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Research Article

Hypolipidemic Activity of *o*-(*N*-Phthalimido)acetophenone in Sprague Dawley Rats

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o-(*N*-Phthalimido)acetophenone has proven to be an effective hypolipidemic agent in rats at 20 mg/kg/day orally. The agent suppressed the activity of the rate-limiting enzyme of the liver involved in de novo synthesis of triglycerides. The synthetic rate-limiting enzyme for cholesterol esters was also inhibited by the drug *in vivo*. *o*-(*N*-Phthalimido)acetophenone lowered cholesterol in the liver and the aorta wall and generally caused an increase in phospholipids in body tissues. Serum lipoproteins were modulated by the drug with a decrease in cholesterol and triglycerides in the chylomicron, very low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) and an increase in high-density lipoprotein (HDL) cholesterol. The phospholipid content was increased in the chylomicron, VLDL, and LDL fractions. In hyperlipidemic rats, *o*-(*N*-phthalimido)acetophenone lowered elevated blood lipid levels at 20 mg/kg/day orally after 3 weeks of administration. The hypolipidemic rat after drug treatment had a lower LDL cholesterol and a higher HDL cholesterol content, which is therapeutically desirable to protect against cardiovascular disease.

KEY WORDS: *o*-(*N*-phthalimide)acetophenone; hypolipidemic agents; cholesterol; high-density lipoprotein (HDL) cholesterol.

INTRODUCTION

Previously studies have shown that *o*-(*N*-phthalimido)acetophenone possessed potent hypolipidemic activity in CF_1 mice from 12.5 to 100 mg/kg/day i.p., lowering serum cholesterol levels 37% and the serum triglyceride level 44%, and in Sprague Dawley rats from 10 to 30 mg/kg/day, lowering serum cholesterol 46% and serum triglyceride 39% (1). The agent suppressed *in vivo* acetyl CoA synthetase, HMG CoA reductase, cholesterol acyl transferase, phosphatidate phosphohydrolase, and *sn*-glycerol-3-phosphate acyl transferase activities in CF_1 mouse liver after 16 days of administration from 10 to 60 mg/kg/day. The present study was undertaken to assess the lipid lowering effects of the drugs for a longer period of time, i.e., 8 weeks, to evaluate the effects of the drug on lipoprotein synthesis as well as the apoprotein content of the lipoprotein fractions, and to determine the drug's ability to lower these same parameters in a hypolipidemic-induced state in rats.

METHODS

o-(*N*-Phthalimido)acetophenone was synthesized according to a literature procedure (1). All isotopes were purchased from New England Nuclear and substrates and cofactors were purchased from Sigma Chemical Co.

Acute Toxicity Studies

CF_1 mice (30 g) were administered *o*-(*N*-phthalimido)acetophenone, from 100 mg to 2 g i.p. The number of deaths was recorded for 7 days.

Blood Lipid Levels in Normal Rats

Sprague Dawley male rats weighing 210 g were commenced on an 8-week study. *o*-(*N*-Phthalimido)acetophenone was orally administered at a dose of 20 mg/kg/day and the control received the vehicle, 1% carboxymethylcellulose. Weekly blood samples were obtained from the tail vein and the serum was obtained by centrifugation (3000*g* × 3 min). The serum cholesterol was determined by a literature procedure (3) and serum triglyceride levels were determined by a commercial kit (Bio/Dynamic/bmc triglycerides). The livers were removed from animals sacrificed in the eighth week and a 10% homogenate was prepared in 0.25 *M* sucrose + 0.001 *M* EDTA, pH 7.2. The following enzyme activities were determined by literature procedures: ATP-dependent citrate lyase (4), acetyl CoA synthetase (5), HMG CoA reductase (6,7), acyl CoA cholesterol acyl transferase (8), cholesterol-7-α-hydroxylase (9), acetyl CoA carboxylase (10), *sn*-glycerol-3-phosphate acyl transferase (11), phosphatidylate phosphohydrolase (12), heparin-induced hepatic lipoprotein lipase (13), acid phosphatase (14), cathepsin (15), and catalase (16,17). Protein for the enzyme assays was determined by the Lowry technique (18).

Liver, Aorta, and Fecal Lipid Extraction

After 8 weeks' dosing of rats the liver, small intestine,

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aorta, and fecal samples (24-hr collection) were removed and 10% homogenates in 0.25 *M* sucrose + 0.001 *M* EDTA were prepared (19). Aliquots (2 ml) of the homogenate were extracted by the Folch *et al.* (20) and Bligh and Dyer (21) methods and the number of milligrams of lipid was determined. The lipid was taken up in ethyl acetate and the cholesterol (3), triglyceride (Bio-Dynamic/bmc triglycerides), neutral lipids (22), phospholipid (23), and protein (18) contents were determined.

Lipoprotein Separation

In Sprague Dawley rats (~210 g), the blood was collected from the abdominal vein after 8 weeks of administration of drug at 20 mg/kg/day orally. The serum was separated from the red blood cells (RBC) by centrifugation (3000*g* × 10 min). The basic ultracentrifuge methods of Hatch and Lees (24) and Havel *et al.* (25) were used for lipoprotein fraction separation, as modified for the rat by Mookerjea *et al.* (26). Each fraction was analyzed for cholesterol (3), triglycerides, neutral lipids (22), phospholipids (23), and protein (18).

Synthetic Rate of Lipoprotein

Sprague Dawley rats (~300 g) were administered *o*-(*N*-phthalmido)acetophenone orally at 20 mg/kg/day for 14 days. 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), [1-^{14}C]palmitic acid (57 mCi/mmol), or buffered ^{32}P (H_2PO_4) (2 mCi) was injected i.v. in the tail vein in isotonic saline, pH 7.4 (27). Twenty-four hours later, the animals were anesthesized with ether and blood was collected from the abdominal vein. The serum lipoprotein fractions were separated by ultracentrifuge techniques, then dialyzed, and aliquots were analyzed for radioactive content. The radioactivity was expressed as disintegrations per minute per milligram of protein isolated.

Apoprotein Analysis

The lipoprotein fraction (5 ml) was dialyzed against 0.1 *M* NaCl, pH 7.3 (18–24 hr), using Spectropor tubing with a molecular weight cutoff of 3500. The lipoprotein fractions were delipidated by extracting with equal volumes of EtO:EtOH (3:2). The aqueous layer was harvested by centrifugation (3500*g* × 10 min) (28). The organic residues were allowed to evaporate off the final aqueous residue. The aqueous layer was removed by rotovap for 10 min at 45°C. The residue was redissolved in 0.1 *M* NaCl, pH 7.3 (1 ml). After analyzing the protein by the Lowry technique (18) the volume was adjusted to 1 μg protein/ml. An aliquot (5–50 μg) was loaded onto a 5–15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) running gel with a 3% acrylamide stacking gel on top which had previously been polymerized (28). Rat serum albumin (10 μg) was used as the standard. The gel was run at a constant current of 20 mA for 4–4.5 hr (negative anode on top and positive anode on bottom). The gel was fixed in 10% trichloroacetic acid (TCA) incubated from 30 to 60 min and then stained in 0.5% Coomassie blue in 20% EtOH diluted 25-fold with 7.5% acetic acid. The gel was destained with 10% acetic acid, 5% MeOH, and 10% glycerol, with 10 to 60 min per rinse.

Radiolabeled Lipid Distribution

Aliquots (20 μCi) of [1,2-^{3}H]cholesterol (40.7 mCi/mmol), [1-^{14}C]palmitic acid (57 Ci/mmol), or H_2 $^{32}PO_4$ (2 mCi) were administered to Sprague Dawley rats (~300 g) i.v. (0.1 ml) in buffered saline, pH 7.4, which had been treated with *o*-(*N*-phthalimido)acetophenone at 20 mg/kg/day or 1% CMC orally for 2 weeks.

Twenty-four hours later, tissue samples of the major organs were excised, homogenized (10%), and combusted (Packard tissue oxidizer) and the radioactivity was counted (29). Aliquots of fecal (24-hr collection) and chyme homogenates were plated on filter paper (Whatman No 1), digested for 24 hr in Hyamine hydroxide (2 ml) at 40°C, and counted.

Hyperlipidemic Rats

Sprague Dawley male rats (~300 g) were maintained on an atherogenic basal diet (32) (U.S. Biochemical Corporation, Atherogenic Test Diet) mixed 1:1 with powdered Wayne Blox Rodent Chow and water *ad libitum* for 4 weeks (29). After 4 weeks the serum cholesterol and triglyceride levels were elevated from 75 to 703 and from 110 to 515 mg%, respectively. *o*-(*N*-Phthalimido)acetophenone treatment at 20 mg/kg/day orally was commenced and continued for the next 4 weeks. Rats were bled by tail vein weekly and serum cholesterol and triglycerides levels were determined. Twenty-four hours prior to sacrifice, [^{3}H]leucine, [^{3}H]cholesterol, [1-^{14}C]palmitic, or H_2 $^{32}PO_4$ was injected i.v. Lipoproteins were separated and the radioactivity content and chemical analysis of lipids were determined on each serum fraction separated. In Tables I through IX, statistical analysis was determined by Student's *t* test on the original data.

RESULTS

o-(*N*-Phthalimido)acetophenone is an effective hypolipidemic agent when administered for 8 weeks in normal rats as well as hyperlipidemic rats. Serum cholesterol and triglycerides were not markedly reduced until the third week for normal rats but the values continued to decline through week 7, where they appeared to stabilize. In one group of rats treated for 8 weeks, drug therapy was discontinued and the serum cholesterol and triglyceride levels returned to control levels within 2 weeks, i.e., the tenth week, and remained constant in the twelfth week (Table I). Treated-animal body weights were not altered from the controls over the 8 weeks, at which time body weights of 410 g were achieved. The daily food intake (g/day/rat) after treatment was unchanged when using paired animals. Organ weights after 8 weeks of treatment were within normal limits of the control values. The LD_{50} in CF_1 mice was 2 g/kg i.p.

Liver enzyme studies after 8 weeks of administration of drug (Table II) showed a significant ($P \leq 0.001$) reduction of 39% in ATP-dependent citrate lyase activity, 28% in acetyl CoA synthetase activity, 32% in acyl CoA cholesterol acyl transferase activity, 37% in *sn*-glycerol-3-phosphate acyl transferase activity, and 23% in phosphatidylate phosphohydrolase activity. Marginal inhibition was observed for HMG CoA reductase ($P \leq 0.05$), acetyl CoA carboxylase ($P \leq 0.001$), and heparin-induced lipoprotein lipase ($P \leq 0.001$) activities. Cholesterol-7-α-hydroxylase activity was signifi-

Table I. Effects of *o*-(*N*-Phthalimido)acetophenone on Serum Cholesterol and Triglyceride Levels in Sprague Dawley Male Rats After 8 Weeks of Oral Dosing at 20 mg/kg/day

Week	Group	Percentage control ($\bar{X}$ ± SD) Serum cholesterol[a]	Serum triglyceride[b]
$N = 8$			
1	Control	100 ± 8	100 ± 7
	Treated	85 ± 10	85 ± 5
2	Control	100 ± 4	100 ± 11
	Treated	81 ± 1*	82 ± 5**
3	Control	100 ± 2	100 ± 6
	Treated	84 ± 6*	80 ± 4*
4	Control	100 ± 2	100 ± 6
	Treated	80 ± 3*	80 ± 1*
5	Control	100 ± 8	100 ± 4
	Treated	64 ± 6*	70 ± 11
6	Control	100 ± 8	100 ± 15
	Treated	66 ± 6*	63 ± 7*
7	Control	100 ± 4	100 ± 11
	Treated	54 ± 12*	59 ± 6*
8	Control	100 ± 4	100 ± 8
	Treated	57 ± 6*	62 ± 6*
		Discontinued treatment	
$N = 2$			
10	Control	100 ± 5	100 ± 3
	Treated	101 ± 4	100 ± 11
12	Control	100 ± 8	100 ± 8
	Treated	95 ± 6	98 ± 8

[a] 75–78 mg%.
[b] 109–110 mg%.
* $P \leq 0.001$.
** $P \leq 0.005$.

Table II. Effect of *o*-(*N*-Phthalimido)acetophenone on Activity of Liver Regulatory Enzymes in Sprague Dawley Male Rats After 8 Weeks of Oral Dosing at 20 mg/kg/day

Enzyme activity ($N = 6$)	Percentage control ($\bar{X}$ ± SD) Control	Treated
ATP-dependent citrate lyase	100 ± 6[a]	61 ± 5*
Acetyl CoA synthetase	100 ± 5[b]	72 ± 5*
HMG CoA reductase	100 ± 7[c]	89 ± 6**
Acyl CoA cholesterol acyl transferase	100 ± 4[d]	68 ± 6*
Cholesterol-7-α-hydroxylase	100 ± 5[e]	146 ± 7*
Acetyl CoA Carboxylase	100 ± 6[f]	83 ± 5*
sn-Glycerol-3-phosphate acyl transferase	100 ± 5[g]	63 ± 3*
Phosphatidylate phosphohydrolase	100 ± 6[h]	77 ± 4*
Heparin-induced lipoprotein lipase	100 ± 4[i]	90 ± 4**
% free acid phosphatase activity	100 ± 5[j]	87 ± 5**
% free acid cathepsin activity	100 ± 6[k]	99 ± 7
Catalase activity	100 ± 4[l]	100 ± 6
Liver protein	100 ± 7[m]	97 ± 5

[a] 9.2 mg citrate hydrolyzed/g wet tissue.
[b] 10.0 mg acetyl CoA formed/g wet tissue.
[c] 103,020 dpm/g wet tissue.
[d] 86,640 dpm/g wet tissue.
[e] 289,450 dpm/g wet tissue.
[f] 43,000 dpm/g wet tissue.
[g] 87,620 dpm/g wet tissue.
[h] 11 μg P_i released/g wet tissue.
[i] 3112 dpm/g wet tissue.
[j] 42.2% free.
[k] 36.6% free.
[l] $K = 0.21$ ml/min.
[m] 4.5 mg protein/g wet tissue.
* $P \leq 0.001$.
** $P \leq 0.005$.

cantly increased, 46%, by drug treatment. Total acid phosphatase and cathepsin activities were not increased from the control value. The percentage free acid phosphatase activity was decreased 13%, while the percentage free cathepsin and catalase activities were unchanged. Liver protein levels of treated animals after 8 weeks of drug administration remained within normal limits.

The lipid content of rat liver (Table III) after 8 weeks of drug treatment showed a significant reduction in cholesterol (22%) ($P \leq 0.001$), phospholipid content (26%), and neutral lipids (11%) ($P \leq 0.05$). There was no statistical increase in the fecal lipid content. Aorta cells showed a reduction in cholesterol (29%), neutral lipids (19%), and triglycerides (15%) ($P \leq 0.001$) (Table III). Rat serum lipoprotein fractions (Table IV) showed a significant reduction in the cholesterol content of the chylomicron (11%), very low-density lipoproteins (VLDL) (16), and low-density lipoproteins (LDL) (48%) but an increase in the cholesterol content of the high-density lipoprotein (HDL) fraction (41%) after 8 weeks of treatment. Neutral lipid and triglyceride contents were reduced in the VLDL, LDL, and HDL lipoprotein fractions. Of particular interest was the reduction in triglycerides in the VLDL (27%) and LDL (37%) fractions. The phospholipid content was elevated significantly in the VLDL fraction. The protein content was not significantly altered in any of the lipoprotein fractions (Table IV).

Apoprotein in the chylomicron, VLDL, and LDL fractions appeared identical in the control and treated samples. However, the HDL apoproteins from the treated animals were altered; the apo B band of the treated animals disappeared and the apo E and apo A I bands appeared more dense for the treated animals compared to the control animals (Fig. 1).

The radiolabeled synthetic rate of protein of the individual lipoprotein fraction demonstrated only minor changes after drug treatment (Table V); however, [^{3}H]cholesterol incorporation into cholesterol esters of the chylomicrons, VLDL, LDL, and HDL were reduced significantly, 26, 33, 24, and 29%, respectively. [^{14}C]Palmitic acid incorporation into triglycerides of the chylomicron and VLDL was markedly increased, but the incorporation was reduced in the LDL and HDL. ^{32}P incorporation into phospholipids was marginally reduced in the chylomicron and VLDL fractions. Radiolabeled distribution studies with [^{3}H]cholesterol i.v. (Table VI) demonstrated lower levels of cholesterol and its metabolites in the liver, heart, and small and large intestines, with elevated levels in the spleen, lung, chyme, and feces after drug administration. [^{14}C]Palmitic acid distribution

Table III. Effect of *o*-(*N*-Phthalimido)acetophenone on Lipid Content of Liver, Feces, and Aorta in Sprague Dawley Male Rats After 8 Weeks of Oral Dosing at 20 mg/kg/day

	Percentage control ($\bar{X}$ ± SD)				
Sample (N = 6)	Cholesterol	Neutral lipid	Triglyceride	Phospholipid	Protein
Liver					
Control	100 ± 8[a]	100 ± 8[b]	100 ± 7[c]	100 ± 17[d]	100 ± 2[e]
Treated	78 ± 6*	89 ± 7***	98 ± 10	74 ± 4***	98 ± 2
Feces					
Control	100 ± 8[f]	100 ± 4[g]	100 ± 8[h]	100 ± 23[i]	100 ± 7[j]
Treated	110 ± 7	103 ± 8	96 ± 6	112 ± 28	85 ± 10
Aorta					
Control	100 ± 7[k]	100 ± 2[l]	100 ± 7[m]	100 ± 19[n]	100 ± 2[o]
Treated	71 ± 9*	81 ± 7*	85 ± 6**	110 ± 35	98 ± 3

[a] 9.18 mg/g tissue; [b]15.70 mg/g tissue; [c]6.37 mg/g tissue; [d]27.19 mg (P_i)/g tissue; [e]12.90 mg/g tissue; [f]2.84 mg/g tissue; [g]3.39 mg/g tissue; [h]1.86 mg/g tissue; [i]5.70 mg/g tissue; [j]6.99 mg/g tissue; [k]11.46 mg/g tissue; [l]79.11 mg/g tissue; [m]4.86 mg/g tissue; [n]4.79 mg/g tissue; [o]2.94 mg/g tissue; *$P \leq 0.001$; **$P \leq 0.01$; ***$P \leq 0.05$.

studies showed higher levels in the liver, spleen, heart, stomach, and feces, with lower levels of radioactivity in the kidney, small and large intestines, and chyme. ^{32}P distribution studies demonstrated a higher content in the liver, kidney, spleen, brain, stomach, small intestine, and chyme but a lower content in the heart, lung, large intestine, and feces. In hyperlipidemic diet-induced rats, *o*-(*N*-phthalimido)acetophenone administration at 20 mg/kg/day resulted in a 42% reduction in serum cholesterol levels by the third week and 48% by the fourth week (Table VII). The serum triglyceride levels were reduced 22% by week 3 and 33% by week 4. The [^{3}H]cholesterol incorporation studies into the serum lipoproteins of hyperlipidemic rats compared to normal rats demonstrated a 17% increase in LDL cholesterol and a 46% reduction in HDL cholesterol incorporation values. [^{14}C]Palmitic acid incorporation into the four lipoprotein fractions was essentially the same for the VLDL fraction, but LDL incorporation was increased 94% and HDL incorporation was decreased 40%. The ^{32}P incorporation was elevated in the hyperlipidemic lipoproteins by 175% in the chylomicrons, 59% in the LDL, and 56% in the HDL lipoprotein fraction (compare footnotes to Tables V, and IX). The chemical analysis of the serum lipoproteins of the hypolipidemic animals compared to the normal rats showed a 307% increase in the cholesterol content of VLDL, a 298% increase in the LDL fraction, and a 762% increase in the HDL fraction (compare control values in the footnotes to Table IV and Table VIII). Triglycerides in the hyperlipidemic diet-treated animals demonstrated increases of 18% in the chylomicron and 45% in the VLDL. Neutral lipids in the diet group were elevated by 32% in the chylomicron, 33% in the VLDL, and 20% in the LDL fractions compared to the normal animals. The phospholipid content was increased 55% in the LDL and 20% in the HDL fractions. The protein contents of the hyperlipidemic lipoprotein fractions were all reduced significantly. The serum lipoprotein fractions from hyperlipidemic drug-treated rats showed a slight decrease in VLDL cholesterol (13%) and a significant elevation in HDL cholesterol (64%) after drug treatment (Table VIII). Triglyceride levels were elevated in the chylomicrons (17%) and LDL (11%) but decreased in the HDL cholesterol (33%). Neutral lipids were not significantly altered from the control animals in any of the serum lipoprotein fractions. Phospholipid levels were elevated slightly in the chylomicrons (9%) and HDL (14%) fraction. Protein levels were not significantly altered excepted in the LDL fraction (13%). The incorporation rates of the protein and lipids into the serum lipoprotein fractions of treated hyperlipidemic rats showed that apoprotein synthesis for all four fractions of the treated animals was reduced (Table IX). Cholesterol incorporation was reduced by drug treatment in the chylomicron (69%), VLDL (26%), and LDL (60%) fractions, but the HDL fraction showed a 39% increase. [^{14}C]Palmitic acid incorporation

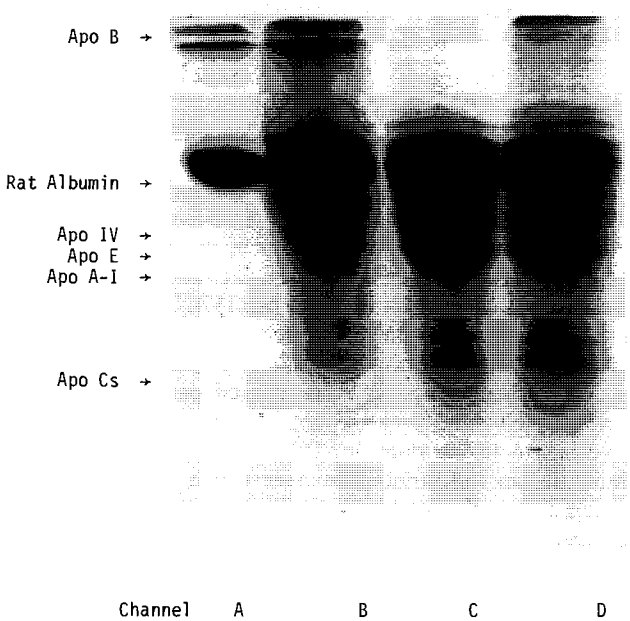

Fig. 1. SDS-PAGE of the HDL fraction from control and *o*-(*N*-phthalimido)acetophenone-treated rats. Channels: A, albumin standard; B, control HDL; C, treated *o*-(*N*-phthalimido)acetophenone HDL; D, treated 2,3-Dihydrophthalazine-1,4-dione HDL.

Table IV. Effect of *o*-(*N*-Phthalimido)acetophenone on Serum Lipoprotein Fractions in Sprague Dawley Male Rats After 8 Weeks of Oral Dosing at 20 mg/kg/day

	Percentage control ($\bar{X}$ ± SD)				
Fractions (N = 6)	Cholesterol	Neutral lipids	Triglycerides	Phospholipids	Protein
Chylomicrons					
Control	100 ± 4[a]	100 ± 2[b]	100 ± 5[c]	100 ± 46[d]	100 ± 2[e]
Treated	89 ± 6**	98 ± 2	74 ± 3*	106 ± 40	99 ± 1
VLDL					
Control	100 ± 7[f]	100 ± 4[g]	100 ± 2[h]	100 ± 35[i]	100 ± 1[j]
Treated	84 ± 5*	93 ± 1**	73 ± 4*	249 ± 40*	100 ± 2
LDL					
Control	100 ± 11[k]	100 ± 5[l]	10 ± 1[m]	100 ± 50[n]	100 ± 1[o]
Treated	54 ± 13*	80 ± 3*	63 ± 2*	125 ± 51	103 ± 2
HDL					
Control	100 ± 6[p]	100 ± 3[q]	100 ± 2[r]	100 ± 36[s]	100 ± 1[t]
Treated	141 ± 10	83 ± 1*	62 ± 7*	86 ± 26	97 ± 1

[a] 337 μg/ml; [b]420 μg/ml; [c]67 μg/ml; [d]149 μg/ml; [e]184 μg/ml; [f]190 μg/ml; [g]221 μg/ml; [h]98 μg/ml; [i]26 μg/ml; [j]50 μg/ml; [k]210 μg/ml; [l]45 μg/ml; [m]10 μg/ml; [n]41 μg/ml; [o]122 μg/ml; [p]544 μg/ml; [q]620 μg/ml; [r]27 μg/ml; [s]153 μg/ml; [t]657 μg/ml; *$P \leq 0.001$; **$P \leq 0.01$.

was reduced in all four lipoprotein fractions of treated animals, with the largest reduction (67%) being in the VLDL fraction. ^{32}P incorporation was significantly increased in all serum lipoprotein fractions of the treated hyperlipidemic rats compared to the hyperlipidemic nontreated rats.

DISCUSSION

o-(*N*-Phthalimido)acetophenone administered over 8 weeks demonstrated potent hypolipidemic activity, reducing significantly both serum cholesterol and triglyceride levels at 20 mg/kg/day orally. A previous study (1) has shown that these parameters were reduced within 2 weeks, rather than 3 weeks. This may be due to the fact that the previous study was performed on older rats than used in the present study (1). The older rats probably had higher basal serum lipid levels. The hypolipidemic effects of the drug were reversible after the termination of drug therapy, indicating that damage to the liver would probably not be the mode of action of the agent resulting in reduction in the synthesis of serum lipids or lipoproteins. Over the 8 weeks of drug administration, there was no morphological or clinical chemistry evidence of liver, kidney, or hemopoietic damage (30). There was no indication in the liver that the drug caused the release of hepatic lysosomal hydrolytic enzymes, i.e., acid phosphatase or acid cathepsin activities, or caused peroxisome formation, i.e., elevated catalase activity, either of which may have led to tissue damage. Previously di(2-ethylhexylo)-phthalate, an industrial plasticizer which possesses hypolipidemic action, was shown to cause peroxisome damage to the liver (31).

After 8 weeks of drug administration, the agent suppressed the activities of de novo lipid regulatory enzymes of rat liver. Suppression of cytoplasmic ATP-dependent citrate lyase and acetyl CoA synthetase activities leads to less acetyl CoA in the cytoplasm to initiate cholesterol and fatty acid de novo synthesis. Inhibition by the drug of acyl CoA cholesterol acyl transferase activity blocks the conversion of cholesterol to esters and reduces tissue cholesterol ester storage. The acceleration of cholesterol-7-α-hydroxylase activity suggests that a larger amount of cholesterol was being converted to bile acids in the presence of the drug. The rate-limiting enzyme of fatty acid synthesis acetyl CoA carboxylase activity was inhibited marginally. Inhibition of *sn*-glycerol-3-phosphate acyl transferase and phosphatidylate phosphohydrolase, the rate-limiting enzymes for the triglyceride pathway by the drug, would explain the reduction in serum triglyceride levels observed. Marginal inhibition of heparin-induced hepatic lipoprotein lipase by the drug would reduce the release of triglyceride from lipoprotein for uptake by the tissues.

[^{14}C]Cholesterol and [^{14}C]palmitic acid distribution studies and lipid analysis of the tissues after 8 weeks of drug therapy demonstrated that the various lipids were not being deposited in the organs, e.g., liver, and aorta, but rather the cholesterol and its metabolites may be removed from the body via the feces. A previous study (1) did show that the drug caused an accelerated excretion of bile cholesterol and its metabolites in rats. Aorta tissue showed a significant reduction in cholesterol and triglyceride content, suggesting that the drug may also reduce lipid deposition in the arterial wall. The lipid distribution of the serum lipoproteins after 8 weeks of drug administration was promising. First, lowering the triglyceride content in the LDL and VLDL fractions is important in reducing the delivery of triglycerides to the peripheral tissues. Second, the LDL cholesterol content was reduced significantly after drug treatment. In humans, LDL is responsible for cholesterol delivery to the plaque cells. Third, HDL cholesterol was elevated 41% after 8 weeks of drug administration. The HDL lipoprotein returns cholesterol from peripheral tissues to the liver, where it undergoes catabolism to bile salts and is excreted in the bile (32). In previous studies, after 2 weeks of drug treatment, cholesterol was not elevated in the HDL fraction (1). The [^{3}H]cholesterol incorporation into HDL of treated rats confirms this

Table V. The Effects of *o*-(*N*-Phthalimido)acetophenone on Incorporation of Precursors into Sprague Dawley Rat Lipoprotein Fractions at 20 mg/kg/day for 2 Weeks ($N = 6$)

	Percentage control ($\bar{X} \pm$ SD)			
	[^{3}H]Leucine into apoprotein	[^{3}H]Cholesterol into cholesterol esters	[^{14}C]Palmitic acid into triglyceride	^{32}P into phospholipids
Chylomicrons[q]				
Control	100 ± 7[a]	100 ± 6[b]	100 ± 7[c]	100 ± 7[d]
Treated	95 ± 6	74 ± 5*	184 ± 8*	88 ± 7
VLDL[r]				
Control	100 ± 5[e]	100 ± 6[f]	100 ± 5[g]	100 ± 8[h]
Treated	97 ± 6	66 ± 4*	328 ± 8*	86 ± 7
LDL[s]				
Control	100 ± 4[i]	100 ± 6[j]	100 ± 6[k]	100 ± 5[l]
Treated	97 ± 3	76 ± 5*	82 ± 6	110 ± 5
HDL[t]				
Control	100 ± 4[m]	100 ± 4[n]	100 ± 5[o]	100 ± 4[p]
Treated	91 ± 3	71 ± 5*	29 ± 2*	96 ± 6

[a] 11,492 dpm/ml; [b]2091 dpm/ml; [c]764 dpm/ml; [d]211 dpm/ml; [e]4722 dpm/ml; [f]434 dpm/ml; [g]386 dpm/ml; [h]424 dpm/ml; [i]2909 dpm/ml; [j]403 dpm/ml; [k]110 dpm/ml; [l]140 dpm/ml; [m]3866 dpm/ml; [n]424 dpm/ml; [o]379 dpm/ml; [p]362 dpm/ml; [q]Total vol, 4 ml; [r]Total vol, 2 ml; [s]Total vol, 2 ml; [t]Total vol, 6.5 ml; $*P \leq 0.001$.

Table VI. The Effects of *o*-(*N*-Phthalimido)acetophenone on Sprague Dawley Rats at 20 mg/kg/day Orally for 2 Weeks on Radiolabel Lipid Distribution in Organs and Fecal Materials ($N = 6$)

	dpm per total organ					
	[^{3}H]Cholesterol i.v.		[^{14}C]Palmitic acid i.v.		^{32}P i.v.	
	Control	Treated	Control	Treated	Control	Treated
Liver	100,016 ± 962	89,121 ± 742	374,036 ± 1692	430,032 ± 1051	399,410 ± 579	504,004 ± 639
Kidney	26,052 ± 112	21,799 ± 302	214,195 ± 918	188,183 ± 1172	76,307 ± 421	90,462 ± 583
Spleen	5,579 ± 494	25,838 ± 506	34,750 ± 284	68,571 ± 393	24,854 ± 135	39,236 ± 302
Heart	17,618 ± 102	9,140 ± 214	24,780 ± 177	68,431 ± 406	35,425 ± 227	20,581 ± 147
Lung	12,155 ± 98	18,721 ± 302	36,904 ± 212	41,019 ± 390	71,991 ± 173	66,718 ± 237
Brain	15,785 ± 107	13,305 ± 157	21,167 ± 133	22,824 ± 174	27,141 ± 97	79,236 ± 116
Stomach	18,775 ± 209	23,288 ± 176	29,973 ± 108	36,991 ± 227	85,219 ± 604	377,120 ± 687
Small intestine	99,580 ± 908	83,812 ± 532	201,066 ± 354	109,882 ± 556	181,257 ± 313	213,180 ± 466
Large intestine	70,962 ± 477	45,344 ± 333	79,669 ± 487	62,347 ± 599	114,134 ± 163	85,990 ± 320
Chyme	193,188 ± 847	336,142 ± 597	98,992 ± 168	49,010 ± 215	101,788 ± 406	141,462 ± 244
Feces	220,283 ± 308	371,854 ± 631	50,939 ± 1032	140,615 ± 2074	325,994 ± 274	210,776 ± 181

Table VII. Effect of *o*-(*N*-Phthalimido)acetophenone at 20 mg/kg/day Orally on Serum Lipids of Hyperlipidemic Diet-Induced Rats ($N = 6$)

	Percentage control ($\bar{X} \pm$ SD)			
	Serum cholesterol		Serum triglyceride	
Week	Control[a]	Treated	Control[b]	Treated
2	100 ± 6	87 ± 5	100 ± 7	77 ± 5*
3	100 ± 5	58 ± 4*	100 ± 6	78 ± 6*
4	100 ± 6	52 ± 5*	100 ± 8	67 ± 3*

[a] 703 mg%.
[b] 515 mg%.
* $P \leq 0.001$.

Table VIII. The Effects of *o*-(*N*-Phthalimido)acetophenone on Lipid Levels of Rat Lipoproteins of Hyperlipidemic Diet-Induced Animals Treated Orally at 20 mg/kg/day for 4 Weeks ($N = 6$)

	Percentage control ($\bar{X} \pm$ SD)				
	Cholesterol	Triglyceride	Neutral lipids	Phospholipids	Protein
Chylomicrons					
Control	100 ± 5[a]	100 ± 5[b]	100 ± 6[c]	100 ± 7[d]	100 ± 5[e]
Treated	104 ± 5	117 ± 3*	105 ± 7	109 ± 7	108 ± 6
VLDL					
Control	100 ± 4[f]	100 ± 6[g]	100 ± 8[h]	100 ± 5[i]	100 ± 4[j]
Treated	87 ± 5*	104 ± 7	101 ± 5	102 ± 4	105 ± 4
LDL					
Control	100 ± 5[k]	100 ± 6[l]	100 ± 4[m]	100 ± 6[n]	100 ± 5[o]
Treated	109 ± 6	111 ± 6	101 ± 6	102 ± 6	83 ± 4
HDL					
Control	100 ± 7[p]	10 ± 5[q]	100 ± 3[r]	100 ± 4[s]	100 ± 6[t]
Treated	164 ± 6*	67 ± 4*	106 ± 7	114 ± 3*	106 ± 7

[a] 371 μg/ml; [b]79 μg/ml; [c]554 μg/ml; [d]231 μg/ml; [e]96 μg/ml; [f]773 μg/ml; [g]142 μg/ml; [h]294 μg/ml; [i]78 μg/ml; [j]15 μg/ml; [k]836 μg/ml; [l]6.2 μg/ml; [m]54 μg/ml; [n]231 μg/ml; [o]73 μg/ml; [p]4689 μg/ml; [q]16 μg/ml; [r]397 μg/ml; [s]184 μg/ml; [t]315 μg/ml.

Table IX. The Effects of *o*-(*N*-Phthalimido)acetophenone on Incorporation of Precursors into Serum Lipoproteins in 4-Week Hyperlipidemic Diet-Induced Sprague Dawley Rats Treated at 20 mg/kg/day Orally for an Additional 2 Weeks ($N = 6$)

	Percentage control ($\bar{X} \pm$ SD)			
	[^{3}H]Leucine into apoprotein	[^{3}H]Cholesterol into cholesterol esters	[^{14}C]Palmitic acid into triglyceride	^{32}P into phospholipids
Chylomicrons				
Control	100 ± 5[a]	100 ± 6[b]	100 ± 7[c]	100 ± 8[d]
Treated	68 ± 4*	31 ± 3	66 ± 5*	169 ± 7*
VLDL				
Control	100 ± 6[e]	100 ± 3[f]	100 ± 6[g]	100 ± 5[h]
Treated	66 ± 2*	74 ± 5*	33 ± 2*	189 ± 6*
LDL				
Control	100 ± 5[i]	100 ± 4[j]	100 ± 5[k]	100 ± 6[l]
Treated	61 ± 5*	40 ± 5*	70 ± 6*	303 ± 9*
HDL				
Control	100 ± 5[m]	100 ± 5[n]	100 ± 3[o]	100 ± 6[p]
Treated	56 ± 3*	139 ± 6*	64 ± 5*	254 ± 10*

[a] 10092 dpm/ml; [b]420 dpm/ml; [c]705 dpm/ml; [d]582 dpm/ml; [e]4131 dpm/ml; [f]376 dpm/ml; [g]395 dpm/ml; [h]413 dpm/ml; [i]2838 dpm/ml; [j]471 dpm/ml; [k]213 dpm/ml; [l]223 dpm/ml; [m]4334 dpm/ml; [n]197 dpm/ml; [o]229 dpm/ml; [p]566 dpm/ml; *$P \leq 0.001$.

elevation in both normal and hyperlipidemic diet-induced rats. In humans it has been shown that high HDL cholesterol levels protect against myocardial infarction. Interestingly apo B, which disappeared in the HDL fraction of treated animals, is the major apoprotein responsible for cholesterol transport into plaque cells. The HDL of rats is different from that of humans in that this fraction is responsible for 73% of the transport of cholesterol to peripheral tissues (32). Apo E and apo A I on HDL are responsible for receptor binding in the liver for serum clearance of HDL cholesterol by the liver (32). Drug treatment with *o*-(*N*-phthalimido)acetophenone caused a heavy banding in the region where apo E and apo A I appear in the gel, suggesting the possibility of an enhanced concentration of these apoproteins in treated rats. Thus, uptake of HDL cholesterol by the HDL receptors of the liver may assist the clearance of cholesterol from the blood and peripheral tissue. The lipoprotein fractions of treated rats appeared to have a higher content of phospholipids. Remnants of the chylomicron and VLDL which are devoid of phospholipids are cleared by the liver at a faster rate (33). The drug was effective in lowering serum cholesterol and triglyceride in hyperlipidemic treated rats, demonstrating significant activity after the third week of drug administration. The HDL cholesterol was elevated in the hyperlipidemic treated rats when analyzed by chemical means as well as by radioactive incorporation of cholesterol into the HDL fraction. High HDL cholesterol with a low LDL cholesterol content, which appears to be afforded by the *o*-(*N*-phthalimido)acetophenone treatment, supposedly protects against myocardial infarction related to atherosclerosis in humans (34).

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