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DISSOCIATION OF INCREASED LAURIC ACID ω-HYDROXYLASE ACTIVITY FROM THE ANTILIPIDEMIC ACTION OF CLOFIBRATE

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Abstract—Clofibrate, an antilipidemic drug that acts by a still obscure mechanism, is known to specifically increase up to 30-fold the activity of the hepatic cytochrome P-450 isozyme that ω-hydroxlates lauric acid. The thesis that accelerated catabolism of medium-length fatty acids initiated by ω-hydroxylation contributes significantly to the antilipidemic action of clofibrate has been examined by measuring the impact on serum triglyceride levels of coadministering clofibrate and a suicide substrate that inactivates the hydroxylase. The results suggest that the antilipidemic action of clofibrate does not depend critically on the enhanced ω-hydroxylation of fatty acids.

Clofibrate† is the prototype of a class of antilipidemic drugs used clinically to depress elevated serum triglyceride levels in patients that do not respond to dietary regimens. A variety of mechanisms, documented in the extensive literature that has accumulated on clofibrate [1], have been proposed for the antilipidemic action of this agent and its analogues. These include inhibition of cholesterol biosynthesis [2–4], accelerated catabolism of cholesterol [5], inhibition of fatty acid transport and biosynthesis [4, 6], and increased mitochondrial and/or peroxisomal β-oxidation of fatty acids [7–10]. The relative importance of these and other mechanisms to the antilipidemic action of clofibrate, however, has yet to be determined.

Clofibrate administration has been found recently to elevate (up to 30-fold) the activity of the cytochrome P-450 isozyme that specifically ω-hydroxylates medium-length fatty acids [11–14]. In contrast, clofibrate only marginally increases the activity of cytochrome P-450 isozymes involved in drug metabolism [15–17]. The magnitude and specificity of the increase in ω-hydroxylase activity suggest that ω-hydroxylation may play an important role in the accelerated catabolism of fatty acids associated with the antilipidemic action of clofibrate. The efficient oxidation of ω-hydroxylated fatty acids to dicarboxylic acids [18, 19], in conjunction with the fact that medium-chain dicarboxylic acids are more rapidly degraded by β-oxidation than the parent monocarboxylic acids [20, 21], provides an attractive route for the ω-hydroxylation-dependent acceleration of fatty acid catabolism. The operation of such a mechanism has been invoked for the antilipidemic action of clofibrate [13, 14].

We have recently demonstrated *in vitro* that 10-UDYA and 11-DDYA are highly specific mechanism-based irreversible inhibitors of the cytochrome P-450 isozymes from uninduced rat liver that catalyze the ω- and ω-1-hydroxylation of lauric acid [22]. The design of these isozyme-specific inhibitors derives from the finding that xenobiotics with terminal π-bonds inactivate cytochrome P-450 enzymes by subverting their normal oxidative mechanisms. Catalytic turnover of the triple bond thus yields a species that N-alkylates the prosthetic heme group [23–27]. The inactivation of fatty acid hydroxylases by the unsaturated fatty acids is highly isozyme-specific because the inactivation is not accompanied by significant decreases in the total cytochrome P-450 concentration or in the rates of metabolism of xenobiotics [22].

1-Aminobenzotriazole, in contrast with the acetylenic fatty acids, irreversibly inhibits a broad spectrum of cytochrome P-450 enzymes [28, 29]. It inactivates the isozymes induced in liver by phenobarbital and 3-methylcholanthrene [28, 29], is active against cytochrome P-450 enzymes in plants [30] and insects [31], and irreversibly inhibits lauric acid hydroxylation by liver microsomes from uninduced rats [22]. Recent work indicates that 1-aminobenzotriazole destroys approximately 80% of the hepatic cytochrome P-450 enzyme complement when administered to uninduced rats and, when administered daily, maintains this low enzyme level without discernible toxicological consequences [32]. 1-Aminobenzotriazole causes no measurable changes in serum transaminase levels, liver membrane peroxidation, microsomal cytochrome b_5 content, cytochrome P-450 reductase activity, or heme biosyn-

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† Abbreviations: clofibrate, 2-(4-chlorophenoxy)-2-methylpropanoic acid (literature citations to clofibrate usually refer to the ethyl ester); 10-UDYA, 10-undecynoic acid; 11-DDYA, 11-dodecynoic acid; and ABT, 1-aminobenzotriazole.

thesis [28, 32, 33]. We describe here studies of the ability of these agents to inactivate the lauric acid ω-hydroxylase in clofibrate-pretreated animals and the use of 1-aminobenzotriazole to determine whether the clofibrate-mediated depression of serum triglyceride levels is contravened by inactivation of the ω-hydroxylases. The results indicate that the marked rise in fatty acid ω-hydroxlase activity associated with clofibrate administration is not essential for the antilipidemic action of this drug.

EXPERIMENTAL

Materials

1-Aminobenzotriazole, synthesized as reported by Campbell and Rees [34], was provided by Dr. K. Langry of this laboratory. The synthesis and characterization of 11-DDYA and 11-hydroxylauric acid have been reported [22]. Lauric acid was purchased from Nu Check Prep and 10-UDYA from Farchan Laboratories. Radiolabeled [1-^{14}C]lauric acid (32 mCi/mmole) was obtained from Amersham and Aquasol from New England Nuclear. NADPH, glucose-6-phosphate dehydrogenase, glucose-6-phosphate, diethylenetriaminepentaacetic acid, 12-hydroxylauric acid, clofibric acid, and Serum Triglyceride Kits were from the Sigma Chemical Co. Clofibric acid rather than its ethyl ester was used for all the experiments described in this paper. *N*-Methyl *p*-chloroaniline was purchased from Calbiochem. *N*-Ethylmorphine was provided by Dr. M. A. Correia. Protein concentrations were determined with kits from BioRad. Fluosol-43 was purchased from the Grand Island Biological Co.

Clofibrate induction

Male Sprague–Dawley rats (250–300 g) were fed a protein test rat diet (ICN Nutritional Biochemicals) supplemented with 0.4% (w/w) clofibrate for 10–12 days. Control rats were treated in the same manner except that their diet was not supplemented with clofibrate. The diet was prepared by directly mixing clofibric acid with the powdered diet. Microsomes were prepared from the livers of control and clofibrate-treated rats as reported before for phenobarbital-pretreated rats [29].

Lauric acid ω- and ω-1-hydroxylation

The concentrations of ω- and ω-1-hydroxylauric acids subsequent to incubation of radiolabeled lauric acid with hepatic microsomes were determined individually by high pressure liquid chromatography and liquid scintillation counting as described earlier [22].

Perfused liver studies

The basic liver perfusion technique employed in these studies has been reported in detail [35, 36]. A control or clofibrate-induced rat (250–300 g) was anesthetized with diethyl ether. The abdomen of the anesthetized rat was shaved, a longitudinal cut was made through the abdominal musculature, and the common bile duct, portal vein, and intrathoracic portion of the inferior vena cava were cannulated. Immediately after cannulation of the portal vein, *in situ* perfusion was begun with Krebs–Henseleit solution prewarmed to 37° and bubbled with 95% O_2 and 5% CO_2. The liver was then excised and transferred to a thermostatically controlled perfusion cabinet maintained at 37°. The perfusion solution employed was a fluorocarbon emulsion (Fluosol-43). The perfusate was circulated with a peristaltic pump at a constant rate of 25 ml/min through a membrane oxygenator [37], a temperature sensitive probe, a stainless steel filter screen, a flow-through pH electrode, a bubble trap/pressure gauge, and, finally, through the cannulated portal vein, the liver. The pH of the perfusion solution was maintained between 7.35 and 7.45 by changing the pCO_2 or by adding bicarbonate to the perfusate. The livers were perfused for at least 30 min to allow the system to reach equilibrium before experiments were initiated. A solution of 10-UDYA in 5 ml of Fluosol-43, filtered through a Whatman filter, was added to the 55 ml of Fluosol-43 in the liver perfusion reservoir. Sufficient 10-UDYA was added in this manner to bring the final concentration in the perfusion solution to 600 μM. The bile flow and perfusion pressure were monitored continuously during the perfusion and remained constant. The perfusions were terminated 60 min after the preliminary equilibration period and the livers were disconnected from the perfusion system and placed in ice-cold 150 mM phosphate buffer (pH 7.4) containing 1.5 mM diethylenetriaminepentaacetic acid, and 150 mM KCl. Microsomes were prepared from the livers as described previously [22] and their ability to hydroxylate lauric acid and to N-dealkylate ethylmorphone and *N*-methyl-*p*-chloroaniline was assayed.

In vivo *inactivation of lauric acid hydroxylases*

Diet. Control animals received a diet supplemented with 5 g of lauric acid per 100 g of diet, whereas the experimental groups received 50, 500, or 5000 mg of 10-UDYA and the amount of lauric acid required to bring the total to 5 g of fatty acids per 100 g of diet. This method of administration was discontinued, however, because the experimental groups refused to eat the food.

Gavage. Control animals received 1000 mg of lauric acid in 1 ml of corn oil. The experimental groups received 1000, 500, 100, or 10 mg/rat of 10-UDYA in the same amount of corn oil. Animals receiving the higher doses, however, died within 3 hr. Only with the lowest dose were no deaths observed. Lauric acid itself caused no deaths.

Intraperitoneal injection. 10-UDYA or 1-aminobenzotriazole in dimethyl sulfoxide (200 mg/5 ml) was administered by intraperitoneal injection to male Sprague–Dawley (250–300 g) rats that had been maintained on a control diet or on a diet supplemented with clofibrate (0.4%) for 14 days prior to the experiment. The rats were decapitated 2 or 4 hr after administration of the agents, and microsomes were prepared from their livers.

Serum triglyceride levels

The animals were starved for 24 hr prior to serum triglyceride determinations. Blood (approximately 200 μl) was drawn from each rat by nicking its tail. The blood samples were drawn into heparin-coated capillary tubes that were subsequently centrifuged

for 20 min at 3000 rpm in an International centrifuge. A 50-μl sample of the plasma thus collected was incubated in 2.0 ml of the Sigma Triglyceride Kit reagent for 10 min at 37°. The absorbance of the samples at 340 nm compared to a water blank was then determined. The Sigma tryglyceride assay is based on that reported by Bucolo and David [38].

Three groups of five rats were placed on a diet supplemented with sucrose (60% of the total) and clofibrate (0.4%). After 16 days, at which time their triglyceride levels had stabilized, one group of control animals was injected intraperitoneally with water (250 μl) for the duration of the experiment. A second control group was placed on a diet without clofibrate but was injected with ABT (10 mg in 250 μl of water per rat) on the same schedule as the experimental group. The experimental group was kept on the clofibrate diet but was injected intraperitoneally with ABT on days 16, 18, 21, 24 and 27. The animals were starved for 24 hr prior to determinations of their triglyceride levels.

RESULTS

Induction of the ω and ω-1-lauric acid hydroxylases by clofibrate

Clofibrate has been reported to cause a major elevation in hepatic ω-hydroxylase activity but only a minor elevation of the ω-1-hydroxylase activity [11–13]. The preferential induction of the ω-hydroxylase was confirmed in a preliminary study (Fig. 1). Sprague–Dawley male rats on a diet supplemented with 0.4% clofibrate exhibited a 9-fold higher ω-hydroxylase activity but only a 2-fold elevation of the ω-1-hydroxylase activity when compared to rats on the unsupplemented diet. The elevation in the ω-hydroxylase was less than the reported 30-fold [11, 12], probably because a small difference in the basal activity greatly alters the calculated ratio of the activities.

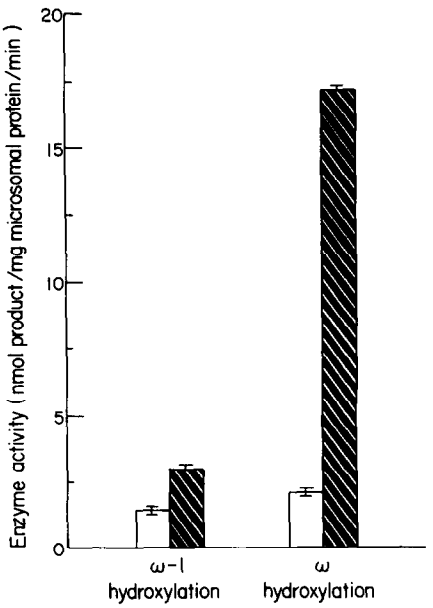

Fig. 1. Induction of ω- and ω-1-lauric acid hydroxylase activities by clofibrate. The activities are shown for control rats (open bars) and for rats fed a diet containing 0.4% clofibrate for 10 days (slashed bars). Standard deviations are indicated by the error bars.

In vitro *inactivation of the clofibrate-induced lauric acid ω-hydroxylase*

10-UDYA, 11-DDYA, and 1-aminobenzotriazole were shown recently to be mechanism-based inhibitors of the hepatic lauric acid ω-hydroxylase [22]. The abilities of these agents to inactivate the ω-hydroxylase induced by clofibrate were checked, however, because the induced enzyme may or may not be identical to that in uninduced microsomes. The incubation of 10-UDYA, 11-DDYA, or 1-aminobenzotriazole with hepatic microsomes from clofibrate-induced rats, as shown in Figs. 2 and 3, suppressed most of the ω-hydroxylase and much of the ω-1-hydroxylase activity. The loss of these enzyme activities was both NADPH and time dependent. Up to 20% inhibition was observed when the preincubation was carried out in the absence of NADPH, but this inhibition was time independent and presumably reflected competitive inhibition and inactivation that occurred during the incubation in which the hydroxylase activity was actually measured (the inhibitor was present during the assay incubation).

Inactivation of the ω-hydroxylase in the perfused liver

The livers of clofibrate-induced rats were surgically transferred to a perfusion cabinet in which they were perfused with oxygen-carrying fluorcarbon emulsion

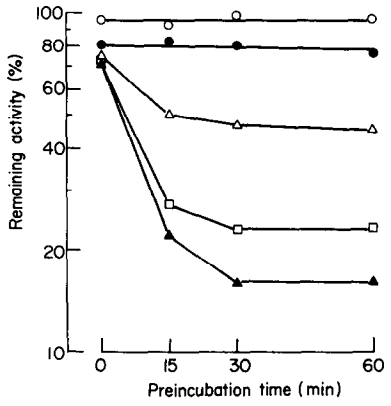

Fig. 2. Inactivation of the ω- and ω-1-hydroxylases in clofibrate-induced rats by 11-DDYA (150 μM). Hepatic microsomes from clofibrate-treated rats were preincubated for the indicated periods before the hydroxylation of lauric acid was assayed in a 5-min incubation. The fraction of the activity observed in the absence of preincubation with inhibitors is given for formation of the unresolved 11- and 12-hydroxylauric acids (□), 12-hydroxylauric acid (▲), and 11-hydroxylauric acid (△). Control experiments were carried out in which the unresolved 11- and 12-hydroxylauric acids were quantitated after preincubation of the microsomes without 11-DDYA or NADPH (○) or in the presence of 11-DDYA but not NADPH (●). The control activities in the absence of inhibitor were 3 and 17 nmoles/mg protein/min, respectively, for ω-1 and ω-hydroxylation. The values in the plots are averages of two independent incubations.

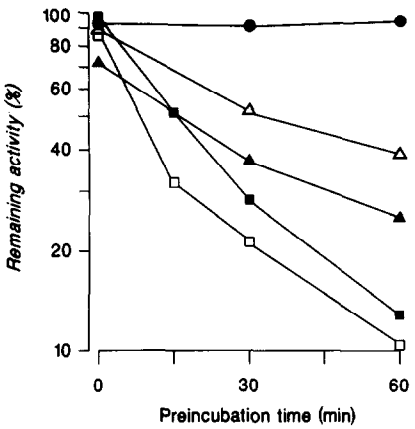

Fig. 3. Inactivation of the ω- and ω-hydroxylases in clofibrate-induced rats by 10-UDYA and ABT. Hepatic microsomes from clofibrate-treated rats were preincubated for the indicated periods with 10-UDYA (triangles) or ABT (squares) before the hydroxylation of lauric acid was assayed. The concentrations of 10-UDYA and ABT in the preincubation mixture were 150 and 1100 μM respectively. The fraction of the activity observed in the absence of preincubation with either inhibitor is given for formation of 12-hydroxylauric acid (closed symbols) and 11-hydroxylauric acid (open symbols). Control experiments were carried out in which the unresolved 11- and 12-hydroxylauric acids were quantitated after preincubation of the microsomes in the presence of 10-UDYA or ABT but in the absence of NADPH: the data for the 10-UDYA experiment is presented (●). The ABT data were essentially identical. The control activities in the absence of inhibitors were 3 and 17 nmoles/mg protein/min, respectively, for ω-1- and ω-hydroxylation. The values in the plots are averages of two independent incubations.

(Fluosol-43). Bile flow, pH and perfusion pressure, monitored continuously, were found to remain constant after an initial 30-min equilibration period. After the perfused livers reached a state of equilibrium, 10-UDYA was added to the perfusion solution (nominal concentration 600 μM). The activity of the lauric acid hydroxylases in microsomes prepared 60 min after initial introduction of the unsaturated fatty acid was compared to the activity of microsomes obtained from perfused livers not exposed to this agent (Fig. 4). The results demonstrate that 10-UDYA reached the endoplasmic reticulum in the hepatocytes and inactivated the ω-hydroxylase, although only partial inactivation of the enzyme was observed under these conditions. The ω-1-hydroxylase activity also appeared to decrease but the magnitude of the change was too small to be significant. The effect of adding 10-UDYA to the perfusion medium on the ability of the microsomes to N-demethylate *N*-methyl *p*-chloroaniline and ethylmorphine was also evaluated to determine if the loss of hydroxylase activity reflected general loss of cytochrome P-450 metabolic capacity. The two *N*-demethylation activities were not altered by 10-UDYA (Fig. 4).

In vivo *ω-hydroxylase inactivation*

Administration of 10-UDYA by gavage at doses above 400 mg/kg to clofibrate-induced rats proved lethal. No deaths were observed among rats receiving 40 mg/kg of 10-UDYA or up to 1000 mg/kg of lauric acid. The decrease in lauric acid hydroxylation was insignificant, however, when rats were treated with a non-lethal (40 mg/kg) dose of 10-UDYA (Fig. 5). In contrast, 1-aminobenzotriazole (40 mg/kg), which is less isozyme-specific but is nontoxic, lowered the *in vivo* ω- and ω-1-hydroxylase activities to less than half of control values (Fig. 5).

Effect of inactivating fatty acid hydroxylases on clofibrate-mediated reduction of serum triglyceride levels

The serum triglyceride levels of Sprague–Dawley rats were not decreased when they were fed a clofibrate-supplemented normal diet. The serum triglyceride concentration for 300 g Sprague–Dawley male rats on a normal Simonsen diet was approximately 100 ± 10 mg/dl, a value similar to the 102 mg/

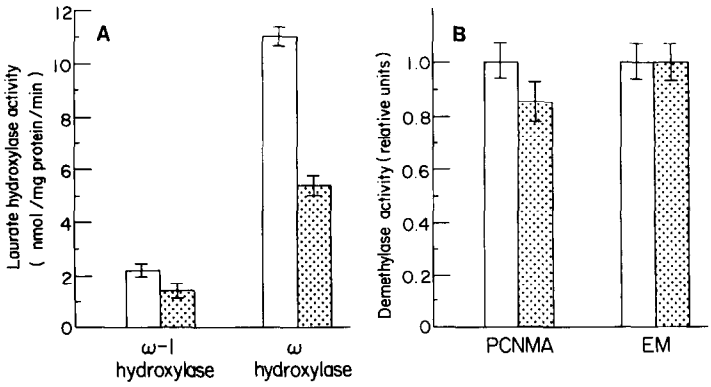

Fig. 4. Loss of cytochrome P-450-catalyzed activities after exposure of perfused livers to 10-UDYA. The formation of 11- and 12-hydroxylauric acids by microsomes prepared from livers of clofibrate-treated rats subsequent to perfusion with Fluosol-43 (open bars) or with the same solution containing 600 μM 10-UDYA (dotted bars) was measured. The N-demethylations of *N*-methyl *p*-chloroaniline (PCNMA) and ethylmorphine (EM) were measured in the same microsomal preparations. Standard deviations are indicated by the error bars.

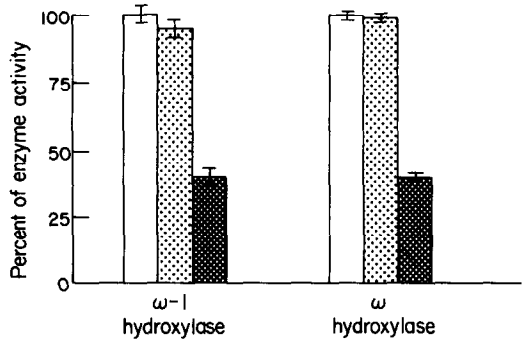

Fig. 5. *In vivo* inactivation of ω- and ω-1-hydroxylases by 10-UDYA and 1-aminobenzotriazole. Control animals (open bars) received 200 μl of dimethyl sulfoxide. Test animals received either 10 mg of 10-UDYA in 200 μl of dimethyl sulfoxide (dotted bars) or 10 mg of 1-aminobenzotriazole in 200 μl of dimethyl sulfoxide (hatched bars). All the rats were clofibrate-induced. Liver microsomes were prepared 4 hr later, and the hydroxylation of lauric acid was assayed as described in the Experimental section. Standard deviations are indicated by the error bars.

dl reported in the literature [5]. In order to see a measurable effect of clofibrate on serum triglyceride levels, it was necessary to make the rats hyperlipidemic by placing them on a diet of 60% sucrose and 40% normal feed [39, 40]. The resulting 80% elevation of serum triglyceride levels was suppressed when clofibrate was added to this special diet (Fig. 6). Clofibrate supplementation decreased the serum triglycerides to the levels of rats on the normal (i.e. sucrose-unsupplemented) diet within 10 days. Administration of 1-aminobenzotriazole on days 16, 18, 21, 24, and 27 to rats stabilized on the sucrose- and clofibrate-supplemented diet caused, if anything, a marginal decrease in serum triglyceride levels (Fig. 6). In contrast, removal of clofibrate from the diet caused a rapid rise of the triglycerides to the levels found in the sucrose-fed control rats even if 1-aminobenzotriazole was administered simultaneously (Fig. 6).

DISCUSSION

To establish whether the marked enhancement of fatty acid ω-hydroxylation caused by clofibrate contributes significantly to the concomitant lowering of serum triglyceride levels, we investigated whether: (a) mechanism-based inhibitors, shown previously to inactivate constitutive fatty acid ω-hydroxylases, would also inactivate the clofibrate-induced enzyme, (b) mechanism-based inhibitors would reach and inactivate the enzyme *in vivo*, and (c) inactivation of the fatty acid ω-hydroxylase in hyperlipidemic animals would interfere with the antilipidemic action of clofibrate.

Clofibrate, as expected [11–13], caused a major increase in the hepatic fatty acid ω-hydroxylase activity of Sprague–Dawley male rats (Fig. 1). The elevation of lauric acid ω-hydroxylation caused by clofibrate in our experiments ranged from 5- to 15-fold. Because it is not known if the clofibrate-induced enzyme is identical to the uninduced enzyme, the ability of agents that inactivate the uninduced enzyme to inactivate the clofibrate-induced enzyme

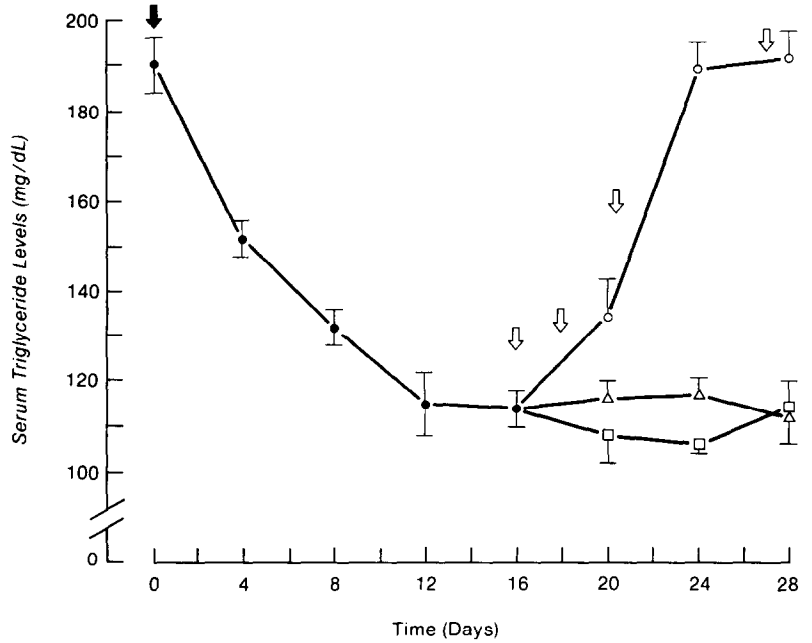

Fig. 6. Modulation of serum triglyceride levels in rats caused by clofibrate treatment and by subsequent administration of 1-aminobenzotriazole. The experimental protocol is outlined in the Experimental section. Rats received clofibrate until their triglyceride levels stabilized (●); one group of rats continued on clofibrate (△), a second group of rats continued on clofibrate and also received ABT (□), and a third group of rats received ABT but no further clofibrate (○). The times at which ABT was injected are indicated by the arrows except that an arrow is not shown for the injection at 24 hr. Standard deviations are indicated for the data points by the error bars.

was examined. The time- and NADPH-dependent loss of the ω- and ω-1-hydroxylase activities of microsomes from clofibrate-induced rats when incubated with 11-DDYA, 10-UDYA, or ABT (Figs. 2 and 3) indicates that the clofibrate-induced enzyme is vulnerable to inactivation by these agents. Parallels exist between the inactivation of the induced and constitutive enzymes: (a) the ω-hydroxylase was inactivated more effectively than the ω-1-hydroxylase, (b) the more effective of the two acetylenic fatty acids was 11-DDYA, (c) inactivation of the ω-1-hydroxylase was biphasic and incomplete, and (d) the ω- and ω-1 activities were inactivated in parallel by 10-UDYA but in a divergent manner by 11-DDYA (Figs. 2 and 3 and Ref. 22). These parallels suggest that the constitutive and induced hydroxylases are similar, but do not establish their identity. More pertinent to the present study is the demonstration that the inactivators are active against the ω-hydroxylase induced by clofibrate.

The inactivation of fatty acid hydroxylases was investigated in the perfused liver to determine if the fatty acid analogues are taken up by liver cells. 10-UDYA rather than 11-DDYA was employed for these studies because it is commercially available. As shown in Fig. 4, 10-UDYA added to the perfusion medium inactivated the lauric acid ω-hydroxylase although not as effectively as it did in an *in vitro* incubation. The inactivation was not the result of general damage to the cytochrome P-450 isozyme pool because the activities of isozymes that *N*-dealkylate ethylmorphine and *N*-methyl *p*-chloroaniline were not affected. The unsaturated fatty acid analogue thus clearly enters liver cells and reaches the targeted enzyme.

The inherently more complex pharmacokinetics in the intact animals, and the toxicity of 10-UDYA, which restricts the dose, prevented delivery of the unsaturated fatty acid to the endoplasmic reticulum in amounts sufficient to reproducibly inactivate the fatty acid hydroxylase (Fig. 5). The construction of unsaturated fatty acid analogues that circumvent these pharmacokinetic limitations is being pursued. The *in vivo* utility of 1-aminobenzotriazole, however, was not constrained by pharmacokinetic considerations (Fig. 5). Administration of a single 40 mg/kg dose of 1-aminobenzotriazole resulted in loss of approximately 60% of the hepatic ω- and ω-1-hydroxylase activities. Cytochrome P-450 isozymes involved in the metabolism of xenobiotics are also inactivated by this treatment, but other enzymes and processes appear not to be affected [28, 29, 32, 33]. Recovery of the ω-hydroxylase activity in uninduced rats after its destruction by 1-aminobenzotriazole has been shown in independent studies to be very slow.* The slow recovery means that fatty acid hydroxylation is depressed after the administration of ABT for a period of at least 48 hr. 1-Aminobenzotriazole was therefore employed to study the link between ω-hydroxylation and the depression of serum triglyceride concentrations.

Control experiments demonstrated that clofibrate does not reproducibly and significantly lower serum triglyceride levels in Sprague–Dawley male rats maintained on a normal diet. This limitation of the Sprague–Dawley rat model was circumvented by placing the rats on a diet of 60% sucrose and 40% normal feed, a regimen that significantly (30–80%) elevates serum triglyceride levels [39, 40]. Administration of clofibrate to rats on the sucrose diet depressed serum triglyceride levels within 10 days to approximately the values found for rats on the sucrose-free diets (Fig. 6). The administration of 1-aminobenzotriazole to clofibrate-treated rats on the sucrose diet, however, did not engender a significant change in serum triglyceride levels. If ω-hydroxylation of medium-length fatty acids were important for the antilipidemic action of clofibrate, a detectable increase in serum triglyceride concentrations should have been associated with destruction of 60% of that activity. The experiment in which clofibrate was withdrawn demonstrates that triglyceride concentrations return to elevated values rapidly enough for the change to have been observed if ABT had interfered with the action of clofibrate. The time required for serum triglyceride levels to return to control values in rats when clofibrate is withheld is comparable to that required in man (5–6 days) [41]. These results indicate that ω-hydroxylation of medium-length fatty acids is not critical for the antilipidemic activity of clofibrate. This conclusion rests on the assumption that any upward pressure on serum triglyceride concentrations caused by suppression of fatty acid hydroxylases is not countered by changes associated with concomitant inactivation of other cytochrome P-450 isozymes.

In summary, inactivation of a major fraction of the fatty acid ω-hydroxylase induced by clofibrate had no discernible impact on the ability of this agent to lower serum triglyceride levels. This suggests that elevation of the fatty acid ω-hydroxylase activity by clofibrate is independent of the antilipidemic action.

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* The ω-hydroxylase activity (as a percentage of the control value) observed in uninduced Sprague–Dawley rats receiving 1-aminobenzotriazole (50 mg/kg) at time zero was 23% at 24 hr, 49% at 72 hr, and 59% at 160 hr (P. R. Ortiz de Montellano and A. K. Costa, manuscript submitted for publication).

REFERENCES

1. D. T. Witiak, H. A. I. Newman and H. P. Feller, *Clofibrate and Related Analogs*. Marcel Dekker, New York (1977).
2. D. L. Azarnoff, D. R. Tucker and G. A. Barr, *Metabolism* **14**, 959 (1965).
3. D. R. Avoy, E. A. Swyryd and R. G. Gould, *J. Lipid Res.* **6**, 369 (1965).
4. L. W. White, *J. Pharmac. exp. Ther.* **178**, 361 (1971).
5. H. Hayashi, K. Fukuyama and F. Yamasaki, *Chem. pharm. Bull., Tokyo* **31**, 653 (1983).
6. R. G. Gould, E. A. Swyryd, D. Avoy and B. Coan, *Prog. Biochem. Pharmac.* **2**, 345 (1965).
7. C. R. Mackerer, *Biochem. Pharmac.* **26**, 2225 (1977).
8. P. B. Lazarow and C. DeDuve, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2043 (1976).

9. P. B. Lazarow, *J. biol. Chem.* **253**, 1522 (1978).
10. D. M. Capuzzi, C. M. Intenzo, . D. Lackman, A. F. Whereat and D. M. Scott, *Biochem. Pharmac.* **32**, 2195 (1983).
11. T. C. Orton and G. L. Parker, *Drug Metab. Dispos.* **10**, 110 (1982).
12. G. C. Gibson, T. C. Orton and P. P. Tamburini, *Biochem. J.* **203**, 161 (1982).
13. P. P. Tamburini, H. A. Masson, S. K. Bains, R. J. Makowski, B. Morris and G. G. Gibson, *Eur. J. Biochem.* **139**, 235 (1984).
14. R. M. Welch, A. R. Brown, R. L. Hawks and H. W. Marr, *Fedn Proc.* **43**, 563 (1984).
15. E. Hietanen, K. Linnainmaa and H. Vaino, *Acta pharmac. tox.* **53**, 103 (1983).
16. R. A. Salvador, S. Haber, C. Atkins, B. W. Gommi and R. M. Welch, *Life Sci.* **9**, 397 (1970).
17. N. J. Lewis, D. T. Witiak and D. R. Feller, *Proc. Soc. exp. Biol. Med.* **145**, 281 (1974).
18. B. Preiss and K. Bloch, *J. biol. Chem.* **239**, 85 (1964).
19. J. A. Peterson, Y. Ishimura and B. W. Griffin, *Archs Biochem. Biophys.* **149**, 197 (1972).
20. P. B. Mortensen, S. Kolvaraa, N. Gregersen and K. Rasmussen, *Biochim. biophys. Acta* **713**, 393 (1982).
21. F. Wada and M. Usami, *Biochim. biophys. Acta* **487**, 261 (1977).
22. P. R. Ortiz de Montellano and N. O. Reich, *J. biol. Chem.* **259**, 4136 (1984).
23. I. N. H. White and U. Muller-Eberhard, *Biochem. J.* **166**, 57 (1977).
24. P. R. Ortiz de Montellano and K. L. Kunze, *J. biol. Chem.* **255**, 5578 (1980).
25. P. R. Ortiz de Montellano and K. L. Kunze, *Biochemistry* **20**, 7266 (1981).
26. K. L. Kunze, B. L. K. Mangold, C. Wheeler, H. S. Beilan and P. R. Ortiz de Montellano, *J. biol. Chem.* **258**, 4202 (1983).
27. P. R. Ortiz de Montellano, in *Bioactivation of Foreign Compounds* (Ed. M. W. Anders), pp. 121–55. Academic Press, New York (1985).
28. P. R. Ortiz de Montellano and J. M. Mathews, *Biochem. J.* **195**, 761 (1981).
29. P. R. Ortiz de Montellano, B. A. Mico, J. M. Mathews, K. L. Kunze, G. T. Miwa and A. Y. H. Lu, *Archs Biochem. Biophys.* **210**, 717 (1981).
30. D. Reichhart, A. Simon, F. Durst, J. M. Mathews and P. R. Ortiz de Montellano, *Archs Biochem. Biophys.* **216**, 522 (1982).
31. R. Feyereisen, K. C. Langry and P. R. Ortiz de Montellano, *Insect Biochem.* **14**, 19 (1984).
32. A. K. Costa and P. R. Ortiz de Montellano, *Fedn Proc.* **44**, 652 (1985).
33. P. R. Ortiz de Montellano, J. M. Mathews and K. C. Langry, *Tetrahedron* **40**, 511 (1984).
34. C. D. Campbell and C. W. Rees, *J. chem. Soc.* (C), 742 (1969).
35. M. K. Kushlan, J. L. Gollan, W-L. Ma and R. K. Ockner, *J. Lipid Res.* **22**, 431 (1981).
36. J. Gollan, L. Hammaker, V. Licko and R. Schmid, *J. clin. Invest.* **67**, 1003 (1981).
37. R. L. Hamilton, M. N. Berry, M. C. Williams and E. M. Severinghaus, *J. Lipid Res.* **15**, 182 (1974).
38. G. Bucolo and H. David, *Clin. Chem.* **19**, 476 (1973).
39. P. Segal, P. S. Roheim and H. A. Eder, *J. clin. Invest.* **51**, 1632 (1972).
40. T. S. Schiff, P. S. Roheim and H. A. Eder, *J. Lipid Res.* **12**, 596 (1971).
41. M. F. Oliver, J. *Atheroscler. Res.* **3**, 427 (1963).