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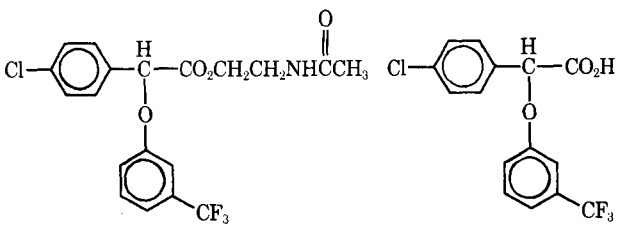
Effect of Halofenate on Binding of Various Drugs to Human Plasma Proteins and on the Plasma Half-Life of Antipyrine in Monkeys

HOWARD B. HUCKER▲, SUSAN C. STAUFFER, and SAMUEL E. WHITE

Abstract □ Halofenate free acid was shown to reduce markedly the binding of salicylic acid and aspirin in human plasma. Similar, but much smaller, effects were observed on binding of chlorothiazide, tolbutamide, digitoxin, and phenylbutazone. No effect was seen on binding of warfarin, dicumarol, or indomethacin. Halofenate administration did not alter the plasma half-life of antipyrine in rhesus monkeys.

Keyphrases □ Halofenate—effect on binding of various drugs to human plasma proteins and on the plasma half-life of antipyrine in monkeys □ 2-Acetamidoethyl (*p*-chlorophenyl)(*m*-trifluoromethylphenoxy)acetate (halofenate)—effect on binding of various drugs to human plasma proteins and on the plasma half-life of antipyrine in monkeys □ Plasma protein binding—effect of halofenate on various drugs □ Half-life, antipyrine—effect of halofenate administration, monkeys □ Antipyrine half-life—effect of halofenate administration

Halofenate[1] is a new drug which reduces concentrations of several lipid parameters in the rat (1) and is currently in clinical trial for treatment of patients with hypercholesterolemia and/or hypertriglyceridemia (2–6). The new agent also has been shown to have hypouricemic activity in man (2). Halofenate is completely metabolized in man by hydrolysis to the halofenate free acid, which is extensively bound to plasma proteins (7).



halofenate halofenate free acid

[1] 2-Acetamidoethyl (*p*-chlorophenyl)(*m*-trifluoromethylphenoxy)acetate.

The pharmacological activity of many drugs is altered by coadministration of drugs that compete for binding sites on plasma proteins and inhibit or induce drug metabolism or excretion (8). We have studied two aspects of possible drug interactions with halofenate, namely, its effects on the binding of other drugs to plasma proteins and its effects on the plasma half-life of antipyrine in rhesus monkeys. The latter technique was recommended as a means to detect induction of drug metabolism (9); for this reason the present report also includes studies with clofibrate and phenobarbital.

EXPERIMENTAL

Materials—The following drugs were used in the study: aspirin-^{14}C (carboxyl)[2], 10.4 μc./mg.; salicylic-7-^{14}C acid[2], 34.8 μc./mg.; digitoxin-^{3}H (generally labeled)[2], 5.8 mc./mg.; dicumarol-^{14}C (methylene)[2], 83 μc./mg.; warfarin-^{14}C[3], 23 μc./mg.; and tolbutamide-^{35}S[3], 21 μc./mg. Chlorothiazide-^{3}H (12 μc./mg.), indomethacin (8.4 μc./mg.), and phenylbutazone-^{3}H (36 μc./mg.) were synthesized[4]. All compounds were found to be radiochemically pure by paper or thin-layer chromatography.

Binding—Fresh blood from human donors was citrated on collection and centrifuged, and the plasma was separated. Solutions of the various drugs were prepared as follows: indomethacin in 0.1 *M* phosphate buffer, pH 8.0; halofenate free acid, salicylic acid, chlorothiazide, warfarin, dicumarol, and phenylbutazone in 0.01 *N* NaOH; aspirin in water; digitoxin in ethanol; and tolbutamide in 5% aqueous K_2CO_3. All drugs were added in the smallest possible volume to 70 ml. of plasma, the pH of which was not affected by addition of the drug.

Binding was measured by ultrafiltration as described by Borga *et al.* (10). Six samples were prepared for each concentration used. After final centrifugation, 0.2 ml. of the ultrafiltrate and 1 ml. of the plasma inside the dialysis tubing were pipeted directly into polyethylene counting vials containing 20 ml. of counting medium. The counting medium consisted of 7 g. diphenyloxazole, 0.23 g. of 1,4-

[2] New England Nuclear Corp.
[3] Amersham-Searle.
[4] By Dr. Mertel, Dr. Ellsworth, and Dr. Meriwether of the Merck Sharp & Dohme Research Laboratories.

bis-2-(5-phenyloxazolyl)benzene, 100 g. naphthalene, and 30 g. of a thixotropic gel[5] in 1 l. of dioxane. Radioactivity was measured in a liquid scintillation spectrometer[6], and the counting efficiency was determined by addition of an internal standard.

Effect on Antipyrine Plasma Half-Life in the Monkey—*Clofibrate*—Eight rhesus monkeys were immobilized with phencyclidine, weighed, and placed in chairs. The animals were divided into two groups of four each (two of each sex). On the following day, the animals were given 100 mg./kg. i.v. of antipyrine. Blood samples were drawn at various times into heparinized syringes, and plasma was analyzed for antipyrine by a previously described method (11). One group was then given empty gelatin capsules for 4 days, beginning on the 2nd day after antipyrine administration; the other group received clofibrate, 120 mg./kg. p.o. in polyethylene glycol for 4 days. On the following day, both groups were then given antipyrine as before and plasma levels were measured.

Phenobarbital—Eight rhesus monkeys (four of each sex) were weighed and placed in chairs on Day 1. On Day 2, four animals (two of each sex) were given 100 mg./kg. i.v. of antipyrine and plasma concentrations were measured at various times. On Day 3, the remaining animals were given antipyrine. Then all animals received 20 mg./kg. of sodium phenobarbital (capsule) daily for 4 days. After the 4-day period, the animals were given antipyrine as before and plasma levels were determined.

Halofenate—Eight rhesus monkeys (four of each sex) were selected, weighed, and placed in restraining chairs (Day 0). On Day 1, all animals received 100 mg./kg. i.v. of antipyrine. Blood samples were taken at 0.5, 1, 2, 3, and 4 hr., and plasma was assayed for antipyrine. No treatment was given for an additional day (Day 2). On Days 3, 4, 5, and 6, four animals received 60 mg./kg. p.o. of halofenate (per day) in gelatin capsules. Empty capsules were administered to a second group during this time. On Day 7, both groups were given 100 mg./kg. i.v. of antipyrine and plasma levels were measured as before.

All animals remained in chairs for the following 5 days with no treatment, after which a crossover phase was initiated. Both groups were given antipyrine as before (Day 1), and plasma levels were measured. The second group then received halofenate (60 mg./kg. p.o.) daily for 4 days, with the first group getting empty capsules. Following this treatment, all monkeys were given antipyrine as before and plasma levels were measured.

RESULTS AND DISCUSSION

The trend toward multiple-drug therapy in medicine necessitates a thorough understanding of the consequences of possible drug interactions. In the present investigation, two types of possible interactions were studied for halofenate, a new hypolipidemic drug. Effects of halofenate free acid, the form in which halofenate is present in the blood, on plasma protein binding of various other drugs are shown in Table I. The range of halofenate free acid concentrations employed was similar to that observed in plasma of human subjects given the drug (500–2000 mg./day) for 4–8 weeks[7].

The presence of halofenate free acid had no effect on the binding of warfarin, dicumarol, or indomethacin; slightly reduced the binding of digitoxin; moderately lowered the binding of chlorothiazide, tolbutamide, and phenylbutazone; and markedly reduced the binding of salicylic acid and aspirin.

It is of interest that halofenate free acid has been reported to displace ^{131}I-labeled thyroxine from thyroid-binding globulin (5) and to displace fatty acid from strong to weaker albumin binding sites (12).

α-(*p*-Chlorophenoxy)isobutyric acid, the free acid derived from clofibrate, has been reported (13, 14) to inhibit binding of warfarin to human albumin. Thus, it is possible that the therapeutic activity of warfarin may be influenced to a lesser degree by halofenate than by clofibrate. Clofibrate caused only a small reduction in digitoxin binding to albumin (15), similar to that observed in the present study with halofenate.

The effect of halofenate administration on the plasma half-life of antipyrine in rhesus monkeys was also determined; the results are shown in Table II. As indicated, no significant effect was found, suggesting that halofenate did not induce liver microsomal enzymes that metabolize antipyrine. Clofibrate also gave negative results, although it has been reported to stimulate testosterone metabolism by rat liver microsomes (16). Man apparently differs from the monkey in this respect, since halofenate was recently reported to shorten the half-life of antipyrine in human subjects (17).

Phenobarbital, a potent inducer of drug-metabolizing enzymes, markedly lowered the plasma half-life. The results with phenobarbital are in agreement with previously reported findings in monkeys (9), but the present study shows that induction with the barbiturate is demonstrable after a much shorter treatment period than 3 weeks.

In conclusion, results of the present study show that halofenate can inhibit binding of some drugs to plasma proteins, but that it does not stimulate the metabolism of antipyrine in the rhesus monkey; halofenate is metabolized similarly in man and the monkey (7).

At this time, it is difficult to estimate the pharmacological significance of these results. No reports of drug interaction with halofenate or halofenate free acid have been received, other than several instances in which it was found necessary to reduce the dosage of warfarin or dicumarol to patients receiving halofenate[8]. However,

Table I—Effect of Halofenate Free Acid on Binding of Various Drugs in Human Plasma

Drug	Drug Concentration, mcg./ml.	Halofenate Free Acid Concentration, mcg./ml.	Percent Drug Bound[a]
Warfarin	10	0	97.2 ± 2.3
		50	98.9 ± 0.3
		200	98.6 ± 0.3
		500	96.9 ± 0.5
Dicumarol	10	0	99.8 ± 0.2
		50	99.7 ± 0.2
		200	99.8 ± 0.2
		500	99.5 ± 0.2
Indomethacin	10	0	96.7 ± 0.2
		50	96.6 ± 0.1
		100	96.6 ± 0.1
		200	97.2 ± 1.2
		500	97.3 ± 1.1
Salicylic acid	100	0	84.4 ± 0.8
		50	81.6 ± 0.9[b]
		100	77.4 ± 0.4[b]
		200	74.0 ± 1.0[b]
		500	44.2 ± 0.8[b]
Aspirin	25	0	60.5 ± 1.7
		50	58.4 ± 1.0[b]
		100	57.9 ± 1.0[b]
		200	50.2 ± 1.2[b]
		500	9.9 ± 0.7[b]
Chlorothiazide	10	0	94.6 ± 1.3
		50	92.9 ± 0.7[b]
		100	91.4 ± 0.6[b]
		200	85.9 ± 1.4[b]
		500	75.5 ± 1.0[b]
Tolbutamide	100	0	95.4 ± 0.1
		50	94.5 ± 0.2[b]
		200	89.4 ± 0.5[b]
		500	74.3 ± 1.2[b]
Digitoxin	0.1	0	91.0 ± 0.4
		50	91.6 ± 0.9
		100	90.2 ± 0.4[b]
		200	88.7 ± 0.7[b]
		500	90.3 ± 1.8[b]
Phenylbutazone	100	0	99.1 ± 0.05
		50	98.8 ± 0.08[b]
		100	98.8 ± 0.05[b]
		500	94.0 ± 0.42[b]

[a] Mean ± *SD* for five to six analyses at each concentration. [b] Significant difference, $p = 0.05$ or less; the value for digitoxin in the presence of 500 mcg./ml. of halofenate free acid was done separately and differed significantly from the control value repeated at the same time (94.4 ± 0.7).

[5] Cab-O-Sil.
[6] Packard.
[7] Dr. D. J. Tocco and Dr. C. C. Porter, unpublished results.
[8] Dr. G. E. Maha, personal communication.

Table II—Effect of Clofibrate, Phenobarbital, Halofenate, and Placebo Treatment on Plasma Half-Life of Antipyrine in Rhesus Monkeys[a]

Group	Period	Treatment	Half-Life, hr.	Antipyrine Concentration, mcg./ml. 0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.
I	Before	None	1.0 ± 0.1	80.4 ± 8.6	63.6 ± 4.2	27.2 ± 4.3	12.9 ± 3.5	7.3 ± 2.1
I	After	Clofibrate	1.3 ± 0.2	74.7 ± 3.8	53.4 ± 4.7	32.8 ± 3.1	18.8 ± 3.0	11.0 ± 3.9
II	Before	None	1.2 ± 0.2	80.2 ± 8.2	61.6 ± 8.0	31.8 ± 5.2	15.9 ± 4.7	13.1 ± 4.5
II	After	Phenobarbital	0.6 ± 0.2[b]	54.5 ± 7.0	31.4 ± 6.1	13.2 ± 3.6	6.7 ± 2.8	1.4 ± 1.6
III	Before	None	1.2 ± 0.2	90.9 ± 7.5	65.7 ± 5.7	37.7 ± 13.4	22.0 ± 9.2	12.4 ± 4.8
III	After	Halofenate	1.3 ± 0.4	88.8 ± 9.9	60.5 ± 4.4	37.4 ± 11.0	24.0 ± 9.7	13.5 ± 7.6
IV	Before	None	1.2 ± 0.5	90.3 ± 6.1	65.7 ± 7.7	38.2 ± 11.6	23.6 ± 11.7	11.0 ± 8.7
IV	After	Placebo	1.3 ± 0.2	88.8 ± 12.0	64.9 ± 13.6	38.6 ± 6.4	24.8 ± 7.6	12.5 ± 4.9

[a] The half-life was estimated by means of a GE Mark I FORTRAN computer program. All values given are the mean ± *SD*. For Group I, $n = 4$; for Group II, $n = 8$; for Group III, $n = 8$; and for Group IV, $n = 12$. [b] Difference was highly significant ($p < 0.001$). All other $t_{1/2}$ values after treatment were not significantly different from pretreatment values.

studies specifically designed to reveal possible clinical interactions between halofenate and the drugs used in this study are currently being planned.

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Rapid Gastric Absorption of Sodium Nitrite in Mice

MARVIN A. FRIEDMAN*, ELLIOTT J. GREENE†, and SAMUEL S. EPSTEIN▲

Abstract □ The concentration of available gastric sodium nitrite and the major pathways involved in its disappearance in mice following single administration were determined. At 10 min. after oral administration, 85% of the available sodium nitrite was lost from the mouse stomach. Ligation of the gastroduodenal junction had no effect on nitrite loss. Following 30 min. incubation *in vitro*, where loss of nitrite by absorption was prevented, there was 63% loss of sodium nitrite, of which 40% had been converted to sodium nitrate. The authors concluded that the major pathway of loss of available gastric nitrite is absorption directly from the stomach into the bloodstream.

Keyphrases □ Sodium nitrite—gastric absorption and metabolism, mice □ Nitrite/nitrate levels—sodium nitrite absorption, metabolic pathways, mice □ Absorption, gastric—sodium nitrite, mice

Technological advances both in agriculture and food preservation have led to increased concern over intake of nitrite and nitrate salts (1). The primary toxicological response to these salts is methemoglobinemia. Nitrate salts do not directly cause methemoglobinemia but rather are converted to nitrite salts by gastric flora (1).

Of perhaps greater significance than methemoglobinemia is the reaction of nitrite with secondary amines