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Effect of AY-25,712 and Other Lipid-Lowering Agents on Liver Catalase and Liver Carnitine Acetyltransferase in Rats[1] (41658)

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Abstract. The effect of the hypolipidemic agent AY-25,712 on liver catalase and carnitine acetyltransferase was studied in rats. At 250 mg/kg/day for 2 or 4 weeks, i.e., at least 125 times the minimum effective hypolipidemic dose, AY-25,712 had no effect on liver weight or liver catalase. Liver catalase was elevated after a 2-week treatment with clofibrate (+30%), bezafibrate (+71%), and fenofibrate (+77%) at doses of 250 mg/kg/day, and with ciprofibrate (+111%) at 25 mg/kg/day. Gemfibrozil at 250 mg/kg/day for 4 weeks increased catalase by 86%. The relative increase in liver weight induced by these compounds showed a good correlation to increased catalase. Nicotinic acid (250 mg/kg/day for 2 weeks) did not alter liver weight or catalase. Clofibrate increased carnitine acetyltransferase by 176% while AY-25,712 had no effect. The results show that AY-25,712 and nicotinic acid did not induce changes in the livers of rats which are associated with treatment by various other hypolipidemic agents.

We have recently reported on the lipid-lowering properties of AY-25,712 [4,5-dihydro-5-methyl-4-oxo-5-phenylfuran-2-carboxylic acid] (Ayerst Laboratories, Montreal, Quebec) in normal and hyperlipidemic rats (1) and on its effect on various aspects of free fatty acid metabolism (2). AY-25,712 at 2–5 mg/kg/day was found to lower serum triglyceride, free fatty acid and LDL[2] cholesterol concentrations (1, 2). We have concluded that the mode of action of AY-25,712 in rats resembles that of nicotinic acid (2).

Unlike clofibrate and its congeners (3–6), AY-25,712 does not elevate liver weight (1). Liver enlargement induced in rodents by certain lipid-lowering agents is associated with proliferation of peroxisomes and, ultimately, with hepatocarcinogenicity (7–12). The increase in peroxisomes induced by clofibrate in rodents is paralleled by an increase in the activity of catalase (13), a principle enzyme of these organelles (14). Similar increases have been observed with fenofibrate (15, 16) and gemfibrozil (16).

Clofibrate (17–19) and gemfibrozil (19) in rats also markedly increases liver carnitine acetyltransferase, particularly in the mitochondria. A change in this enzyme may reflect other ultrastructural changes (20–22) that occur with clofibrate.

An evaluation of the effect of AY-25,712 on liver catalase is presented in this paper. Comparisons are made with the following lipid-lowering agents: clofibrate, gemfibrozil, fenofibrate, ciprofibrate, bezafibrate, and nicotinic acid. The effect of AY-25,712 on liver carnitine acetyltransferase is also reported.

Materials and Methods. *Animals and diets.* Male albino Sprague–Dawley rats (Charles River Canada, Inc., St. Constant, Quebec), weighing about 100 g were fed Purina Laboratory Chow and kept under observation for 2–3 days prior to each study. Compounds were suspended in 2% Tween-80 and administered by gastric intubation. Animals were decapitated and were not fasted prior to killing.

Liver catalase. Groups of eight rats were given po daily for 2 weeks either AY-25,712, clofibrate, fenofibrate, bezafibrate, or nicotinic acid at a dose of 250 mg/kg or ciprofibrate at a dose of 25 mg/kg. AY-25,712 and gemfibrozil were given also at a dose of 250 mg/kg daily for 4 weeks. Control animals were given vehicle (2% Tween 80) only. The rats were decapitated 2 hr after the last dose. Serum was collected and frozen for triglyceride analysis.

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[2] Abbreviations: DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); CoA, Coenzyme A; LDL, low-density lipoprotein.

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Livers were perfused for 5–6 min *in situ* by gravity with cold, isotonic phosphate buffer, pH 7.4, then quickly immersed in ice-cold buffer. Homogenates were prepared and liver catalase was determined colorimetrically as described by Cohen *et al.* (23). The assay involves the reaction of the hydrogen peroxide remaining after incubation with potassium permanganate and measuring the residual color spectrophotometrically; the latter corresponds to the rate of hydrogen peroxide consumption by the catalase by first order kinetics.

Liver carnitine acetyltransferase. Groups of eight rats were given po daily for 2 weeks either AY-25,712 or clofibrate at a dose of 250 mg/kg. Control animals were given vehicle only. The rats were decapitated 2 hr after the last dose. Livers were perfused *in situ* by gravity with cold saline for 5–6 min and then quickly immersed in ice-cold saline. Livers were then minced and homogenized in five volumes of ice-cold Tris buffer, pH 8.0 (0.25 *M* sucrose, 0.11 *M* Tris–hydrochloride, and 2.5 m*M* EDTA). The homogenates were centrifuged at 2°C for 7 min at 700*g* to remove nuclei and cellular debris; the fatty layer was removed and the supernatant decanted. Ten microliters of supernatant was used per assay. Liver carnitine acetyltransferase was determined colorimetrically (24, 25). The assay is based on the nonenzymatic disulfide exchange between DTNB and CoA (formed by the enzyme) to give 5-thio-2-nitrobenzoate, the rate of formation of which is followed spectrophotometrically and is equivalent to the rate of CoA formation.

Triglyceride and protein analyses. Serum triglycerides were determined either by a semiautomated method (26) or by an automated method using a commercial enzyme preparation (Dow Diagnostics, Indianapolis, Ind.) Liver homogenate protein was measured by a semiautomated technique (27).

Statistical analyses. One way analysis of variance (ANOVA) was used to determine subsequent testing for statistical significance. The two-tailed *t* test was then used to determine the significance of difference between group means.

Results. AY-25,712, given for 2 or 4 weeks at a dose of 250 mg/kg daily, had no effect on liver weight (Table I). Nicotinic acid also had no effect on liver weight after 2 weeks administration of the same dose (Table I). In contrast, clofibrate, fenofibrate, and bezafibrate, at a dose of 250 mg/kg daily and ciprofibrate at a dose of 25 mg/kg daily for two weeks, caused statistically significant increases in liver weight (Tables I and II) as did gemfibrozil at a dose of 250 mg/kg daily for 4 weeks (Table I). All drugs lowered serum triglycerides. Fenofibrate increased liver catalase (77%) as did bezafibrate (71%) and ciprofibrate (111%). A 4-week treatment with gemfibrozil increased liver catalase (86%). In contrast, no

TABLE I. EFFECT OF AY-25,712, NICOTINIC ACID, GEMFIBROZIL, AND CLOFIBRATE AT DOSES OF 250 mg/kg/DAY ON LIVER WEIGHT, SERUM TRIGLYCERIDES, AND LIVER CATALASE ACTIVITY[a]

Group	Weeks of treatment	Liver wt (g/100 g body wt)	Serum triglycerides (mg/dl)	Liver catalase (μmole H_2O_2 consumed/min/mg protein)	Liver catalase Percentage change
Control		4.84 ± 0.12	82 ± 7.2	349 ± 16.3	—
AY-25,712	2	4.86 ± 0.11	52 ± 4.0[c]	380 ± 27.0	+8.9
Clofibrate		6.12 ± 0.15[d]	37 ± 4.3[d]	470 ± 21.4[d]	+34.7
Control		3.76 ± 0.06	100 ± 7.4	358 ± 10.6	—
AY-25,712	4	3.83 ± 0.07	74 ± 8.0[b]	362 ± 25.3	+1.1
Gemfibrozil		6.19 ± 0.12[d]	56 ± 5.6[d]	666 ± 29.4[d]	+86.0
Control		4.15 ± 0.08	99 ± 8.2	357 ± 13.2	—
Nicotinic acid	2	4.06 ± 0.12	41 ± 3.1[d]	391 ± 23.3	+9.5
Clofibrate		5.48 ± 0.21[d]	47 ± 2.7[d]	426 ± 10.0[c]	+19.3

[a] Data are presented as means ± SEM for six to eight rats/group for three studies.
[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$.

TABLE II. EFFECT OF 2-WEEK TREATMENT WITH FENOFIBRATE, CIPROFIBRATE, BEZAFIBRATE, AND CLOFIBRATE ON LIVER WEIGHT, SERUM TRIGLYCERIDES, AND LIVER CATALASE ACTIVITY IN RATS[a]

Group	Dose (mg/kg/day)	Liver wt (g/100 g body wt)	Serum triglycerides (mg/dl)	Liver catalase (μmole H_2O_2 consumed/ min/mg protein)	Liver catalase Percentage change
Control	—	4.73 ± 0.11	64 ± 4.1	330 ± 41.9	—
Clofibrate	250	5.84 ± 0.20[d]	48 ± 5.4[b]	459 ± 33.3[b]	+39.1
Fenofibrate	250	7.18 ± 0.13[d]	35 ± 3.0[d]	583 ± 48.9[c]	+76.7
Control	—	4.76 ± 0.24	112 ± 8.3	334 ± 12.6	—
Clofibrate	250	5.71 ± 0.13[c]	57 ± 5.2[d]	434 ± 15.5[d]	+30.0
Ciprofibrate	25	8.00 ± 0.22[d]	44 ± 2.9[d]	706 ± 47.5[d]	+111
Control	—	4.51 ± 0.11	96 ± 13.1	329 ± 18.4	—
Clofibrate	250	6.50 ± 0.13[d]	53 ± 4.3[c]	446 ± 14.1[d]	+35.6
Bezafibrate	250	7.77 ± 0.16[d]	37 ± 3.2[d]	563 ± 29.1[d]	+71.1

[a] Data are presented as means ± SEM for seven to eight rats/group for three studies.
[b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$.

effect was observed in rats given AY-25,712 for 2 or 4 weeks or nicotinic acid for 2 weeks. As expected, clofibrate increased liver catalase activity (about 30%).

In rats given clofibrate at a dose of 250 mg/kg daily for 2 weeks, liver carnitine acetyltransferase activity was increased by 176% (Table III). In contrast, AY-25,712 had no effect on liver carnitine acetyltransferase.

Discussion. The effects of AY-25,712 on various aspects of lipid metabolism have been described and compared to those of nicotinic acid and clofibrate (1, 2). It was concluded that the mode of action of AY-25,712 more resembles that of nicotinic acid than of clofibrate (2). The lipid-lowering activity of clofibrate, its congeners and gemfibrozil has been found to be accompanied by various liver changes, most notably an increase in liver weight and an increase in liver peroxisomes (3–6). We have confirmed that clofibrate increases the activities of catalase (13) and carnitine acetyltransferase in rat liver (17, 18). We have also confirmed that fenofibrate (15, 16) and gemfibrozil (16) increase liver catalase activity in rats. It has been speculated that increased activities of these enzymes could contribute to the hypolipidemic activity (17, 18, 25, 28–30), although this view has been questioned (19, 31, 32). It has also been suggested that hypolipidemic agents that increase liver weight and induce peroxisome proliferation in rats can also cause liver tumorigenesis (7, 9–12).

In the studies reported here, a good correlation was found between the relative increases in liver weight and liver catalase activity. Fenofibrate, ciprofibrate, gemfibrozil, and bezafibrate produced a marked increase in both liver weight and liver catalase activity,

TABLE III. EFFECT OF 2-WEEK TREATMENT WITH AY-25,712 AND CLOFIBRATE AT DOSES OF 250 mg/kg/DAY ON LIVER WEIGHT AND LIVER CARNITINE ACETYLTRANSFERASE IN RATS[a]

Group	Liver wt (g/100 g body wt)	Liver carnitine acetyltransferase (nmole CoA/min/ mg protein)	Liver carnitine acetyltransferase Percentage change
Control	4.56 ± 0.10	22.1 ± 2.76	—
AY-25,712	4.59 ± 0.10	20.2 ± 1.72	−8.6
Clofibrate	5.93 ± 0.11[b]	61.1 ± 3.96[b]	+176

[a] Data expressed as means ± SEM for eight rats/group.
[b] $P < 0.001$.

while the effect of clofibrate was less pronounced. Like nicotinic acid, AY-25,712 whether administered for 2 or 4 weeks, had no effect on liver weight or liver catalase activity. In addition, AY-25,712 did not increase liver carnitine acetyltransferase activity. It is pertinent that AY-25,712 was administered at a dose that was at least 125 times higher than required to lower serum triglycerides in normal rats (1). Such a dose was used in order to assess whether AY-25,712 has the capability of elevating these liver enzymes. None of the other drugs was administered at such a high dose in relation to their triglyceride-lowering potency (4). In conclusion, the results show that AY-25,712, like nicotinic acid, does not induce changes in the livers of rats that take place upon treatment with various other hypolipidemic agents.

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