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METABOLISM OF ETHANOL AND SORBITOL IN CLOFIBRATE-TREATED RATS

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Abstract—The rates of ethanol and sorbitol removal and the cytoplasmic and mitochondrial redox states of the liver were determined in female rats pretreated with clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate) for 2–15 days. The drug significantly increased the rate of elimination of ethanol even within the first two days. A significant increase in liver mass took place within a week but cannot explain the initial increase in ethanol removal. Although the liver mass was increased by clofibrate, the rate of sorbitol removal was significantly decreased. A decrease in liver sorbitol dehydrogenase activity was also observed. The sum of the removal of ethanol and sorbitol, when they were simultaneously metabolized, was significantly decreased in clofibrate-treated rats as compared with control ones. Sorbitol inhibition of ethanol elimination was increased but ethanol inhibition of sorbitol elimination was abolished by clofibrate administration. Ethanol and sorbitol caused similar changes in cytoplasmic (lactate/pyruvate) and mitochondrial (β-OH-butyrate/acetoacetate) redox states of clofibrate-treated rat liver, as has been earlier observed in livers of control animals.

Both ethanol and sorbitol are mainly oxidized in the liver cytoplasm by NAD-dependent dehydrogenases: ethanol to acetaldehyde by alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1) and sorbitol to fructose by sorbitol dehydrogenase (L-iditol: NAD oxidoreductase, EC 1.1.1.14). In each reaction one mole of NADH is generated from one mole of substrate oxidized. Sorbitol and ethanol depress each other's oxidation rates [1, 2]. It has been suggested that the inhibition originates from the competition of the respective dehydrogenases for NAD^+ and that the rate of reoxidation of NADH is the regulative factor in the oxidative reactions [3].

The reduced coenzyme (NADH) formed during the oxidation of ethanol and sorbitol is mainly oxidized in the mitochondrial respiratory chain. Since the mitochondrial membrane is quite impermeable to NADH [4], the reducing equivalents must be transferred from cytoplasm to mitochondria by specific shuttle mechanisms. At least three shuttle systems appear to function in liver cells. These are the malate–aspartate shuttle, the α-glycerophosphate shuttle and the fatty-acid elongating shuttle [5–7]. The nature of the substrate from which the hydrogen to be transferred originates may determine the shuttle via which the transport to mitochondria occurs [8]. The main route for the reducing equivalents formed by alcohol dehydrogenase reaction seems to be the malate–aspartate shuttle [9, 10], but other routes may be involved if this shuttle is inhibited [11]. On the other hand, the equivalents originating from sorbitol are apparently transferred via both the α-glycerophosphate shuttle and the malate–aspartate shuttle [8, 12, 13].

Clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate) is known to enhance the rate of ethanol elimination in rats by increasing the functional liver mass in relation to body weight [14, 15], but other mechanisms, the catalatic peroxidation of ethanol and the increased oxidation of NADH, are reported to contribute to the effect as well [16, 17]. To our knowledge, clofibrate has not been reported to affect the rate of sorbitol removal. However, there is evidence that clofibrate inhibits mitochondrial respiration in rat liver [18], and therefore the rate of reoxidation of the reducing equivalents formed during metabolism of sorbitol and ethanol is probably also affected by the drug.

The present paper reports on the liver metabolism of ethanol and sorbitol in clofibrate-treated rats. The time-course effects of clofibrate were determined since some effects of the drug may precede others. Increases in hepatic protein, mitochondria and smooth endoplasmic reticulum, for example, evidently precede the elevation in liver weight [19, 20]. Sorbitol–ethanol interactions were also studied since it seemed possible that the metabolism of sorbitol and ethanol could be differently inhibited by clofibrate.

MATERIALS AND METHODS

Female Sprague–Dawley rats, aged 4 months and weighing 200–300 g, were given free access to tap water and ordinary laboratory food during the whole experiment. Pure clofibrate (Medica, Pharmaceutical Co., Helsinki, Finland) was administered to rats by subcutaneous (s.c.) injections, 0.2 g/kg body wt per day. Body weight of the animals did not change significantly during the treatment.

Ethanol elimination and the effect of sorbitol. Four groups of rats, nine animals in each, were used. One group served as a control group and the three other groups received clofibrate for 3, 9 or 15 days. The rate of ethanol elimination was tested by giving an injection of 10 m-moles/kg body wt of a 2 M ethanol solution to the femoral vein under a light aether anaesthesia [21]. On the following day the same animals

were tested again but this time ethanol was given together with sorbitol. The solution was made 2 M with respect to both ethanol and sorbitol and the dose injected was 5 ml (10 m-moles of ethanol and 10 m-moles of sorbitol)/kg body wt. These amounts of ethanol and sorbitol were sufficient to saturate the corresponding enzymes for at least 1 hr. The clofibrate-treated groups of rats were tested on the 2nd and 3rd, 8th and 9th, and 14th and 15th days after the beginning of the drug treatment.

Blood samples were drawn every half an hour from the tip of the tail into tubes containing ice-cold perchloric acid (0.6 M), and the blood ethanol concentrations were measured with a Perkin–Elmer F 40 gas chromatograph [21]. The rate of ethanol elimination was calculated as described elsewhere [21].

After the inhibitory effect of sorbitol on the rate of ethanol removal had been tested, all rats were decapitated and the livers were weighed. Since clofibrate increased the liver mass and ethanol is oxidized almost exclusively in the liver, the rate of ethanol elimination was also calculated on the basis of liver weight.

Sorbitol elimination and the effect of ethanol. Three groups of rats, nine animals in each, were used. The control group was first tested on two successive days for the rate of sorbitol elimination. On the first day sorbitol was given alone and on the second day it was given together with ethanol. The other two groups were tested in a similar fashion on the 2nd and 3rd and the 14th and 15th days of clofibrate treatment: sorbitol was given on the first of these two days and sorbitol plus ethanol on the second.

Sorbitol, 10 m-moles of a 2 M solution/kg body wt, was administered i.v. to rats under a light aether anaesthesia. When the effect of ethanol on sorbitol metabolism was tested, the solution given was 2 M with respect to both ethanol and sorbitol.

Blood samples were drawn from the tip of the tail into tubes containing ice-cold perchloric acid (0.6 M). The amount of sorbitol in the urine was also taken into account in calculating the rate of elimination; for this purpose urine was collected during the 12 hr following sorbitol injection. Sorbitol concentrations from the blood and urine were measured colorimetrically [22].

Cytoplasmic and mitochondrial redox states. Rats received clofibrate (0.2 g/kg daily) for 2 or 14 days. Ethanol, sorbitol, or ethanol plus sorbitol were given i.v. to rats as described above. Control animals received saline instead of sorbitol and ethanol. Livers were sampled by means of the freeze-stop technique 15 min after the injections [23]. From the liver samples, which were treated as described elsewhere [24], lactate and pyruvate were determined enzymatically [25] and β-hydroxybutyrate and acetoacetate by gas chromatograph [26].

Sorbitol dehydrogenase. Ten per cent (w/v) liver homogenate from control rats and from rats pretreated for 14 days with clofibrate was prepared in ice-cold 0.25 M sucrose. The homogenate was centrifuged for 10 min at 5000 *g* and the enzyme activity was determined from the supernatant [27] by Boehringer test kits (Mannheim, Germany).

Statistics. The statistical differences were calculated with Student's *t*-test.

RESULTS

Ethanol elimination. Administration of clofibrate for two days caused a significant increase in the capacity of liver tissue to eliminate ethanol when calculated per g of liver fresh weight (Table 1). However, this effect disappeared when clofibrate treatment was continued for longer periods. Within the first two days of clofibrate treatment no significant increase in liver mass took place, but when the drug was administered for more than two days a substantial increase was observed (Table 1).

The initial increase in the rate of ethanol elimination may be related to the increased content of mitochondria in the liver. This effect of clofibrate is known to appear after treatment of only two days [20] and is accompanied by a transient increase in the activity of NADH: cytochrome *c* reductase. After treatment with clofibrate for more than 3 days the liver mass and the functional liver tissue in relation to body weight were significantly increased. A similar increase occurred in the elimination rate of ethanol when calculated per kg body weight (Table 1). Since the rate of ethanol removal as calculated per g of liver fresh weight decreased back to the control level when clofibrate treatment was continued, we, as others [15], concluded that the increase in the rate of ethanol elimination during prolonged clofibrate treatment is solely due to the increase in the liver mass.

Sorbitol elimination. Unlike ethanol, which is mainly eliminated by the liver, a substantial portion of injected sorbitol is excreted via the urine [28]. In our present experiments about half of the amount of sorbitol injected was found in the urine (52, 53 and 57 per cent after clofibrate treatment of 0, 2 and 14

Table 1. Ethanol elimination in clofibrate-treated rats

Pretreatment with clofibrate	Liver wt (g/100 g)	Rate of ethanol elimination: Without sorbitol (μmole g liver wt/min)	Rate of ethanol elimination: Without sorbitol (μmol/100 g body wt/min)	Rate of ethanol elimination: Sorbitol present (μmol/100 g body wt/min)	Inhibition by sorbitol (%)
	3.23 ± 0.31	3.33 ± 0.26	10.70 ± 0.51	8.05 ± 0.39	25
2–3 days	3.32 ± 0.21	3.57 + 0.20*	11.74 ± 0.84*	8.15 ± 0.43	31
8–9 days	3.71 ± 0.17†	3.15 ± 0.28	11.69 ± 1.09*	5.66 ± 0.53†	51
14–15 days	3.52 ± 0.12*	3.43 ± 0.36	12.07 ± 1.49*	6.31 ± 0.24†	47

Subcutaneous injections of clofibrate (0.2 g/kg) were given to rats on 0–15 successive days. The rate of ethanol removal was measured 2, 8 and 14 days after initiation of the treatment. The inhibitory effect of sorbitol on ethanol removal was tested on the 3rd, 9th and 15th days of the treatment. Ethanol and sorbitol were given intravenously under ether anaesthesia as described in the Methods section. All the figures represent the mean ± S.D. of 9 animals.

* $P < 0.05$, † $P < 0.001$, for difference from corresponding controls.

Table 2. Sorbitol elimination in clofibrate-treated rats

Pretreatment with clofibrate	Rate of sorbitol elimination (μmole/100 g body wt/min) Without ethanol	Ethanol present	Inhibition by ethanol (%)	Sorbitol dehydrogenase activity (U/g liver wt)	(U/100 g body wt)
—	8.09 ± 2.27	5.68 ± 1.10	27	7.15 ± 1.48	21.53 ± 3.64
2–3 days	6.33 ± 0.69*	4.18 ± 2.22	36		
14–15 days	4.66 ± 1.37†	5.20 ± 1.05	—	5.77 ± 0.64*	18.77 ± 2.35

Subcutaneous injections of clofibrate (0.2 g/kg) were given to rats on 0–15 successive days. The rate of sorbitol removal was measured 2 and 14 days after initiation of the clofibrate treatment. On the 3rd and 15th days of the treatment the inhibitory effect of ethanol on sorbitol removal was tested. Ethanol and sorbitol were given intravenously under ether anaesthesia as described in the Methods section. All the figures represent the mean ± S.D. of 9 animals.
* $P < 0.05$, † $P < 0.01$, for difference from corresponding controls.

days respectively). A slightly greater amount of sorbitol was excreted in the urine when ethanol was present, except in the case of animals given clofibrate for two weeks (64, 63 and 63 per cent after clofibrate treatment of 0, 3 and 15 days). The rate of sorbitol removal was then significantly ($P < 0.01$) lower than in the control animals (Table 2). Clofibrate did not affect the capacity of kidneys to excrete sorbitol but rather it depressed the elimination of sorbitol by the liver. Since clofibrate increases liver mass (Table 1) the decrease in sorbitol elimination would have been even higher if calculated per g of liver tissue.

Pretreatment of rats with clofibrate for two weeks also decreased the liver sorbitol dehydrogenase activity as compared with the controls, but when the liver weight was taken account the difference was insignificant (Table 2). The decrease in the elimination rate of sorbitol seems not to be due to the decrease in sorbitol dehydrogenase activity.

Mutual inhibition of ethanol and sorbitol elimination. Sorbitol inhibited significantly ($P < 0.001$) the rate of elimination of ethanol in both control and clofibrate-treated rats (Table 1). Pretreatment of rats with clofibrate increased the inhibitory effect of sorbitol. The inhibition was slightly higher after clofibrate treatment of only three days and rose to its maximum of about 50 per cent within nine days (Table 1). Thereafter no further increase was observed.

In the presence of ethanol the rate of elimination of sorbitol was also significantly ($P < 0.01$) decreased in control rats (Table 2). Pretreatment with clofibrate for three days did not significantly alter the inhibition per cent (from 27 to 36), but after two weeks of the drug treatment the ethanol inhibition had vanished.

In control rats sorbitol inhibited ethanol elimination about as much as ethanol inhibited sorbitol elimination (25 and 27 per cent, respectively). Thus, the sum of their removal rates was smaller when both substrates were simultaneously present than when substrates were oxidized separately. This sum was further decreased by pretreatment of rats with clofibrate, suggesting that the drug decreases the capacity of the liver tissue to get rid of these substances when they are present simultaneously. The clofibrate-induced increase in the inhibition of ethanol removal by sorbitol evidently contributed to this decrement.

Effect of ethanol and sorbitol on the liver redox state. After administration of clofibrate for two days, the lactate/pyruvate and β-OH-butyrate/acetoacetate ratios used to measure the redox state of the free $NAD^+/NADH$ couple in the liver cytoplasm and mitochondria, respectively, were about the same as those reported for normal untreated rats [3, 29]. Ethanol had the well-known reducing effect on the liver redox state [30] and the increase in the lactate/pyruvate ratio was as great as seen in normal ethanol-treated rats [3, 15]. In the case of the β-OH-butyrate/acetoacetate ratio, the shift to the reduced state was so small as to be insignificant (Table 3). This may be due to the increase in the content of liver mitochondria [20].

Table 3. The cytoplasmic and mitochondrial redox states in clofibrate-treated rats

Injection	NaCl	Ethanol	Sorbitol	Ethanol + Sorbitol
Pretreatment				
Clofibrate 2 days				
Lactate	840 ± 300	734 ± 94	—	—
Pyruvate	76 ± 33	39 ± 41	—	—
L/P	12.0 ± 4.4	30.1 ± 15.1‡	—	—
β-OH-butyrate	170 ± 30	143 ± 49	—	—
Acetoacetate	123 ± 29	96 ± 21	—	—
B/A	1.3 ± 0.6	1.5 ± 0.5		
Clofibrate 14 days				
Lactate	1000 ± 115	1988 ± 901†	1995 ± 236‡	2290 ± 653‡
Pyruvate	178 ± 81	73 ± 25†	125 ± 62	48 ± 21‡
L/P	6.6 ± 2.7	27.4 ± 8.4‡	19.1 ± 8.3‡	60.7 ± 28.2‡
β-OH-butyrate	118 ± 90	225 ± 43†	193 ± 25*	179 ± 29
Acetoacetate	139 ± 20	123 ± 24	105 ± 27*	126 ± 44
B/A	0.9 ± 0.7	1.9 ± 0.6†	1.9 ± 0.4†	1.7 ± 0.9

The contents of lactate, pyruvate, β-OH-butyrate and acetoacetate in liver are expressed as nmole/g of liver and all the figures represent the mean ± S.D. of 9 animals. Subcutaneous injections of clofibrate (0.2 g/kg/day) were given to rats for 2 or 14 successive days. The substrates were injected intravenously as described in the Methods section.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, for differences from the corresponding controls.

As compared with two-day treatment, prolonged clofibrate treatment decreased the L/P ratio in rats significantly ($P < 0.05$). Also the B/A ratio decreased, though not significantly. Ethanol increased L/P and B/A ratios in rats treated with clofibrate for two weeks, as was found earlier *in vivo* [15] but not *in vitro* [17]. Likewise, sorbitol increased L/P and B/A ratios in clofibrate-treated rats.

When ethanol and sorbitol were administered together, the shift in the L/P ratio was significantly greater than that caused by ethanol ($P < 0.01$) or sorbitol ($P < 0.001$) alone. In spite of the marked change in the L/P ratio, the administration of sorbitol and ethanol together did not alter the redox level of the mitochondria more than ethanol or sorbitol alone (Table 3).

DISCUSSION

Results of the present study confirm earlier findings that rats treated with clofibrate for one week or longer eliminate ethanol faster than untreated controls [15, 17] and that the increase in the elimination rate is solely due to the increase in the liver mass in relation to body weight [14, 15]. However, the increase found in the ethanol elimination rate in the early stage of the clofibrate treatment cannot totally be explained by the increase in liver mass, because the liver mass is not significantly changed until after two days. One possibility is that the oxidation rate of NADH, which apparently regulates the elimination rate of ethanol in fed rats [31], is temporarily increased for some reason. Gear *et al.* [20] report that, after clofibrate treatment of only two days, the content of liver mitochondria is increased and the activity of rotenone-sensitive mitochondrial NADH: cytochrome *c* reductase is 168 per cent higher than in control rats. Because NADH is mostly oxidized in mitochondria, the liver capacity to reoxidize NADH is probably increased. However, while the content of liver mitochondria continues to increase in the course of the clofibrate treatment, the activity of NADH: cytochrome *c* reductase decreases to a somewhat lower level than in untreated controls [20]. So it seems unlikely that the increase in the content of liver mitochondria contributes to the enhancement of ethanol elimination. We therefore suggest that the initial increase in the ethanol removal by clofibrate is mostly due to an increase in the oxidation rate of NADH, which reflects the increased activity of mitochondrial NADH: cytochrome *c* reductase. No such effect of clofibrate on the elimination rate of sorbitol was found after treatment of two days, suggesting that α-glycerophosphate shuttle may be more important in sorbitol metabolism than during ethanol oxidation.

Clofibrate inhibits mitochondrial state 3 (ADP present) respiration and also oxidative phosphorylation [18, 32, 33]. The first site in the respiratory chain inhibited by clofibrate is in complex I where NADH is reoxidized (Fig. 1). Accordingly, the removal of NADH-dependent substrates, such as ethanol, should be more effectively inhibited than the oxidation of substrates like sorbitol, which are supposed, at least partly, to enter the respiratory chain beyond the level of NADH [8, 12, 13]. However, as seen in Table 1, prolonged clofibrate-treatment did not decrease the elimination rate of ethanol when this substrate was present alone. Accordingly it is concluded that the inhibitory effect of clofibrate on the mitochondrial respiratory chain is not very strong and that the rate of NADH oxidation is probably regulated by the general control of energy metabolism [31, 34, 35]. The two-week treatment with clofibrate decreased both the elimination rate of sorbitol and the activity of sorbitol dehydrogenase in the liver but not the total capacity of the liver to eliminate sorbitol. We cannot conclude whether the decreased enzyme activity had any effect on the decreased elimination rate or whether the inhibitory actions of clofibrate on the mitochondrial function contributed to the decrement.

The situation was different when both ethanol and sorbitol were present simultaneously. During clofibrate treatment the inhibitory effect of sorbitol on ethanol elimination was increased and, at the same time, the inhibitory effect of ethanol on sorbitol elimination was decreased. The different changes may reflect the different sites where reducing equivalents from each reaction enter the respiratory chain.

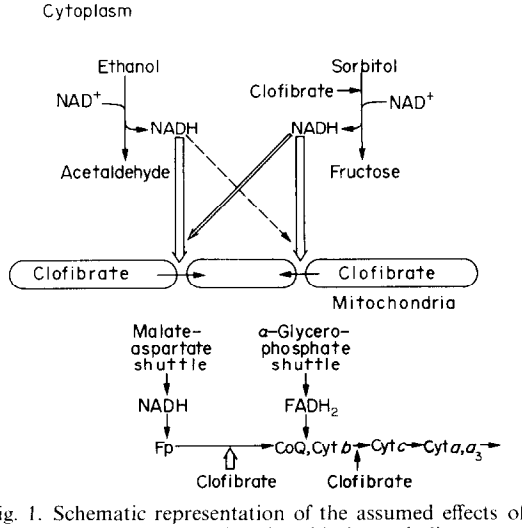

Fig. 1. Schematic representation of the assumed effects of clofibrate on ethanol and sorbitol metabolism.

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