Differential Effects of Fibrates on the Metabolic Activation of 2-Phenylpropionic Acid in Rats

Chunze Li,1 Mark P. Grillo,2 Ilaria Badagnani,3 Kimberly L. Fife, and Leslie Z. Benet

Department of Biopharmaceutical Sciences, University of California, San Francisco, California

Received July 23, 2007; accepted January 9, 2008

ABSTRACT:

A series of studies were conducted to explore the inductive potential of different fibric acid derivatives on the two alternative metabolic activation pathways of 2-phenylpropionic acid (2-PPA) (a model substrate for profen drugs), namely acyl-CoA formation and acyl glucuronidation, in vivo in rats, and to evaluate whether such treatment could potentially modulate the covalent binding of profens to hepatic protein. After administration of a single dose of 2-PPA (130 mg/kg) to rats pretreated with equimolar doses of clofibrirate (160 mg/kg/day), fenofibrate (260 mg/kg/day), or gemfibrozil (180 mg/kg/day) for 7 days, rat livers were collected and analyzed for covalent binding and hepatic levels of the two reactive metabolites over a 2-h period. Results showed that the three fibrates exhibited very different effects on the hepatic levels of 2-PPA-S-acyl CoA (2-PPA-CoA) in vivo, even though all three significantly increased acyl-CoA synthetase activity in vitro in liver homogenate. Treatment with clofibrirate markedly increased the hepatic exposure of 2-PPA-CoA by 2.9-fold and led to a 25% increase (p < 0.05) in covalent binding of 2-PPA to liver protein. In contrast, significant decreases of the hepatic levels of 2-PPA acyl glucuronide and/or 2-PPA-CoA by fenofibrate and gemfibrozil significantly lowered the covalent binding of 2-PPA to hepatic protein. Together, these results suggest that fibrates exhibit markedly different abilities to alter the extent of covalent binding of 2-PPA to hepatic protein by differentially modulating the hepatic exposure of the two reactive metabolites of 2-PPA, namely 2-PPA-CoA thioester and acyl glucuronide.

2-Arylpropionic acids (profen drugs) are a widely used class of nonsteroidal antiinflammatory drugs, which have a chiral center at the carbon α to the carbonyl group. They are dosed therapeutically as racemic mixtures, with the notable exception of naproxen. In vitro studies suggested that anti-inflammatory activity resides almost exclusively in the (S)-enantiomer (Williams, 1990). Yet, the two enantiomers of profen drugs have been shown to exhibit similar anti-inflammatory properties in vivo, because the inactive (R)-enantiomers are unidirectionally inverted in vivo to the active (S)-enantiomers (Hutt and Caldwell, 1983; Caldwell et al., 1988). The mechanism of this inversion is believed to involve the enantioselective formation of acyl-CoA thioester followed by enzymatic epimerization and hydrolysis to regenerate free acids (Fig. 1) (Nakamura et al., 1981; Hall and Quan, 1994). Enantioselective activation of the (R)-enantiomers to their acyl-CoA thioesters is believed to be the key step in such chiral inversion, because it accounts for the apparent unidirectional nature of the process (R to S, but not vice versa) in humans and several animal species (Caldwell et al., 1988).

The activated acyl-CoA derivatives of profen drugs also serve as obligatory intermediates for the formation of amino acid conjugates, acyl carnitine derivatives, and hybrid triacylglycerides (Fears, 1985; Hutt and Caldwell, 1990) (Fig. 1). Because of the electrophilic nature of the thioester bond, acyl-CoA thioesters of a number of xenobiotic carboxylic acids, including nafenopin (Sallustio et al., 2000), clofibrate acid (Grillo and Benet, 2002), 2-phenylpropionic acid (2-PPA) (Li et al., 2002b), naproxen (Olsen et al., 2002), 2,4-dichlorophenoxyacetic acid (Li et al., 2003a), tolmetin (Olsen et al., 2003, 2007), and zomepirac (Olsen et al., 2005), have been demonstrated to be chemically reactive and capable of acylating biological nucleophiles, such as glutathione and proteins. Studies with eight structurally diverse carboxylic acids showed that, like acyl glucuronides (Benet et al., 1993), the chemical reactivity of acyl-CoA thioesters depends on the electronic nature and degree of substitution at the α-carbon and thus could be predicted from their chemical structures (Sidenius et al., 2004).

Most profens are metabolized to acyl glucuronides and acyl-CoA thioesters, both of which have been demonstrated to be chemically reactive and are believed to mediate covalent adduct formation in vivo (Fig. 1) (Boelsterli, 2002; Li and Benet, 2002). Comparative in vitro studies with several carboxylic acids, including clofibrate acid (Shore et al., 1995; Grillo and Benet, 2001), 2-PPA (Li et al., 2002b), and naproxen (Olsen et al., 2002), showed that acyl-CoA thioesters are 40-

ABBREVIATIONS: 2-PPA, 2-phenylpropionic acid; DTT, dithiothreitol; HPLC, high-performance liquid chromatography; 2-PPA-CoA, 2-PPA-S-acyl CoA; AUC, area under the curve.
to 70-fold more reactive toward glutathione, forming acyl glutathione conjugates, than the respective acyl glutathiones in vitro in buffer. In the case of 2-PPA, metabolic activation by acyl-CoA formation has been shown to contribute more to covalent binding than the corresponding acyl glucuronidation in vitro in hepatocytes (Li et al., 2002a) and in vivo in rats (Li et al., 2003b).

Considering the potential importance of acyl-CoA thioesters in covalent binding, we hypothesized that the extent of covalent adduct formation of profen drugs might be modulated by agents that regulate acyl-CoA formation. Our previous studies showed that the extent of covalent binding of 2-PPA to hepatic protein was markedly decreased when the corresponding acyl-CoA formation was inhibited by trimethylacetic acid (Li et al., 2003b). It is unknown, however, whether the agents that potentially up-regulate acyl-CoA pathways could enhance the extent of covalent binding.

Fibric acid derivatives, or so-called fibrates, are drugs widely used in the treatment of hyperlipidemia (Rader and Haffner, 1999). Administration of these fibrates is known to induce several hepatic enzymes associated with fatty acid metabolism, including acyl-CoA synthetases, the enzymes that catalyze the formation of acyl-CoA thioesters (Schoonjans et al., 1993; Alegret et al., 1994). A significant increase of free CoA, a cofactor that is essential for acyl-CoA formation, was observed in the livers of rats treated with fibrates (Horie et al., 1993). In addition, Shirley et al. (1994) have reported that ibuprofen-CoA formation was significantly higher in hepatocytes from clofibric acid-treated rats than the corresponding controls. Based on these data, we used 2-PPA as a model compound to evaluate the effect of fibrates on the two alternative metabolic activation pathways of profens, namely acyl-CoA formation and acyl glucuronidation, and ultimately on the extent of covalent binding of 2-PPA to liver protein. Among the three fibrates examined in the present studies, clofibric acid is the pharmacologically active form of clofibrate, the first and most extensively studied fibrate, whereas fenofibrate and gemfibrozil are the only two fibrates that are currently prescribed in the United States.

Materials and Methods

Materials. (R.S)-2-PPA, (S)-2-PPA, perchloric acid (70%), EDTA, clofibric acid, fenofibrate, gemfibrozil, CoA, ATP, MgCl₂, dithiothreitol (DTT), Triton X-100, Tris-HCl, and trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO); NaHCO₃ and diethyl ether were from Fisher Scientific Co. (Fair Lawn, NJ). Hionic-Fluor scintillation fluid was purchased from Packard BioScience (Meriden, CT). (R.S)-[1-¹⁴C]2-PPA and [1-¹⁴C]palmitic acid were purchased from American Radiolabeled Chemicals, Inc (St. Louis, MO), and purity was confirmed by HPLC analysis. Synthetic 2-PPA-S-acyl CoA (2-PPA-CoA) and the biosynthetic 2-PPA acyl gluturonide were available from previous studies in this laboratory (Li et al., 2002b). All solvents used for HPLC analysis were of chromatographic grade.

Animals. Male Sprague-Dawley rats (200–250 g) were purchased from B and K Universal (Liverpool, CA), maintained in a controlled housing environment with 12-h light/dark cycles, and received standard laboratory chow and water ad libitum. Rats were allocated at least 3 days to become acclimated to the housing conditions before use in experiments. All animal studies were approved by the University of California San Francisco Committee on Animal Research.

Enzyme Activities in Rat Liver Homogenate. Rats were randomly assigned to treatment or vehicle control groups with five animals per group. The treated groups received 160 mg/kg/day clofibric acid prepared in sodium bicarbonate buffer (0.1 M, pH 7.8, i.p.), 260 mg/kg/day fenofibrate prepared in 0.5% methylcellulose (oral gavage), or 180 mg/kg/day gemfibrozil in 0.5% methylcellulose (oral gavage) for 7 days. The doses of these three fibrates were chosen in such a way as to be approximately equivalent to each other (~0.7 mmol/kg/day) and also within the dose range commonly used in fibrate induction studies (Berge et al., 1981; Bremer et al., 1981; Schoonjans et al., 1993). Alegret et al. (1994) showed that maximal induction of palmitoyl-CoA synthetase was achieved after a 7-day fibrate treatment, and, thus, a 7-day treatment was used in the present study. An additional 10 rats, 5 for each treatment, received the corresponding dosing vehicles, namely 0.1 M sodium bicarbonate (pH 7.8, i.p.) or 0.5% methylcellulose (oral gavage). Data analysis showed that there were no significant differences in the percentage of increase in body weight, liver weight, and activities of acyl-CoA synthetases between the two vehicle treatments. Consequently, the data from these two vehicle treatments were combined and reported as one group.

On day 8, the rats were anesthetized with ether, the abdominal cavities were...
Rats (5 animals per group) were treated with 160 mg/kg/day clofibrate acid prepared in 0.1 M sodium bicarbonate, 260 mg/kg/day fenofibrate prepared in water in 0.5% methylcellulose (oral gavage), or 180 mg/kg/day gemfibrozil in methylcellulose (oral gavage) for 7 days. The control group includes 10 animals, 5 each for each dosing vehicle, namely 0.1 M sodium bicarbonate (i.p., pH 7.8), and 0.5% methylcellulose (oral gavage). Data analysis showed that there were no significant differences in the percent increase in body weight, liver weight, and activities of acyl-CoA synthetases between the two vehicle treatments. Consequently, the data from these two vehicle treatments were combined and reported as one group. Values are expressed as the mean ± S.D.

opened, and the livers were perfused with ice-cold saline. Perfused livers were rapidly removed, weighed, and frozen immediately in liquid nitrogen. Rat liver homogenate was prepared as described previously (Li et al., 2003c). To determine (R)-2-PPA-CoA synthetase activity, (R)-2-PPA (1 mM) was incubated with 0.25 mg of liver homogenate protein, 0.05% Triton X-100, 1 mM DTT, 2 mM EDTA, 1.2 mM CoA, 6.2 mM MgCl₂, and 2.5 mM ATP in a final volume of 0.5 ml (Li et al., 2003c). After a 3-min preincubation, reactions were initiated by addition of ATP, allowed to proceed for 30 min at 37°C, and then stopped by addition of 50 μl of perchloric acid (7%). Under the above conditions, the formation of 2-PPA-CoA was linear with incubation time and protein concentration, whereas enzymes were saturated with cofactors CoA and ATP. After centrifugation at 10,000g for 5 min, the supernatants were neutralized with 1 M NaOH and analyzed by reverse-phase HPLC.

Palmitoyl-CoA synthetase activity in rat liver homogenate was determined with [1-14C]palmitic acid by the previously described method (Krisans et al., 1980) with a minor modification. Briefly, incubations of [1-14C]palmitic acid (100 μM, 5.2 Ci/mol) with rat liver homogenate (5 μg) were performed in triplicate in 150 mM Tris-HCl buffer (pH 7.4) containing 0.05% Triton X-100, 1 mM DTT, 2 mM EDTA, 1.2 mM CoA, 6.2 mM MgCl₂, and 2.5 mM ATP in a final volume of 0.2 ml. Samples were preincubated at 37°C for 3 min, and the reactions were then initiated by addition of ATP. Control incubations were performed without the addition of ATP. The incubations were terminated after 5 min and [1-14C]palmitoyl-CoA was quantified as described by Krisans et al. (1980).

In Vivo Studies with 2-PPA. Clofibrate acid was administered i.p. as a sodium bicarbonate solution, whereas fenofibrate and gemfibrozil were dosed as suspensions in 0.5% methylcellulose via oral gavage, because clofibrate acid was reported to be completely absorbed orally in rats (Cayen et al., 1977), and there was no significant difference in the in vitro and in vivo observations between the two control groups, namely sodium bicarbonate-treated (i.p.) and 0.5% methylcellulose-treated (oral gavage) groups.

One group of rats (n = 10) was dosed i.p. with 2-PPA (160 mg/kg/day in 0.1 M sodium bicarbonate, pH 7.8) for 7 days. The control group (n = 10) was treated with the same amount of vehicle (0.1 M sodium bicarbonate, pH 7.8). On day 8, the rats were given a single i.p. dose of (R,S)-2-PPA (130 mg/kg in 0.9% saline). After administration of 2-PPA, two rats were decapitated at times 0, 0.25, 0.5, and 2 h for both clofibrate acid and control pretreatment groups. An additional six rats, three for each treatment, received (R,S)-[1-14C]-2-PPA (0.1 μCi/mmol, 130 mg/kg in 0.9% saline) and were sacrificed 2 h later. Their livers were removed, rinsed of blood, and immediately frozen in liquid nitrogen.

Three groups of rats (four per group) received either 260 mg/kg/day fenofibrate in 0.5% methylcellulose, 180 mg/kg/day gemfibrozil in 0.5% methylcellulose, or the dosing vehicle (0.5% methylcellulose) via daily oral gavage for 7 days. One day 8, the rats were dosed with (R,S)-[1-14C]-2-PPA (0.1 μCi/mmol, 130 mg/kg in 0.9% saline) and were killed 2 h after 2-PPA administration. Their livers were removed and immediately frozen in liquid nitrogen.

Cova lent binding of 2-PPA to tissue proteins was measured by scintillation counting of exhaustively washed protein precipitates as described previously (Li et al., 2003b). 2-PPA-CoA and acyl glucuronide formation in rat livers was determined by the previously described method (Li et al., 2003b).

HPLC Analysis. HPLC analysis was carried out on a Shimadzu gradient system (autosampler model SIL-10A and HPLC pumps model LC-10AT; Shimadzu Corp., Kyoto, Japan) with a Shimadzu SPD-10A UV-visible detector. HPLC analysis of 2-PPA-CoA formation and acyl glucuronidation in rat livers was performed as described previously (Li et al., 2003b). Quantitative measurements of 2-PPA-CoA and 2-PPA acyl glucuronide were made using a standard curve generated from absolute peak areas, by spiking liver samples of untreated rats with synthetic 2-PPA-CoA and biosynthetic 2-PPA acyl glucuronide standards, followed by processing as described above.

Statistical Analysis. Data were analyzed by unpaired Student's t test when there were only two treatment groups. Analysis of variance was used when there are three or more treatment groups. In this case, when significant differences were found, pairwise multiple comparisons were performed using the Student-Newman-Keuls method with significance set at p < 0.05.

Results

All the animals showed a steady increase in body weight during the 7-day treatment. Except for fenofibrate-treated rats, there were no significant differences in the percentage increase in body weight after a 7-day treatment with gemfibrozil, clofibrate acid, and control vehicles (Table 1). Rats treated with fenofibrate for 7 days exhibited significantly less percent body weight gain compared with the other three groups (p < 0.05) (Table 1). In contrast, there was a significant increase in liver weight in fibrate-treated animals (31, 24, and 51% increase in gemfibrozil-, clofibrate acid-, and fenofibrate-treated animals, respectively, compared with controls, p < 0.05) (Table 1). These observations were in agreement with the typical hepatomegaly of fibrates observed previously (Skrede and Halvorsen, 1979; Hawkins et al., 1987).

Studies with rat liver homogenate showed that treatment with the three fibrates led to significant increases (1.3- to 2.6-fold) in long-chain acyl-CoA synthetase activity (measured by palmitoyl-CoA formation) (Table 1), consistent with the previous mRNA (Schoonjans et al., 1993) and protein (Scheuerer et al., 1998) findings. Marked increases (4- to 8-fold) of (R)-2-PPA-CoA formation were also observed in liver homogenates treated with these fibrates (Table 1). On an equimolar basis, clofibrate acid appeared to exhibit greater inductive effects than fenofibrate and lesser effects than fenofibrate.

In agreement with the increased 2-PPA-CoA formation in rat liver homogenate, clofibrate acid-treated rats exhibited much higher levels of hepatic 2-PPA-CoA thioester than controls over a 2 h period (Fig.

**TABLE 1**

Effects of fibrates on the percent increase in body weight, rat liver weights, and enzyme activities of palmitoyl-CoA formation and (R)-2-PPA-CoA formation in rat liver homogenate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals</th>
<th>Increase in Body Weight</th>
<th>Liver Weight</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>g</td>
<td>Palmitoyl-CoA Formation</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>21 ± 3</td>
<td>11.5 ± 0.6</td>
<td>39.8 ± 3</td>
</tr>
<tr>
<td>Gemfibrozil-treated</td>
<td>5</td>
<td>18 ± 2</td>
<td>15.1 ± 1.8</td>
<td>51.7 ± 5.2</td>
</tr>
<tr>
<td>Clofibrate-acid-treated</td>
<td>5</td>
<td>20 ± 3</td>
<td>14.3 ± 1.3</td>
<td>69.1 ± 9.1</td>
</tr>
<tr>
<td>Fenofibrate-treated</td>
<td>5</td>
<td>12 ± 6abc</td>
<td>17.4 ± 1.8abc</td>
<td>102 ± 3abc</td>
</tr>
</tbody>
</table>

* Significantly different from control using the Student-Newman-Keuls method.

* Significantly different from gemfibrozil-treated animals using the Student-Newman-Keuls method.

* Significantly different from clofibrate-acid-treated animals using the Student-Newman-Keuls method.

Significantly different from clofibrate acid-treated animals using the Student-Newman-Keuls method.

**Results**

All the animals showed a steady increase in body weight during the 7-day treatment. Except for fenofibrate-treated rats, there were no significant differences in the percentage increase in body weight after a 7-day treatment with gemfibrozil, clofibrate acid, and control vehicles (Table 1). Rats treated with fenofibrate for 7 days exhibited significantly less percent body weight gain compared with the other three groups (p < 0.05) (Table 1). In contrast, there was a significant increase in liver weight in fibrate-treated animals (31, 24, and 51% increase in gemfibrozil-, clofibrate acid-, and fenofibrate-treated animals, respectively, compared with controls, p < 0.05) (Table 1). These observations were in agreement with the typical hepatomegaly of fibrates observed previously (Skrede and Halvorsen, 1979; Hawkins et al., 1987).

Studies with rat liver homogenate showed that treatment with the three fibrates led to significant increases (1.3- to 2.6-fold) in long-chain acyl-CoA synthetase activity (measured by palmitoyl-CoA formation) (Table 1), consistent with the previous mRNA (Schoonjans et al., 1993) and protein (Scheuerer et al., 1998) findings. Marked increases (4- to 8-fold) of (R)-2-PPA-CoA formation were also observed in liver homogenates treated with these fibrates (Table 1). On an equimolar basis, clofibrate acid appeared to exhibit greater inductive effects than gemfibrozil and lesser effects than fenofibrate.

In agreement with the increased 2-PPA-CoA formation in rat liver homogenate, clofibrate acid-treated rats exhibited much higher levels of hepatic 2-PPA-CoA thioester than controls over a 2 h period (Fig.
2A; Table 2). Compared with control rats (vehicle-treated), the exposure [AUC \( (0-2\ h) \)] of 2-PPA-CoA to liver protein over a 2-h period was markedly increased by 2.9-fold by clofibric acid (Fig. 2A; Table 2). The fold increases observed in vivo, however, were much less than that observed in vitro in liver homogenate (6.8-fold increase in 2-PPA-CoA formation compared with controls) (Table 1). Clofibrate acid treatment did not significantly change the hepatic concentration-time profile of 2-PPA and its corresponding acyl glucuronide compared with controls (Fig. 2, B and C; Table 2).

The effects of clofibrate acid on the metabolic activation of 2-PPA and the extent of covalent binding of 2-PPA to liver protein were further examined 2 h after \((R,S)-[1-^{14}C]2-PPA\) administration. As shown in Table 2, the hepatic levels of 2-PPA-CoA and acyl glucuronide 2 h after administration of \((R,S)-[1-^{14}C]2-PPA\) were consistent with those levels 2 h after nonradiolabeled \((R,S)-2-PPA\) treatment (Fig. 2). Because of the limited availability of \((R,S)-[1-^{14}C]2-PPA\) in our laboratory, time-dependent studies with \((R,S)-[1-^{14}C]2-PPA\) were not performed. However, the metabolite concentration-time profiles (AUC) obtained from nonradiolabeled \((R,S)-2-PPA\) studies appear to be a good estimate of the exposure of reactive metabolites of \([1-^{14}C]2-PPA\) to liver protein in \((R,S)-[1-^{14}C]2-PPA\)-treated rats. As indicated in Table 2, treatment with clofibrate acid, which markedly increased the hepatic exposure of 2-PPA-CoA by 2.9-fold, significantly increased covalent binding to hepatic protein by 25% \((p < 0.05)\) 2 h after administration of \((R,S)-[1-^{14}C]2-PPA\).

In contrast to clofibrate acid, treatment with fenofibrate did not significantly change 2-PPA-CoA concentrations in rat livers, whereas gemfibrozil significantly decreased the hepatic levels of 2-PPA-CoA thioester \((p < 0.05)\) (Fig. 3A). The hepatic concentrations of 2-PPA acyl glucuronide 2 h after 2-PPA treatment were markedly decreased by both fenofibrate- and gemfibrozil-treatment, compared with controls \((0.5\%\ methylcellulose-treated)\) (Fig. 3B). Both treatments led to significant decreases (25 and 29% decreases in fenofibrate- and gemfibrozil-treated rats, respectively, compared with controls, \(p < 0.05)\) in covalent binding of 2-PPA to hepatic protein (Fig. 3C).

**Discussion**

The primary goal of the present investigation was to evaluate the inductive effects of fibrates on the two alternative metabolic activation pathways of 2-PPA in vivo in rats. These studies were undertaken as a first step to understand whether prolonged treatment with fibrates could potentially modulate the extent of covalent binding of profen drugs to hepatic protein. Three fibrates were evaluated in the present studies.

The in vitro results with liver homogenates demonstrated that all three fibrates are capable of inducing \((R)-2-PPA-CoA\) formation as well as long-chain acyl-CoA synthetase. To our knowledge, this is the first report on the inductive effects of the three fibrates on \((R)-2-PPA-CoA\) formation activity. Our findings that fibrates induced the enzyme activity of long-chain acyl-CoA synthetase are consistent with previous reports on mRNA, protein, and functional activity results (Schoonjans et al., 1993; Alegret et al., 1994; Scheuruer et al., 1998). Interestingly, all three fibrates appear to show greater effects on the enzyme activity of \((R)-2-PPA-CoA\) formation than on long-chain acyl-CoA formation (Table 1), indicating that long-chain acyl-CoA synthetase might not be the only enzyme responsible for the 2-PPA-CoA formation. Other acyl-CoA synthetases that are more susceptible to fibrate induction may also play a role. This is consistent with our previous kinetic studies in rat liver homogenate, which indicated that more than one acyl-CoA synthetase is involved in 2-PPA-CoA formation (Li et al., 2003c).

The results of the in vivo studies, however, demonstrated that increases in the hepatic capacity of catalyzing 2-PPA-CoA formation in vitro do not necessarily lead to significant increases of hepatic exposure to 2-PPA-CoA thioester in vivo in rats. In fact, the present results show that the three fibrates differ in their ability to modulate the hepatic levels of 2-PPA-CoA thioester in vivo, although all three fibrates significantly increased 2-PPA-CoA formation in vitro. Treatment with clofibrate acid resulted in significantly higher levels of hepatic 2-PPA-CoA thioester than those in controls, exhibiting a 2.9-fold increase in the hepatic exposure of 2-PPA-CoA over a 2-h period [AUC \( (0-2\ h) \)] (Fig. 2A; Table 2). In contrast, on an equimolar basis, treatment with fenofibrate appeared to have no effect on 2-PPA-CoA concentrations in rat livers, whereas gemfibrozil treatment led to a significant decrease in the hepatic levels of 2-PPA-CoA thioesters \((p < 0.05)\) 2 h after 2-PPA administration (Fig. 3A). The good reproducibility of these studies suggests that these unexpected observations are unlikely to be the result of experimental artifacts.

We do not, at this point, understand the mechanism for the apparent discrepancy observed between in vitro and in vivo results among the
Effects of clofibric acid on 2-PPA-CoA formation, acyl glucuronidation, and covalent binding in vivo in rat livers

Rats were pretreated i.p. with clofibric acid (160 mg/kg/day) or vehicle (0.1 M sodium bicarbonate) for 7 days. On day 8, rats received an i.p. injection of [RS]-[1-14C]-2-PPA (130 mg/kg in 0.9% saline, 0.5 ml/rat). Livers were collected 2 h after [RS]-[1-14C]-2-PPA administration. Values are expressed as the mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2-PPA-CoA (nmol/g liver)</th>
<th>2-PPA glucuronide (nmol/g liver)</th>
<th>Covalent binding (pmol/mg protein)</th>
<th>AUC(0-2 h) Ratio (Treated/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.6 ± 13.0</td>
<td>589 ± 27</td>
<td>195 ± 16</td>
<td>2-PPA-CoA</td>
</tr>
<tr>
<td>Clofibric Acid-Treated</td>
<td>171 ± 7 *b</td>
<td>534 ± 57</td>
<td>243 ± 21 *b</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* AUC(0-2 h) in clofibric acid treated and control animals (n = 2 for each time points) was calculated from Fig. 2.

* P < 0.05 versus control, using unpaired Student’s t test.

Effects of three fibrates, and this will be an area of future investigation. As depicted in Fig. 1, 2-PPA-CoA thioester is a metabolic intermediate, which not only mediates the chiral inversion of 2-PPA but also readily undergoes enzyme-mediated hydrolysis and may readily conjugate with amino acids and carnitine and incorporate into lipids. Therefore, the hepatic level of 2-PPA-CoA thioester depends not only on the formation of 2-PPA-CoA thioester but also on its subsequent metabolism. In fact, it has been documented that fibrates also up-regulate acyl-CoA hydrolase (Berge and Bakke, 1981; Alegret et al., 1994) and carnitine acyl transferase, the enzyme that catalyzes the formation of acyl-carnitine from acyl-CoA thioester (Tosh et al., 1989), in addition to their effects on acyl-CoA synthetase. Scheuerer et al. (1998) demonstrated that clofibrate treatment significantly increased the distribution of radiolabeled ibuprofen to several tissues, including liver and fat, and concluded that such an increase in tissue distribution is probably due to the increase in the incorporation of radiolabeled ibuprofen into long-lived hybrid lipids. Therefore, the lack of in vitro and in vivo correlation probably results from a combination of inductive effects of fibrates on the multiple elimination pathways of 2-PPA-CoA thioester, in addition to their inductive effects on 2-PPA-CoA formation. Thus, caution should be taken to extrapolate the effect of modulating agents on the in vivo exposure of a reactive intermediate and its consequent toxic effects. The effects on both formation and elimination pathways of the reactive intermediate should be evaluated. The differential effect of fibrates on the hepatic levels of CoA may also contribute partly to the apparent in vitro and in vivo discrepancy. It was shown that clofibrate and its structural analog ciprofibrate significantly elevated hepatic CoA level by increasing the biosynthesis of CoA (Skrede and Halvorsen, 1979; Horie et al., 1986; Bhuiyan et al., 1988). The effects of fenofibrate and gemfibrozil treatment on the hepatic content of CoA, however, are still unknown. It is possible that availability of hepatic CoA may limit the formation of 2-PPA-CoA thioester in vivo and thus contribute to the apparent in vitro and in vivo disconnect.

In contrast to 2-PPA-CoA, the three fibrates appeared to exhibit similar effects on the hepatic levels of 2-PPA acyl glucuronide. Treatment with both fenofibrate and gemfibrozil led to significant decreases in the hepatic levels of 2-PPA acyl glucuronide 2 h after 2-PPA administration (Fig. 3B), whereas clofibrate acyl acid slightly, although not significantly, decreased the hepatic exposure of 2-PPA acyl glucuronide by 21% over the 2-h period (Fig. 2B). These observations are consistent with previous reports that clofibrate had no inductive effects on acyl glucuronidation of acidic drugs (Fournel-Gigleux et al., 1988; Ritter, 2000). In fact, a small reduction of 2-PPA acyl glucuronidation has been reported in vitro in liver microsomes from clofibrate-acid treated rats (Fournel-Gigleux et al., 1988). However, it is possible that the decrease in hepatic levels of 2-PPA acyl glucuronide could also result from increased hepatic excretion of 2-PPA acyl glucuronide, in addition to reduction of formation.

The effects of these three fibrates on the extent of covalent binding of 2-PPA to hepatic protein were different (Table 1; Fig. 3C). Treatment with clofibrate acyl acid led to a significant increase in covalent binding of 2-PPA to liver protein, when a marked increase in the hepatic exposure of 2-PPA-CoA was observed. Conversely, significant decreases of the hepatic levels of 2-PPA acyl glucuronide and/or 2-PPA-CoA by fenofibrate and gemfibrozil significantly lowered the extent of covalent binding of 2-PPA to hepatic protein in vivo in rats. These observations are consistent with our previous observations that both metabolic activation pathways, namely acyl-CoA formation and acyl glucuronidation, are involved in the 2-PPA covalent adduct formation (Li et al., 2002a,b). From these findings, it is not possible to determine whether 2-PPA-CoA or 2-PPA acyl glucuronide was chiefly responsible for covalent binding under fibrate-induced conditions. That would require concomitant binding measurements under...
fibrate-induced conditions in the presence of inhibitors of each pathway, following the methods we described in Li et al. (2002a,b).

In conclusion, this is the study to demonstrate that the three commonly studied fibrates, namely clofibrate acid, fenofibrate, and gemfibrozil, differ in their ability to alter the extent of covalent binding of 2-PPA to hepatic protein by differentially modulating the hepatic exposure of the two reactive metabolites of 2-PPA, namely 2-PPA-CoA thioester and acyl glucuronide. Additionally, the results suggest that the potential risk of fibrate treatment to increase the hepatic toxicity of profen drugs might vary with the fibrates used and differ among individual subjects, considering the complexity of the inductive effects of these fibrates on hepatic formation and elimination of the two reactive intermediates of profen drugs.

Acknowledgments. We thank Milagros Hann for assistance in performing HPLC analyses.

References


Address correspondence to: Dr. Leslie Benet, Department of Biopharmaceutical Sciences, 533 Panassus Ave., U-68, University of California, San Francisco, CA 94143-0446. E-mail: leslie.benet@ucsf.edu