two weeks administration on liver enzyme activities, are shown in Table III. Acetyl CoA carboxylase activity was inhibited significantly, with a dose of 10 mg/kg/day causing a maximal reduction of 72% of enzyme activity. *sn*-Glycerol-3-phosphate acyl transferase activity was also inhibited greatly at all doses, with a maximal reduction of enzyme activity of 54% occurring at a dose of 20 mg/kg/day. This agent essentially has no effect on fatty acid synthetase activity; however, an increase in phosphatidate phosphohydrolase activity of 37% was observed at 20 mg/kg/day. Acetyl CoA synthetase activity was maximally inhibited by 45% at 20 mg/kg/day, and only a slight reduction of cholesterol synthesis activity of 14% was noted at 10 mg/kg/day. Administration of 3-iminophthalimidine over the dose range studied, had little effect on DNA, RNA, or protein synthesis, with the exceptions that ^{3}H-thymidine incorporation into DNA was inhibited 36% and there was an increase of 22% of ^{3}H-leucine incorporation into protein at 50 mg/kg/day.

Table II. The Effects of 3-Iminophthalimidine (20 mg/kg/day) Administered Intraperitoneally to Hyperlipidemic CF_1 Male Mice

Compound (N = 6)	Percent Control, $\bar{X}\pm$ S.D. Serum Cholesterol		Serum Triglyceride	
	2-Week Diet	Day 14 Drug	2-Week Diet	Day 14 Drug
Control (1% CMC)	100 ± 6[b]	100 ± 7[c]	100 ± 5[d]	100 ± 4[e]
Control Atherogenic Diet-1% CMC	289 ± 9[a]	290 ± 9[a]	131 ± 3[a]	131 ± 5[a]
3-Iminophthalimidine	289 ± 8[a]	215 ± 8[a] (26)*	131 ± 7[a]	98 ± 9 (33)*

*These figures represent the per cent decrease in plasma cholesterol or triglyceride levels from the maximum levels attained after two weeks of feeding the atherogenic diet.

[a] $p = 0.001$, significant differences from control value; [b] 118 mg%; [c] 122 mg%; [d] 136 ± 7 mg%; [e] 139 ± 5 mg%.

The *in vitro* effects of 3-iminophthalimidine at 2.5 to 10 mM on a number of mouse liver enzyme activities are shown in Table IV. Acetyl CoA carboxylase and acyl transferase activities were inhibited significantly with 10 mM of drug, by 68% and 47%, respectively. Fatty acid synthetase activity was inhibited by 32% with 5 mM of drug. Phosphatidate phosphohydrolase activity was not affected *in vitro* for all drug concentrations. Acetyl CoA synthetase activity was inhibited 19% with 10 mM of this agent. The cholesterol synthesis was not inhibited by 3-iminophthalimidine; indeed, an increase of 24% was observed with 10 mM of drug. Mitochondrial citrate exchange was markedly inhibited by 64% with 2.5 mM of the drug. Cytoplasmic citrate lyase activity was slightly inhibited by 17% at 5 mM of drug. Cholesterol side chain oxidation and 7-α-hydroxylase activity were reduced by 77% and 52%, respectively, at 10 mM of drug. Acyl : CoA cholesterol acyl transferase activity was also inhibited by 41% with 10 mM of drug.

Administration of 3-iminophthalimidine to mice from 5 to 50 mg/kg/day resulted in a reduction of liver lipids (Table V). Total liver lipid content was reduced 41% and liver cholesterol levels were reduced by 22% at 20 mg/kg/day. Neutral lipid and phospholipid levels were reduced 47% and 31%, respectively, at 20 mg/kg/day. The effects of the administration of 3-iminophthalimidine at 20 mg/kg/day for 16 days to Holtzman male

Table III. *In vivo* Effects of 3-Iminophthlalimidine on CF_1 Male Mouse Liver Enzyme Activities After Dosing for 16 Days

Enzyme Activity Measured (N = 6)	Control (1% CMC)	Percent control, $\bar{X}$ ± S.D. 3-Iminophthalimidine (mg/kg/day)			
		5	10	20	50
Acetyl CoA Synthetase	100 ± 7	98 ± 7	85 ± 5	54 ± 7	84 ± 6[b]
HMG-CoA Reductase	100 ± 5	89 ± 7	86 ± 7[b]	89 ± 8	88 ± 6[b]
Acetyl CoA Carboxylase	100 ± 6	54 ± 4[a]	28 ± 3[a]	46 ± 4[a]	50 ± 4[a]
Fatty Acid Synthetase	100 ± 6	92 ± 8	88 ± 8	79 ± 7[a]	107 ± 9
Phosphatidate phosphohydrolase	100 ± 5	103 ± 10	110 ± 11	137 ± 11[a]	106 ± 10
sn-Glycerol-3-phosphate Acyl Transferase	100 ± 14	49 ± 2[a]	47 ± 4[a]	46 ± 4[a]	49 ± 2[a]
^{3}H-Thymidine into DNA	100 ± 9	81 ± 7[b]	107 ± 13	111 ± 11	64 ± 1[a]
^{3}H-Uridine into RNA	100 ± 14	108 ± 1	87 ± 8	112 ± 4	120 ± 5
^{3}H-Leucine into Protein	100 ± 3	98 ± 6	123 ± 1[a]	114 ± 1[a]	122 ± 1[a]

[a] $p \leq 0.001$; [b] $p \leq 0.005$

Table IV. *In vitro* Effects of 3-Iminophthalimidine (*1*) on CF_1 Male Mouse Liver Enzyme Activities

Drug (N = 6)	mM Drug	Mitochondrial Citrate Exchange	Acetyl CoA Synthetase	Citrate Lyase	HMG-CoA Reductase	Cholesterol Side Chain Oxidation
Control (1 % CMC)	–	100 ± 4[c]	100 ± 6[d]	100 ± 4[e]	100 ± 9[f]	100 ± 8[g]
1	2.5	36 ± 12[a]	94 ± 6	88 ± 2[a]	114 ± 4	62 ± 28
~	5.0	141 ± 7[a]	88 ± 14	83 ± 1[a]	123 ± 12[b]	38 ± 10[a]
	10.0	64 ± 11[a]	81 ± 7[a]	86 ± 1[a]	124 ± 11[b]	23 ± 8[a]

Drug (N = 6)	mM Drug	Cholesterol 7-α-Hydroxylase	Acyl CoA: Cholesterol Acyl Transferase	Acetyl CoA Carboxylase	Fatty Acid Synthetase	*sn*-Glycerol-3-Phosphate Acyl Transferase	Phosphatidate Phosphohydrolase
Control (1 % CMC)	–	100 ± 16[h]	100 ± 9[i]	100 ± 6[j]	100 ± 18[k]	100 ± 12[l]	100 ± 17[m]
1	2.5	94 ± 12	99 ± 11	42 ± 15[a]	69 ± 6[b]	82 ± 5	91 ± 5
~	5.0	76 ± 10	71 ± 5[a]	44 ± 11[a]	68 ± 4[a]	76 ± 12	94 ± 6
	10.0	48 ± 2[a]	59 ± 10[a]	32 ± 1[a]	72 ± 5[b]	53 ± 11[a]	95 ± 6

[a] $p \leq 0.001$; [b] $p \leq 0.025$; [c] 38 % mitochondrial citrate exchange; [d] 28.5 mg acetyl CoA formed/g wet tissue/20 min; [e] 30.5 mg citrate hydrolyzed/g wet tissue/10 min; [f] 906,916 dpm/g wet tissue/60 min; [g] 400,022 dpm CO_2 formed/mg mitochondrial protein/18 h; [h] 224,000 dpm/μg microsomal protein/20 min; [i] 4808 dpm/mg microsomal protein/20 min; [j] 1,159332 dpm/g wet tissue/20 min; [k] 51,637 dpm/g wet tissue/20 min; [l] 15,003 dpm/g wet tissue/30 min; [m] 16.7 μg P_i/g wet tissue/15 min.

Table V. The Effects of 3-Iminophthalimidine on CF_1 Male Mouse Liver Lipids After Dosing at 20 mg/kg/day Intraperitoneally for 16 Days

		Per cent Control, $\bar{X}$ ± S.D.				
Compound (N = 6)	Dose (mg/kg/day)	Total Lipid	Cholesterol	Triglyceride	Neutral Lipid	Phospholipid
Control (1 % CMC)		100 ± 14	100 ± 4	100 ± 6	100 ± 5	100 ± 10
3-Iminophthalimidine	5	69 ± 14	77 ± 6[a]	60 ± 5[a]	84 ± 1[a]	81 ± 1[a]
	10	69 ± 8[a]	97 ± 4	31 ± 3[a]	67 ± 6[a]	85 ± 4[b]
	20	59 ± 5[a]	78 ± 4[a]	39 ± 4[a]	53 ± 14[a]	69 ± 18[b]
	50	61 ± 9[a]	80 ± 7[a]	32 ± 2[a]	95 ± 8	86 ± 18

[a] $p = 0.001$; [b] $p = 0.005$

Table VI. Effects of 3-Iminophthalimidine After 14 Days Dosing on ^{3}H-Cholesterol Distribution in Holtzman Rats 24 h After Administration Orally

	Control		Treated	
Organ	Total Organ dpm	Percent Recovery	Total Organ dpm	Percent Recovery
Brain	42412	1.21	11897	0.34
Heart	37638	1.07	24143	0.69
Lung	100584	2.87	86776	2.48
Liver	901785	25.77	615827	17.60
Spleen	67760	1.93	62632	1.79
Kidney	69192	1.97	38489	1.10
Stomach	127446	3.64	123515	3.53
Small Intestine	851406	24.33	983224	28.10
Large Intestine	246924	7.05	250179	7.17
Chyme	163977	4.68	175301	5.01
Feces	889892	25.43	1126683	32.20

rats on radiolabeled cholesterol distribution are shown in Table VI. The most significant effects were the increase in the amount of radiolabel recovered in the small and large intestine, as well as the chyme and feces. The effects of the drug (20 mg/kg/day) on rat bile excretion showed there was a 24 % increase in the volume of total bile output after six hours of collection with an increase in ^{3}H-cholesterol and its metabolites (83 %), and phospholipid (9 %).

Administration of 3-iminophthalimidine to rats for two weeks at 20 mg/kg/day caused a reduction of lipids in all lipoprotein classes (Table VII). Cholesterol, neutral lipid, and triglyceride levels were reduced in all four lipoprotein classes by 24 to 64 %. Protein levels remained essentially unchanged with the exception of the chylomicron and VLDL protein content after administration of the drug. Phospholipid levels remained unchanged in chylomicrons and VLDL, and were greatly increased in HDL (121 %). The LD_{50} values in CF_1 mice for 3-iminophthalimidine was 300 mg/kg/ I. P.

Table VII. The Effects of 3-Iminophthalimidine on Rat Serum Lipoprotein Fractions After Dosing Intraperitoneally for 14 Days at 20 mg/kg/day

	Percent Control, $\bar{X}$ ± S.D.									
	Chylomicrons					VLDL				
Drug (N = 6)	Cholesterol	Neutral Lipid	Triglyceride	Phospholipid	Protein	Cholesterol	Neutral Lipid	Triglyceride	Phospholipid	Protein
Control (1% CMC)	100 ± 9[c]	100 ± 8[d]	100 ± 6[e]	100 ± 10[f]	100 ± 7[g]	100 ± 8[h]	100 ± 9[j]	100 ± 7[j]	100 ± 7[k]	100 ± 8[l]
3-Iminophthalimidine	45 ± 5[a]	50 ± 4[a]	47 ± 5[a]	100 ± 9	40 ± 3[a]	65 ± 6[a]	58 ± 3[a]	46 ± 4[a]	108 ± 8	45 ± 7[a]
	LDL					HDL				
Drug (N = 6)	Cholesterol	Neutral Lipid	Triglyceride	Phospholipid	Protein	Cholesterol	Neutral Lipid	Triglyceride	Phospholipid	Protein
Control (1% CMC)	100 ± 9[m]	100 ± 7[n]	100 ± 8[o]	100 ± 7[p]	100 ± 8[q]	100 ± 8[r]	100 ± 9[s]	100 ± 4[t]	100 ± 6[u]	100 ± 8[v]
3-Iminophthalimidine	59 ± 3[a]	9 ± 2[a]	42 ± 3[a]	111 ± 8	100 ± 4	65 ± 5[a]	33 ± 4[a]	48 ± 5[a]	221 ± 12[a]	83 ± 7

[a] $p = 0.001$; [b] $p = 0.010$; [c] 337 μg/ml; [d] 67 μg/ml; [e] 420 μg/ml; [f] 149 μg/ml; [g] 3 mg/ml; [h] 190 μg/ml; [i] 98 μg/ml; [j] 221 g/ml; [k] 26 μg/ml; [l] 50 μg/ml; [m] 210 μg/ml; [n] 10 μg/ml; [o] 45 μg/ml; [p] 41 μg/ml; [q] 680 μg/ml; [r] 544 μg/ml; [s] 153 μg/ml; [t] 27 μg/ml; [u] 153 μg/ml; [v] 5.7 mg/ml

Discussion

3-Iminophthalimidine has demonstrated potent hypolipidemic activity in both rats and mice, reducing serum cholesterol and triglyceride significantly at 20 mg/kg/day. Clofibrate was not effective at 20 mg/kg/day in rats, and only marginal reductions were observed in mice at 150 mg/kg. 3-Iminophthalimidine was active as a hypolipidemic agent when administered either orally or intraperitoneally. The agent also demonstrated good hypolipidemic activity in diet-induced hyperlipidemic mice, with reductions in serum cholesterol and triglyceride levels of sufficient magnitude to cause serum lipid levels to approach control values. The agent was able to decrease both serum cholesterol and triglyceride levels to the same extent. The dose required for hypolipidemic activity of 3-iminophthalimidine was within a safe therapeutic range and similar to other cyclic imides, e.g., phthalimide, saccharin, succinimide and 1,8-naphthalimide derivatives (1–4). 3-Iminophthalimidine does not exert its hypolipidemic effect through a reduction in the dietary intake of the animals.

Inhibition of a number of key enzymes involved in lipid metabolism probably plays a major role in the hypolipidemic effect of the agent. Mitochondrial citrate exchange was greatly reduced by 3-iminophthalimidine, which is similar to the effects of other cyclic imides. Reduction in the exchange would cause a reduction of cytoplasmic acetyl CoA levels, and thus a net suppression of cholesterol and fatty acid *de novo* synthesis. Cytoplasmic citrate lyase and acetyl CoA synthetase activities were slightly inhibited, and therefore the inhibition of mitochondrial citrate exchange may be playing a more important role in the suppression of cytoplasmic acetyl CoA level. The accumulated suppressions of both mitochondrial citrate exchange and citrate lyase may severely reduce cytoplasmic acetyl CoA availability for lipid metabolism to a critical level. Other researchers have positively related the ability of 5-(tetradecyloxy)-2-furoic acid and terephthalic acid to suppress mitochondrial citrate transport to its hypotriglyceridemic effect in rodents (28).

Lamb et al. (13) found that a number of 1,3-bis-(substituted)-2-propanones were potent inhibitors of *sn*-glycerol-3-phosphate acyl transferase and phosphatidate phosphohydrolase activities, both *in vitro* and *in vivo*. These agents also reduced serum triglyceride levels in rats, suggesting that a correlation existed between the agents' ability to suppress the activities of these two enzymes and their hypotriglyceridemic activity. A number of cyclic imides have been shown to reduce the activities of these same two enzymes in mice. Clofibrate has also been shown to inhibit rat liver acetyl CoA carboxylase activity and *sn*-glycerol-3-phosphate acyl transferase (29), as well as mouse liver phosphatidate phosphohydrolase and *sn*-glycerol-3-phosphate acyl transferase activities (30). Acetyl CoA carboxylase is the rate limiting enzyme in the formation of fatty acids, while *sn*-glycerol-3-phosphate acyl transferase is a regulatory enzyme involved in triglyceride biosynthesis. The degree of reduction of enzyme activity by the drug would account for the magnitude of reduction of serum triglyceride levels. 3-Iminophthalimidine increased phosphatidate phosphohydrolase activity *in vitro*. This is a unique action for a cyclic imide, since phthalimide, saccharin, and 1,8-naphthalimide have the opposite effect on the activity of this enzyme as does clofibrate. Nevertheless, after treatment with 3-iminophthalimidine, a subsequent decrease in liver phospholipid levels was observed.

Studies using radiolabeled cholesterol indicated that the administration of 3-iminophthalimidine resulted in an increase in radiolabel recovered in the small and large intestine as well as the chyme and fecal matter, suggesting that the agent accelerated the removal of cholesterol or its metabolites from the blood and major organs, or it interfered with the absorption of cholesterol or bile acids by the small intestine. Results from the bile study indicate that a slight increase was observed in bile flow. More importantly, biliary cholesterol concentration increased after administration of this agent. Neither cholesterol 7-α-hydroxylase activity nor cholesterol

side chain oxidation were increased after administration of this agent. The increase in bile cholesterol concentration coupled with the increase in bile flow may be sufficient to account in part for the hypocholesterolemic effect of the agent.

In rats, clofibrate has been shown to increase biliary cholesterol and phospholipid levels and to decrease the bile acid content of bile (31). The latter observation is in agreement with the finding that clofibrate reduced 7-α-hydroxylase activity (32). Such findings indicate that the hypolipidemic effect is exerted by the increase in bile cholesterol excretion with little effect on the elimination of bile acids. Pirinixil, another hypolipidemic agent, has also been observed to decrease 7-α-hydroxylase activity. Like the imide analogs, this agent was found to have no effect on HMG-CoA reductase activity, the regulator enzyme of cholesterol synthesis, and to reduce liver cholesterol and triglyceride content (33).

3-Iminophthalimidine sharply reduced plasma lipoprotein lipid levels by 30 to 50%. Although lipoprotein lipid levels were decreased, essentially no change was observed in the ratios of the various lipids comprising the lipoprotein structure. This suggets that the agent exerts its effect at more than one site in lipoprotein metabolism, particularly in the liver. The liver plays an important role in the formation and secretion of lipoprotein particles, and changes in the ability of the liver to perform this function would cause subsequent changes in the serum lipoprotein composition of lipids.

The metabolism of various lipoproteins is highly integrated and their integrity is contingent upon the ratios of the various lipids and proteins comprising the lipoprotein complex. Alteration of normal lipid metabolism by this agent may show varied effects on the lipoprotein classes. Evidence indicated that the LDL/HDL or LDL cholesterol/HDL cholesterol ratios may be more reliable indicators of the hypolipidemic or lipid modifying effects of various agents. It is believed that increased HDL or HDL cholesterol levels and/or decreased LDL or LDL cholesterol levels are desirable. Administration of 3-iminophthalimidine demonstrates no sginificant change in the LDL/HDL or LDL cholesterol/HDL cholesterol ratios, although the total serum lipid and lipoprotein lipid levels, including cholesterol, are sharply reduced.

In conclusion, 3-iminophthalimidine has demonstrated promise as a hypolipidemic agent, and the agent shows many of the same effects on lipid metabolism as other commercially available hypolipidemic agents.

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