Differential Effects of Fibrates on the Metabolic Activation of

2-Phenylpropionic Acid in Rats

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Abbreviations used: 2-PPA, 2-phenylpropionic acid; 2-PPA-CoA, 2-PPA-S-acyl CoA; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetate.
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 if 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 clofibrate acid (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 clofibrate acid 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.
Introduction

2-Arylpropionic acids (profen drugs) are a widely used class of nonsteroidal antiinflammatory drugs (NSAIDs), which have a chiral center at the carbon alpha to the carboxyl 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, since 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-coenzyme A (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, as well as 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), clofibric acid (Grillo and Benet, 2002), 2-phenylpropionoic 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 and 2007) and zomepirac (Olsen et al., 2005), have been demonstrated to be
chemically reactive and capable of acylating biological nucleophiles, such as glutathione (GSH) and proteins. Studies with 8 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 $\alpha$-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) (Li and Benet, 2002; Boelsterli, 2002). Comparative in vitro studies with several carboxylic acids, including clofibric acid (Grillo and Benet, 2001; Shore et al., 1995), 2-PPA (Li et al., 2002b) and naproxen (Olsen et al., 2002), showed that acyl-CoA thioesters are 40-70 fold more reactive toward GSH, forming acyl glutathione conjugates, than the respective acyl glucuronides 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 upregulate 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 (Alegret et al., 1994; Schoonjans et al., 1993). 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., 1986). In addition, Shirley et al. (1994) have
reported that ibuprofen-CoA formation was significantly higher in hepatocytes from
clofibraric 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, clofibraric acid is the
pharmacological 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 U.S.
**Methods**

**Materials.** (R,S)-2-PPA, (R)-2-PPA, perchloric acid (70%), EDTA, clofibric acid, fenofibrate, gemfibrozil, CoA, ATP, MgCl₂, dithiothreitol (DTT), Triton X-100, Tris-HCl and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Co. (St. Louis, MO); NaHCO₃ and diethyl ether from Fisher Scientific (Fair Lawn, NJ). Hionic-Fluor scintillation fluid was purchased from Packard BioScience Co. (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 glucuronide 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 & K Universal (Livermore, CA), maintained in a controlled housing environment with 12-h light/dark cycles, and received standard laboratory chow and water ad libitum. Rats were allowed 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 (Bremer et al., 1981; Schoonjans et al., 1993; Berge et al., 1981).
Alegret et al. (1994) showed that maximal induction of palmitoyl-CoA synthetase was
achieved after 7-day fibrate treatment, and thus, 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 opened
and the livers 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 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, then stopped by
addition of 50 µL perchloric acid (7%). Under the above conditions, the formation of 2-
PPA-CoA was linear with incubation time and protein concentration, while enzymes
were saturated with cofactors CoA and ATP. After centrifugation at 10,000 g for 5 min, the supernatants were neutralized with 1M 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 carried out 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 conducted 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 suspension in 0.5% methylcellulose via oral gavage due to the apparent insolubility of fenofibrate and gemfibrozil in buffer. No attempt was made to re-evaluate clofibrate acid in 0.5% methylcellulose via oral gavage, since 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 clofibric acid (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, 1 and 2 h for both clofibric acid and control pretreatment groups. An additional 6 rats, 3 each for each treatment, received (R,S)-[1-14C]-2-PPA (0.1 mCi/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 day. One day 8, the rats were dosed with (R,S)-[1-14C]-2-PPA (0.1 mCi/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.

Covalent 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, HPLC pumps model LC-10AT, Shimadzu Corp., Japan) with a Shimadzu SCL-10A controller and a Shimadzu SPD-10A UV-Vis detector.
HPLC analysis of 2-PPA-CoA formation and acyl glucuronidation in rat livers was carried out 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. ANOVA was utilized when the treatment groups are equal to or more than 3. In this case, when significant differences were found, pairwise multiple comparisons were conducted 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 7-day treatment with gemfibrozil, clofibric acid and control vehicles (Table 1). Rats treated with fenofibrate for 7 days exhibited significantly less percentage body weight gain as 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-, clofibric acid- and fenofibrate-treated animals, respectively, as compared to controls, $p < 0.05$) (Table 1). These observations were in agreement with the typical hepatomegaly of fibrates observed previously (Hawkins et al., 1987; Skrede and Halvorsen, 1979).

Studies with rat liver homogenate showed that treatment with the three fibrates led to significant increases (1.3-2.6 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 (4-8 fold) of ($R$)-2-PPA-CoA formation were also observed in liver homogenates treated with these fibrates (Table 1). On an equimolar basis, clofibric acid appeared to exhibit greater inductive effects on these two enzyme activities than gemfibrozil, and less effects than fenofibrate.

In agreement with the increased 2-PPA-CoA formation in rat liver homogenate, clofibric acid-treated rats exhibited much higher levels of hepatic 2-PPA-CoA thioester than controls over a 2 h period (Fig. 2A and Table 2). Compared with control rats (vehicle-treated), the exposure [$\text{AUC}_{(0-2h)}$] of 2-PPA-CoA to liver protein over a 2-h
period was markedly increased by 2.9 fold by clofibric acid (Fig. 2A and 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 as compared to controls) (Table 1). Clofibric acid-treatment did not significantly change the hepatic concentration-time profile of 2-PPA and its corresponding acyl glucuronide as compared to controls (Fig. 2 B and C, and Table 2).

The effects of clofibric 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 post (R,S)-[1-\textsuperscript{14}C]-2-PPA administration. As shown in Table 2, the hepatic levels of 2-PPA-CoA and acyl glucuronide 2 h post-administration of (R,S)-[1-\textsuperscript{14}C]-2-PPA were consistent with those levels 2 h after non-radiolabeled (R,S)-2-PPA treatment (Fig. 2). Due to the limited availability of (R,S)-[1-\textsuperscript{14}C]-2-PPA in our laboratory, time-dependent studies with (R,S)-[1-\textsuperscript{14}C]-2-PPA were not performed. However, the metabolite concentration-time profiles (AUC) obtained from non-radiolabeled (R,S)-2-PPA studies appear to be a good estimate of the exposure of reactive metabolites of [1-\textsuperscript{14}C]-2-PPA to liver protein in (R,S)-[1-\textsuperscript{14}C]-2-PPA-treated rats. As indicated in Table 2, treatment with clofibric 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 post administration of (R,S)-[1-\textsuperscript{14}C]-2-PPA.

In contrast to clofibric acid, treatment with fenofibrate did not significantly change 2-PPA-CoA concentrations in rat livers, whereas gemofibrozil 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 post 2-PPA treatment were markedly
decreased by both fenofibrate- and gemfibrozil-treatment, as compared with controls (0.5% methylcellulose-treated) (Fig. 3B). Both treatments led to significant decreases (25 and 29% decrease in fenofibrate and gemfibrozil-treated rats, respectively, as compared to 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 if 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; Scheuerer et al., 1998). Interestingly, all three fibrates appear to show greater effects on the enzyme activity of (R)-2-PPA-CoA formation than 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 of 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 clofibric acid resulted in significantly higher levels of hepatic 2-PPA-CoA thioester than controls—exhibiting a 2.9 fold increase in the hepatic exposure of 2-PPA-CoA over a 2 h period [AUC (0-2h)] (Fig. 2A and 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 post 2-PPA administration (Fig. 3A). The good reproducibility of these studies suggests that these unexpected observations are unlikely 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 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 upregulate 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
increase of tissue distribution is likely due to the increase of the incorporation of radionuclide ibuprofen into long-lived hybrid lipids. Therefore, the lack of in vitro and in vivo correlation likely 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 discrepancies. It was shown that clofibrate and its structural analog ciprofibrate significantly elevated hepatic CoA level by increasing the biosynthesis of CoA (Horie et al., 1986; Bhuiyan et al., 1988; Skrede and Halvorsen, 1979). 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 post 2-PPA administration (Fig. 3B), whereas clofibric acid also slightly, although not significantly, decreased the hepatic exposure of 2-PPA acyl glucuronide by 21% over the 2-hr 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 clofibrac acid-treated rats (Fournel-Gigleux et al., 1988). However, it is possible that the decrease of 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 and Fig. 3C). Treatment with clofibrac 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; Li et al., 2003b). 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 fibrates induced conditions in the presence of inhibitors of each pathway, following the methods we described in Li et al. (2002a and 2003b).

In conclusion, this is the first study to demonstrate that the three commonly studied fibrates, namely clofibrac 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 the hepatic formation and elimination of the two reactive intermediates of profen drugs.

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References


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Footnotes

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Figure Legends

Figure 1. Schematic representation of 2-PPA metabolism by inversion, via an acyl-CoA thioester intermediate, and noninversion, e.g. acyl glucuronidation pathways.

Figure 2. Effects of clofibric acid on the hepatic concentrations of: (A) 2-PPA-CoA thioester, (B) 2-PPA acyl glucuronide, and (C) 2-PPA \textit{in vivo} in rats. Values are expressed as the mean ± S.D..

Figure 3. Effects of clofibric acid, fenofibrate, and gemfibrozil on the hepatic concentrations of: (A) 2-PPA-CoA thioester, (B) 2-PPA acyl glucuronide, and (C) covalent binding of 2-PPA to liver protein \textit{in vivo} in rats. Values are expressed as the mean ± S.D. “Significantly different from the corresponding controls using unpaired Student’s t test.”
Table 1. Effects of fibrates on the percentage increase in body weight, rat liver weight and enzyme activities of palmitoyl-CoA formation and (R)-2-PPA-CoA formation in rat liver homogenate.

<table>
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<th>Treatment</th>
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<th>Liver weight</th>
<th>Enzyme activity</th>
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<td></td>
<td></td>
<td>%</td>
<td>g</td>
<td>Palmitoyl-CoA formation</td>
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<td></td>
<td></td>
<td></td>
<td>nmol/min/mg protein</td>
<td>pmol/min/mg protein</td>
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<td>51.7 ± 5.2[^b]</td>
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<tr>
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<td>69.1 ± 9.1[^b,c]</td>
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<td>17.4 ± 1.8[^b,c,d]</td>
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</table>

[^a] Rats (5 animals per group) were treated with 160 mg/kg/day clofibric acid prepared in 0.1 M sodium bicarbonate (pH 7.8, i.p.), 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. Control group includes 10 animals, 5 each for each dosing vehicle, namely 0.1 M sodium bicarbonate (pH 7.8, i.p.) and 0.5% methylcellulose (oral gavage). Data analysis showed that there were no significant differences in the percentage 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.

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

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

Significantly different from clofibric acid-treated animals using Student-Newman-Keuls method.
Table 2. Effects of clofibric acid on 2-PPA-CoA formation, acyl glucuronidation and covalent binding in vivo in rat livers.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Clofibric acid-treated</th>
<th>AUC (0-2h) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PPA-CoA</td>
<td>nmol/g liver</td>
<td>73.6 ± 13.0</td>
<td>171 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-PPA glucuronide</td>
<td>nmol/g liver</td>
<td>589 ± 27</td>
<td>534 ± 57</td>
</tr>
<tr>
<td>Covalent binding</td>
<td>pmol/mg protein</td>
<td>195 ± 16</td>
<td>243 ± 21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>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-<sup>14</sup>C]-2-PPA (130 mg/kg in 0.9% saline, 0.5 ml/rat). Livers were collected 2 h post (RS)-[1-<sup>14</sup>C]-2-PPA administration. Values are expressed as the mean ± S.D. (n = 3).

<sup>b</sup>AUC (0-2h) in clofibric acid treated and control animals (n = 2 for each time points) was calculated from Figure 2.

<sup>c</sup>p < 0.05 versus control, using unpaired Student’s t test.
Figure 1

Covalent Binding

\[(R)-2-PPA\] glucuronide

UGT

Hydrolysis

\[(R)-2-PPA\]

Hydrolysis

\[(R)-2-PPA-CoA\]

Hydrolysis

Epimerase

Hybrid Triacylglycerides
Amino acid Conjugates
Carnitine Conjugates

\[(S)-2-PPA\] glucuronide

UGT

\[(S)-2-PPA\]

UGT

\[(S)-2-PPA-CoA\]

Covalent Binding

Hepatic Excretion

Covalent Binding

Covalent Binding

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Figure 2
Figure 3

A. Concentration of 2-PPA-CoA

B. Concentration of 2-PPA acyl glucuronide

C. Covalent binding