PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 142–145 (1977)

Influence of 2-Acetoamidoethyl (*p*-chloro-phenyl) (*m*-trifluoromethylphenoxy) Acetate (Halofenate) on Hepatic Drug Metabolism in Retired Breeder Rats[1] (39623)

LUEAN E. ANTHONY[2] AND ALBERT L. JONES[3]

Cell Biology Section, Veterans Administration Hospital; and the Departments of Medicine and Anatomy, and the Liver Center, University of California, San Francisco, California 94121

The relationship between serum cholesterol level and the prevalence of coronary heart disease (1) has stimulated research on plasma lipid-lowering drugs (2). Halofenate is an investigational drug that is closely related structurally to clofibrate (chlorophenoxisobutyrate). This new agent has been shown to lower serum lipids (3, 4) and uric acid (3, 5) in man and plasma cholesterol in rats (6, 7). The long-term safety of halofenate is not known but it has been suggested that it has several advantages over clofibrate; e.g., lower doses are required (8) and it needs to be administered only once daily (4). The chronic administration of clofibrate to rats has produced elevations in liver weight and the proliferation of hepatic smooth-surfaced endoplasmic reticulum (9, 10), and has enhanced rates of hepatic drug metabolism (11). Since halofenate is being considered for use in man as a hypolipidemic agent, and because cholesterol and steroid hydroxylations are presumed to be mediated via the microsomal mixed function oxidase system (12–14), the present study was initiated to ascertain the effect of chronic halofenate administration on several parameters of hepatic drug metabolism in hyperlipidemic retired breeder male rats. The retired breeder rat has been suggested as an animal model for atherosclerosis (15) and hyperlipidemia (16).

Materials and methods. Recently retired Holtzman male breeder rats (500 g) were housed in individual cages and provided tap water and Purina Laboratory Chow *ad libitum* for at least 1 week prior to use. The rats were fasted for 18 hr before collecting tail vein blood for the initial serum lipid determinations. Serum samples were extracted in chloroform–methanol (2:1) and analyzed for cholesterol according to the method of Rudel and Morris (17) and for triglycerides by the method of Eggstein and Kreutz (18), using the Biochemica test combination for triglycerides (Boehringer-Mannheim Corp.). Halofenate (30 or 60 mg/kg) was administered daily for 10 days by stomach tube as a 30-mg/ml solution in corn oil (Mazola), while control rats received corn oil alone. The rats were fasted 18 hr prior to sacrifice.

The rats were killed by decapitation and serum samples were collected. The livers were excised, weighed, and divided into three portions. One part of the liver was homogenized in ice-cold water (1:19 dilution) and analyzed for protein by the method of Lowry *et al.* (19), and another portion of the liver was homogenized in saline (1:8 dilution), extracted in chloroform–methanol (2:1), and analyzed for cholesterol (17) and triglycerides (18). The remainder of the liver was homogenized in 10 vol of 0.25 *M* sucrose and the microsomal fraction was isolated by differential centrifugation as previously described (20). Microsomal protein was estimated by the method of Gornall *et al.* (21), and cytochrome P-450 and cytochrome b_5 were determined by the method of Omura and Sato (22). NADPH–Cytochrome *c* reductase was measured as described by Masters *et al.* (23). Microsomal *N*-demethylation was measured as previously described (20), using ethylmorphine (6.5 m*M*) as substrate. The formaldehyde produced was assayed using the procedure described by Nash (24). Statisti-

[1] Supported by the Medical Research Service of the Veterans Administration, and by NIH Grants No. HL 16363-01 and (NIAMDD) No. P50-AM 18520.

[2] Present address: VA Hospital, Medical Service, 3801 Miranda Ave., Palo Alto, Calif. 94304.

[3] Address reprint requests to: Dr. A. L. Jones, Cell Biology Section (151E), VA Hospital, 4150 Clement St., San Francisco, Calif. 94121.

cal comparisons were made using Student's t test.

Results. The results summarized in Table I show that halofenate exerted a significant hypocholesterolemic effect on the serum of retired breeder rats. The drug administered at two dose levels showed a dose–response effect. Serum triglyceride levels were unchanged after 60 mg/kg of halofenate, and those of the rats treated with 30 mg/kg of halofenate appeared to increase.

No differences in body weight were found between the halofenate-treated and control retired breeder rats during the course of this experiment. However, throughout the treatment period 4 of the 11 rats treated with 60 mg/kg of halofenate died. They were sick and lethargic for a day prior to death and died toward the end of the treatment period.

As shown in Table II, significant increases were noted in liver weight and liver/body weight ratios in the halofenate-treated rats at both dose levels. Halofenate treatment did not affect the levels of hepatic protein or cholesterol in the retired breeder rats. Livers from halofenate-treated animals contained a significantly higher concentration of triglyceride than did the controls ($P < 0.001$). Microsomal protein was significantly increased in the halofenate-treated livers.

An examination of components of the liver microsomal drug-metabolizing system demonstrated that cytochrome P-450 and cytochrome b_5 in both groups of halofenate-treated rats were significantly increased from the control values (Table III). NAPDH-Cytochrome c reductase levels were significantly increased only in the rats treated with 60 mg/kg of halofenate. The microsomal rates of ethylmorphine N-demethylation were significantly greater in the treated animals in comparison to the control values.

Discussion. The results (Table I) confirm the hypocholesterolemic action of halofenate in rats (6, 7) at dosages (30 and 60 mg/kg) which are 2 and 4 × those usually reported in man (4, 8). However, considerable mortality (4/11) was observed at the higher dosage, which puts the safety of this drug in doubt. Like its parent compound, clofibrate (25), halofenate stimulated hepatomegaly without a concomitant increase in liver protein concentration. In contrast to the findings with clofibrate (25, 26), liver triglyceride concentrations were markedly increased after halofenate treatment, suggesting that the increase in liver weight was

TABLE I. EFFECT OF HALOFENATE ON SERUM LIPID LEVELS OF MALE RETIRED BREEDER RATS.[a]

	Serum cholesterol (mg/100 ml)			Serum triglyceride (mg/100 ml)		
Treatment	Initial	After 10 days of treatment	P[b]	Initial	After 10 days of treatment	P
Control	112 ± 14	114 ± 15	NS	46 ± 8	40 ± 3	NS
Halofenate (30 mg/kg)	118 ± 17	94 ± 10	<0.02	36 ± 4	49 ± 4	<0.05
Halofenate (60 mg/kg)	113 ± 12	74 ± 5	<0.05	39 ± 4	38 ± 2	NS

[a] Values represent the mean ± SE of 7 to 11 rats.
[b] Difference between initial and post-treatment determinations.

TABLE II. EFFECT OF HALOFENATE ON LIVER WEIGHT, LIVER/BODY WEIGHT, AND LIVER COMPOSITION.[a]

		Treatment	
Parameter	Control	Halofenate (30 mg/kg)	Halofenate (60 mg/kg)
Liver weight (g)	11.65 ± 0.23	13.71 ± 0.62*	14.35 ± 0.65**
Liver/body weight (%)	2.4 ± 0.1	2.8 ± 0.1**	3.1 ± 0.1**
Liver protein (mg/g)	204 ± 3	203 ± 3	207 ± 1
Liver cholesterol (mg/g)	0.440 ± 0.017	0.476 ± 0.018	0.416 ± 0.022
Liver triglycerides (mg/g)	11.9 ± 0.6	26.5 ± 2.1**	23.7 ± 0.92**
Microsomal protein (mg/g)	16.7 ± 1.4	26.1 ± 1.2*	23.8 ± 1.2*

[a] Results are expressed as the mean ± SE of 7 to 11 rats. Significance: * $P < 0.01$; ** $P < 0.001$.

TABLE III. MEASUREMENTS OF LIVER MICROSOMAL DRUG METABOLISM IN RETIRED BREEDER RATS TREATED WITH HALOFENATE.[a]

	Control	Treatment: Halofenate (30 mg/kg)	Treatment: Halofenate (60 mg/kg)
Cytochrome P-450 (nmole × mg^{-1} protein)	1.11 ± 0.03	1.31 ± 0.06**	1.79 ± 0.05***
Cytochrome b_5 (nmole × mg^{-1} protein)	0.48 ± 0.01	0.52 ± 0.01*	0.59 ± 0.02***
NADPH-Cytochrome *c* reductase (nmole × min^{-1} × mg^{-1} protein)	52 ± 3	62 ± 4	84 ± 4***
Ethylmorphine demethylation (nmole × min^{-1} × mg^{-1} protein)	1.9 ± 0.1	2.8 ± 0.2**	3.4 ± 0.4***

[a] Results are expressed as the mean ± SE of 7 to 11 rats. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

due, in part, to an increase in lipid content.

Halofenate treatment of rats also increased liver microsomal protein content, stimulated microsomal hydroxylation of ethylmorphine (Table III) and aniline (27), and increased the concentrations of cytochrome P-450 and cytochrome b_5, and increased the activity of NADPH-cytochrome *c* reductase in rat liver microsomes (Table III). These characteristics suggest that halofenate belongs among the more than 200 drugs that are known to stimulate the activity of a variety of hepatic microsomal drug-metabolizing enzymes (28).

These observations suggest that after halofenate treatment in man an increase in the activity of drug-metabolizing enzymes can be expected. This could lead to accelerated biotransformations of drugs and steroid hormones *in vivo* and thus alter the duration and intensity of drug or hormone action.

Only a few reports have attempted to establish the extent of drug-drug interactions associated with the administration of halofenate. Halofenate administration shortened plasma antipyrine and bishydroxycoumarin half-lives but prolonged plasma warfarin half-lives (27) and potentiated the hypoglycemic effect of sulfonylureas (29). Although the mechanism by which halofenate causes these changes is unclear, the present study provides further evidence that this drug, which could be used clinically, both chronically and in combination with other agents, has the potential for altering the metabolism of a coadministered drug.

Summary. Chronic administration of halofenate at two dose levels (30 and 60 mg/kg) to male retired breeder rats caused a significant lowering of serum cholesterol levels. Halofenate treatment also produced hepatomegaly in rats, and this increase in liver weight was due, at least in part, to an increase in the quantity of triglyceride. Considerable mortality (4/11) was associated with the higher dose level. The influence of pretreatment with halofenate on several parameters of hepatic microsomal drug metabolism was investigated. *In vitro* ethylmorphine *N*-demethylation was increased and the concentrations of cytochrome P-450 and cytochrome b_5 were elevated. The specific activity of NADPH-cytochrome *c* reductase was also increased.

The authors thank Merck, Sharp & Dohme Laboratories for a supply of halofenate. We wish to also thank Mrs. Naomi Ross for typing the manuscript.

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Received September 7, 1976. P.S.E.B.M. 1977, Vol. 154.