PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 281–285 (1974)

Influence of Clofibrate (Ethyl-4-Chlorophenoxyisobutyrate) on Hepatic Drug Metabolism in Male Rats[1] (37793)

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It has been established that clofibrate is a hypolipidemic agent which effectively lowers the levels of serum cholesterol and triglycerides in man (1–3) and experimental animals (4–6). Moreover, the chronic administration of this drug to rats has produced elevations in liver weight and a proliferation of the smooth endoplasmic reticulum of liver (7, 8). Few investigators (5, 8) have attempted to examine the influence of pretreatment with clofibrate on drug oxidations in liver microsomes. Since cholesterol and steroid hydroxylations are presumed to be mediated via the microsomal mixed function oxidase system (5, 9, 10), the present study was initiated to ascertain the effect of chronic clofibrate administration on various parameters of hepatic drug metabolism in rats.

Materials and Methods. Unless stated otherwise, male albino Harlan Wistar rats, weighing 90–120 g, were used. Clofibrate (0.4 mmole/kg or 0.8 mmole/kg) or coconut oil vehicle were given orally twice daily for 7 consecutive days. Animals were allowed free access to rat chow and water throughout the pretreatment period. Treated and control groups were fasted for 14–16 hr after the last dose.

Sleeping time. After the injection of pentobarbital sodium (30 mg/kg, ip) or zoxazolamine (100 mg/kg, ip) to groups of pretreated or control groups of animals, the duration of sleep was determined to be the time interval between the loss and restoration of righting reflex.

Liver preparation. Animals were killed by stunning, the livers excised, weighed, and homogenized with a Teflon-glass homogenizer in 4 vol of 20 m*M* Tris–HCl buffer, pH 7.4, containing 1.15% KCl. Homogenates were centrifuged at 9000*g* for 20 min in a refrigerated centrifuge. The 9000*g* supernatant was carefully decanted and saved, or recentrifuged at 105,000*g* for 60 min in a Model L Beckman ultracentrifuge. The microsomal pellet was resuspended in 20 m*M* Tris–HCl–KCl buffer, pH 7.4, and stored on ice (0–4°) until further use.

Enzyme assays. Incubation mixtures contained the following components in a final volume of 3.0 ml: 5 mg of microsomal protein (or 1 ml of 9000*g* supernatant), a NAD PH-generating system (consisting of 2 enzyme units of glucose-6-phosphate dehydrogenase, 15 μmoles of $MgCl_2$, 15 μmoles of glucose-6-phosphate, and 2.4 μmoles $NADP^+$), 150 μmoles of Tris–HCl buffer, pH 7.4, and substrate. Concentrations of drug substrates used were aniline (10 μmoles), zoxazolamine (5 μmoles), pentobarbital (2 μmoles), ethylmorphine (5 μmoles), and aminopyrine (5 μmoles).

Analytical methods. The extent of pentobarbital metabolism and ethylmorphine or aminopyrine *N*-demethylation by liver microsomes were assayed using the procedure described by Nash (11). The conversion of aniline to *p*-aminophenol was assayed as described by Kato and Gillette (12). Zoxazolamine disappearance was determined by the method of Conney *et al.* (13). Cytochrome P-450 was estimated by the procedure of

[1] This work was supported by NIH Grant No. HL 12740 from the U.S. Public Health Service. A preliminary report appeared at the American Chemical Society Meetings (1972).

Omura and Sato (14) and microsomal protein assayed by the method of Lowry *et al.* (15).

Statistical comparisons were made using Student's *t* test.

Results. Preliminary experiments indicated that alterations in hepatic protein and drug metabolism induced by chronic administration of clofibrate occurred within a 7-day period, and this pretreatment with clofibrate on liver weight, liver/body weight ratio, and microsomal protein in male rats is presented in Table I. Immature rats (age 21–24 days) treated with clofibrate showed a significant elevation in microsomal protein content, while older animals (46–50 days) did not exhibit any alteration in microsomal protein after drug pretreatment. Significant increases were only noted in liver weight and liver/body weight ratios of older animals, while these parameters remaind unchanged in the youngest age group used. Subsequent biochemical experiments were conducted with younger rats.

The influence of clofibrate administration on the depressant action of pentobarbital or zoxazolamine in rats is shown in Table II. Pretreatment with clofibrate was found to significantly reduce both pentobarbital and zoxazolamine sleeping times to values 60–74% of that observed for the control animals. The decrease in pentobarbital sleeping time associated with clofibrate pretreatment was observed to be dose dependent. The reduction in sedative action for pentobarbital and zoxazolamine was related to an increase in total hepatic drug metabolism (Table III). The specific activity of the microsomal enzyme(s) for pentobarbital metabolism was significantly increased, whereas zoxazolamine hydroxylation remained unchanged in drug-treated animals. However, the magnitude of hepatic metabolism based upon a total liver protein basis, was 20% and 77% greater for zoxazolamine and pentobarbital, respectively, in clofibrate-treated animals ($P < 0.05$).

A further examination of the changes in microsomal enzyme levels after pretreatment with clofibrate is presented in Table IV. The microsomal rates of ethylmorphine and aminopyrine *N*-demethylation were significantly greater in treated animals when compared to control values, while the microsomal hydroxylation of aniline was unaffected by clofibrate pretreatment. Clofibrate administration was also observed to elevate the microsomal cytochrome P-450 content. Although not presented, the hepatic metabolism of all drug substrates, when estimated on the basis of total protein, was observed to be significantly greater in drug-pretreated rats when compared to control animals.

Discussion. Several investigators (5, 7, 8) have demonstrated an increase in liver weight and microsomal protein content after clofibrate administration. In the present

TABLE I. Effect of Age on the Changes in Liver Weight, Liver/Body Weight, and Microsomal Protein in Rats Pretreated with Clofibrate.[a]

Parameter	Treatment	Age (days)		
		21	36	48
Liver weight (g)[b]	Control	5.53 ± 0.50	7.57 ± 0.34	8.08 ± 0.31
	Clofibrate	5.90 ± 0.27	8.61 ± 0.21[d]	9.46 ± 0.46[d]
Liver/Body Weight (%)[b]	Control	4.82 ± 0.13	3.91 ± 0.07	3.44 ± 0.23
	Clofibrate	4.94 ± 0.10	4.30 ± 0.10[d]	4.13 ± 0.21[d]
Microsomal protein (mg/g)[c]	Control	21.2 ± 0.8	25.2 ± 0.6	21.2 ± 0.8
	Clofibrate	26.6 ± 0.4[d]	29.0 ± 1.8	22.8 ± 1.2

[a]Groups of animals ($N = 6$) were given clofibrate (0.4 mmol/kg) or vehicle orally, twice daily for 7 consecutive days. Animal weights for rats of ages 21, 36, and 48 days ranged from 90 to 120 g, 170 to 200 g and 220 to 250 g, respectively.

[b]Values represent mean ± standard error of $N = 6$.

[c]Values represent mean ± standard error of $N = 3$.

[d]$P < 0.05$.

TABLE II. Effect of Clofibrate Pretreatment on Hexobarbital Sleeping and Zoxazolamine Paralysis Times in Male Rats.[a]

Treatment	Duration of action (min ± standard error)[b] Pentobarbital 0.4 mmol/kg	Pentobarbital 0.8 mmol/kg	Zoxazolamine 0.4 mmol/kg
Control	64 ± 2	88 ± 8	401 ± 26
Clofibrate	48 ± 5[c]	48 ± 7[c]	242 ± 21[c]
% Control	76	54	60

[a]Groups of animals ($N = 6$) were given clofibrate (0.4 or 0.8 mmol/kg) or requisite volume of coconut oil orally twice daily for 7 consecutive days. Experiments were initiated 12–16 hr after the last dose. Values represent the mean ± standard error.

[b]Groups of animals received pentobarbital (30 mg/kg, ip) or zoxazolamine (100 mg/kg, ip) and the sleeping times were recorded.

[c]Significantly different from the control value ($P < 0.05$).

work, consistent elevations in microsomal protein and liver weight were observed to be dependent upon the age of the animals. It has been established that increases in hepatic protein and smooth endoplasmic reticulum occur during the early stages of clofibrate treatment and that the increase in protein synthesis induced via clofibrate precedes the elevation in liver weight (16).

Administration of clofibrate to rats reduced the duration of action for zoxazolamine and pentobarbital *in vivo* (Table II). The decrease in sedation obtained with pentobarbital and zoxazolamine in drug-treated rats was correlated with an elevation in the total hepatic metabolism. Clofibrate pretreatment selectively elevated the activities of microsomal enzymes for the metabolism of Type I substrates (ethylmorphine, aminopyrine, and pentobarbital), whereas the microsomal rates of metabolism for Type II substrates (zoxazolamine and aniline) remained unchanged. These findings are in general agreement with earlier findings on the oxidation of certain drugs in liver microsomes isolated from clofibrate-treated animals (5, 8).

It is noteworthy that the changes in various microsomal enzymes induced via clofibrate were also accompanied by an elevation in cytochrome P-450, a component of the electron transport system usually associated with alterations in drug oxidations (17). Wada *et al.* (18, 19) and Atkin *et al.* (20) have recently shown that the microsomal electron transport system and cytochrome P-450 also participate in the biosynthesis and metabolism of cholesterol *in vitro*. In preliminary studies, we have observed that the rate of cholesterol oxidation in liver microsomes from clofibrate-treated rats was 10–15% faster than the microsomal rate obtained from control animals. We have been unable to discern whether the inductive effects on microsomal drug-metabolizing enzymes associated with clofibrate treatment in this species are accompanied by similar changes in cholesterol

TABLE III. Influence of Clofibrate Pretreatment on the Metabolism of Pentobarbital and Zoxazolamine in Rat Liver Microsomes.[a]

Treatment[b]	Pentobarbital metabolism nmole/mg/20 min	Pentobarbital metabolism μmole/liver/20 min	Zoxazolamine hydroxylation nmole/mg/20 min	Zoxazolamine hydroxylation μmole/liver/20 min
Control	33.2 ± 1.1	4.70	25.2 ± 2.7	3.52
Clofibrate	44.1 ± 1.2[c]	8.40[c]	22.2 ± 2.7	4.23[c]
% of Control	133	177	88	120

[a]Values represent the mean ± standard error of $N = 3$.

[b]Animals were given clofibrate (0.4 mmole/kg, orally) or vehicle twice daily for 7 consecutive days.

[c]$P < 0.05$.

TABLE IV: Influence of Clofibrate Pretreatment on the Metabolism Ethylmorphine, Aminopyrine and Aniline and Level of Cytochrome P-450 in Rat Liver Microsomes.[a]

Treatment[b]	Ethylmorphine *N*-demethylation (nmole/mg/20 min)	Aminopyrine *N*-demethylation (nmole/mg/20 min)	Aniline hydroxylation (nmole/mg/15 min)	Cytochrome P-450 ($\Delta OD_{450-490}$/mg/ml)
Control	57.2 ± 2.4	33.5 ± 0.8	28.9 ± 0.2	0.071 ± 0.002
Clofibrate	77.8 ± 4.5[c]	40.2 ± 1.4[c]	29.2 ± 0.1	0.093 ± 0.004[c]
% of Control	136	120	101	131

[a]Values represent the mean ± standard error of $N = 3$.
[b]Animals were given clofibrate (0.4 mmole/kg, orally) or vehicle twice daily for 7 consecutive days.
[c]$P < 0.05$.

metabolism. Our results suggest the need for further studies on the role of hepatic microsomal induction and cytochrome P-450 on the reduction of cholesterol levels by clofibrate *in vivo*.

Only a few reports have attempted to establish the extent of drug–drug interactions associated with the administration of clofibrate and related analogues in man. Oliver *et al.* (21) and Schrogie *et al.* (22) observed that clofibrate administration does not change the metabolism of coumarin-type drugs and actually enhances the anticoagulant responses. The results of these studies on metabolism may be uncertain because of the multiple interacting actions attributable to clofibrate (23). It is of interest to note that halofenate, a congener of clofibrate, alters the metabolism of certain drugs in man. Vesell and Passanati (24) reported an acceleration of antipyrine and bishydroxycoumarin metabolism, whereas warfarin metabolism was unaffected by chronic halofenate administration. On the basis of these findings, the extrapolation of drug–drug interactions with clofibrate in man based upon the selection of one or two drugs may be misleading.

Summary. Elevations in liver weight, liver/body weight, and microsomal protein content associated with chronic clofibrate administration in male rats were found to be dependent upon the age of the animals. Pretreatment with clofibrate significantly reduced pentobarbital and zoxazolamine sleeping times and enhanced the rates of microsomal metabolism for pentobarbital, ethylmorphine and aminopyrine. Specific activities of microsomal enzymes involved with the hydroxylation of zoxazolamine or aniline remained unaffected via chronic drug administration. The metabolism of all drug substrates were significantly increased via pretreatment with clofibrate when the results were expressed on the basis of total liver protein.

The authors thank Ayerst Laboratories for the generous supply of clofibrate (Atromid-S).

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Received March 29, 1973. P.S.E.B.M., 1974, Vol. 145.