# The Hypolipemic Properties of 2-Acetoamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy)acetate in the Rat (35474)

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(Introduced by D. K. Bosshardt)
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In man, atherosclerosis and its associated complications are the leading causes of death in many countries today. Numerous studies have shown a positive correlation between the blood levels of cholesterol (1, 2) and possibly triglycerides (3), and the incidence of this disease. Therefore, an agent capable of reducing blood levels of cholesterol and triglycerides may be of clinical value in various hypercholesteremic and hyperlipidemic states. In our experimental investigations for hypolipemic drugs, 2-acetoamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate (halofenate) (4) affects a number of important plasma lipid parameters in the rat.

*Methods and Materials.* Holtzman male albino rats weighing 130–140 g were maintained on Purina Laboratory Chow for approximately 1 week. Thereafter, the rats were randomized by weight into groups of 10 rats (each averaging 165 g body weight) and placed on the experimental diets. The rats were housed on wire, 10 to a cage, and were given food and water *ad libitum*.

The control diet consisted of (%): Labco casein, 20, sucrose, 72, cellulose, 2, salt mixture (5), 4, corn oil, 1, and *dl*-methionine, 0.5. A vitamin mixture was added that supplied (mg for each 100 g of diet): vitamin A palmitate, 0.4; calciferol, 0.005; menadione, 1; α-tocopheral acetate, 4; thiamine–HCl, 0.8; riboflavin, 1.6; pyridoxine–HCl, 0.8; niacinamide, 4.0; calcium pantothenate, 4.4; para-aminobenzoic acid, 4.0; inositol, 20; biotin, 0.02; folic acid, 0.2; choline bitartrate, 400; and vitamin $B_{12}$, 0.03; as a 0.1% trituration in mannitol. Test compounds were added at the expense of equal weights of sucrose.

The test diets were fed for 9 days. At the conclusion of the test period, using light nembutal anesthesia, 5 ml of cardiac blood were taken and placed in tubes containing 0.5 ml of 0.4 *M* sodium citrate. Plasma was obtained by centrifugation for the determination of total cholesterol by the method of Abell *et al.* (6), triglycerides by the method of Van Handel and Zilversmit (7) as modified by Carlson (8), phospholipids by the method of Morrison (9), and free fatty acids by the method of Dole and Meinertz (10). Nine-day total body weights and liver weights were also determined.

*Results and Discussion.* Using 10 rats in each group, Table I shows the effect of halofenate at multiple dose levels on four plasma lipid parameters [cholesterol (chol), triglyceride (TG), phospholipids (PL), and free fatty acids (FFA)].

*Effect on plasma lipids.* Halofenate, when administered orally to rats by incorporation into the diet for 9 days, significantly reduced the plasma cholesterol level. The drug was incorporated in the diet at levels ranging from 0.00625 to 0.05% and showed a dose–response effect. A small (12%), but significant reduction was obtained at the 0.00625% level with an average lowering of 40% at the 0.05% drug level.

Halofenate also reduced the plasma triglyceride level. In general, this reduction parallels the plasma cholesterol depression. Plasma triglyceride concentrations are subject to diet, eating habits, etc., and therefore are generally more variable than cholesterol levels. The triglyceride data show less evidence of a dose–response effect and are somewhat more variable from experiment to experiment than the plasma cholesterol responses.

Plasma free-fatty acids and phospholipds were also depressed by halofenate adminis-

TABLE I. Effect of Halofenate on Four Plasma Lipid Parameters and Body Weight Gain from Five Separate Experiments.

| Level of Halofenate in diet (%) | Plasma level[a] (mg/100 ml)[b] | | | | Decrease (%) from control[a] | | | | Body wt gain (g) |
|---|---|---|---|---|---|---|---|---|---|
| | Chol | TG | PL | FFA | Chol | TG | PL | FFA | |
| 0 (control) | 64 | 87 | 146 | 0.20 | — | — | — | — | 48 |
| 0.00625 | 55[e] | 76[c] | 132[e] | 0.19 | 12[e] | 12[c] | 10[e] | 0 | 46 |
| 0.0125 | 49[e] | 71[d] | 119[e] | 0.15[d] | 24[e] | 18[d] | 18[e] | 23[d] | 50 |
| 0.025 | 44[e] | 62[e] | 111[e] | 0.12[c] | 31[e] | 29[e] | 24[e] | 38[e] | 50 |
| 0.05 | 40[e] | 49[e] | 101[e] | 0.13[e] | 38[e] | 43[e] | 31[e] | 34[e] | 49 |

[a] The geometric mean of 5 expts. with 10 rats/group.

[b] Mg/100 ml for all parameters except free-fatty acids which are given as mEq/liter.

[c] Statistically significantly less than value, one-sided $p < 0.05$; [d] one-sided $p < 0.01$; [e] one-sided $p < 0.001$.

tration. The free-fatty acids, as the plasma triglycerides, are subject to variation with less evidence of a dose–response effect and more variability between experiments than is found with plasma cholesterol. As shown by the weight gain data, there was no effect of the drug on growth of the animals over the dose range and time studied.

*Comparison with clofibrate.* Halofenate was compared in the rat with the hypocholesteremic agent clofibrate [CPIB-ester, Atromid-S, (ethyl-*a*-*p*-chlorophenoxyisobutyrate)] (11). Table II shows the mean percentage reduction for three plasma lipid parameters for six separate experiments comparing halofenate and clofibrate. Employing the data from the six experiments, a statistical evaluation (parallel line assay of response against log-dose) for relative potency shows the potency of halofenate relative to clofibrate as a hypocholesteremic agent to be 5.7, with 95% confidence limits of 4.9 to 6.7. For influencing the plasma triglyceride and phospholipid levels, the potency of halofenate vs clofibrate is of the same general order of magnitude. Halofenate produced hepatomegaly in the rat similar to that reported for clofibrate (12).

*Summary.* 2-Acetoamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate (halofenate) has been established as a potent hypolipemic agent in the rat, significantly lowering, in a dose–response relationship, plasma cholesterol, triglycerides, phospholip-

TABLE II. Comparison of Halofenate and Clofibrate on Plasma Lipids from Six Separate Experiments.

| | Decrease (%) from control[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Clofibrate | | | | Halofenate | | | |
| Level in diet (1%) | Chol | TG | PL | Liver wt[b] | Chol | TG | PL | Liver wt[b] |
| 0 (control) | — | — | — | — | — | — | — | — |
| 0.00625 | 0 | 0 | 0 | — | 19 | 10 | 12 | (5) |
| 0.0125 | 7 | 8 | 8 | — | 27 | 16 | 20 | (9) |
| 0.025 | 13 | 19 | 13 | — | 35 | 28 | 30 | (10) |
| 0.05 | 25 | 17 | 14 | (9) | 43 | 50 | 38 | (21) |
| 0.10 | 32 | 20 | 21 | (22) | — | — | — | — |
| 0.20 | 37 | 25 | 22 | (22) | — | — | — | — |
| 0.30 | 40 | 33 | 31 | (24) | — | — | — | — |

[a] The geometric mean of 6 expts. with 10 rats/group.

[b] Numbers in parentheses are percentage increases in liver weight (g/100 g of body wt) over liver weight of controls.

ids, and free-fatty acids.

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