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Chiral Inversion of (*R*)-(–)-Fenoprofen in Guinea-pigs Pretreated with Clofibrate

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ABSTRACT

The influence of clofibrate on the stereoconversion of fenoprofen (FPF) was studied in guinea pigs. This hypolipidaemic agent has been related to some biochemical changes in the liver leading to an increase in the chiral inversion process. Two groups of animals ($n = 6$ per group) were pretreated with oral doses of clofibrate (280 mg/kg per day) for three days and were then given (*R*)- or (*S*)-FPF (5 mg/kg, IV). The FPF enantiomers were extracted from the guinea-pigs' plasma using a solid phase procedure and analysed by HPLC with previous derivatization with L-leucinamide. Pretreatment with clofibrate increased the chiral inversion of (*R*)-FPF in favour of the pharmacologically active (*S*)-FPF enantiomer. Before this metabolic interaction can be applied to therapy with fenoprofen, the toxic effects of (*S*)-(+)-FPF on the gastrointestinal and renal tracts and the interference by (*R*)-(–)-FPF with the metabolism of lipids should be thoroughly evaluated.

Keywords: arylpropionates, chiral inversion, clofibrate, lipid metabolism, profens

Abbreviations: AUC, area under the concentration–time curve; AUC_S, area under the concentration–time curve of the *S*-enantiomer; AUC_R, area under the concentration–time curve of the *R*-enantiomer; FPF, fenoprofen; IV, intravenous

INTRODUCTION

Fenoprofen (FPF) is a nonsteroidal anti-inflammatory drug belonging to the family of the 2-arylpropionic acids or profens. Although it is not licensed to be used in veterinary medicine, it represents very well other molecules of the group that undergo inversion. It has a chiral carbon atom (C-2) and it is marketed as a racemate (50:50, (*R*)–(*S*)-enantiomers) for clinical use in humans. Studies *in vitro* have shown that the (*S*)-enantiomer is the compound responsible for inhibition of prostaglandin synthesis (Rubin *et al.*, 1985; Caldwell *et al.*, 1988). The stereoselective disposition of FPF has been mainly attributed to a unidirectional metabolic chiral inversion of (*R*)-(–)-FPF to its antipode.

The molecular mechanism of chiral inversion of profens involves three steps: (i) stereoselective activation of (*R*)-(–)-profen by formation of the acyl-CoA thioester in the presence of co-enzyme A, ATP and Mg^{2+}; (ii) enzymatic epimerization of the

(*R*)-(–) thioester to the (*S*)-(+) thioester; (iii) release of the free active (*S*)-(+) enantiomer by hydrolysis of the thioester (Figure 1) (Wechter *et al.*, 1974; Nakamura *et al.*, 1981). Berry and Jamali (1991) have demonstrated that the liver is the most important organ in the development of this mechanism. Stereoconversion also takes place in the intestine, kidney, lung, muscle and fat (Cox *et al.*, 1985; Mehvar and Jamali, 1988; Jeffrey *et al.*, 1991; Hall *et al.*, 1992). The chiral inversion of FPF has been studied in humans (Rubin *et al.*, 1985), rabbits (Hayball and Meffin, 1987), rats (Berry and Jamali, 1991), sheep (Soraci *et al.*, 1995), horses and dogs (Soraci *et al.*, 1996) and cats (Castro *et al.*, 1998). The results showed large interspecies variations in the magnitude of inversion related to the expression of long-chain acyl-CoA synthetase (EC 6.2.1.3).

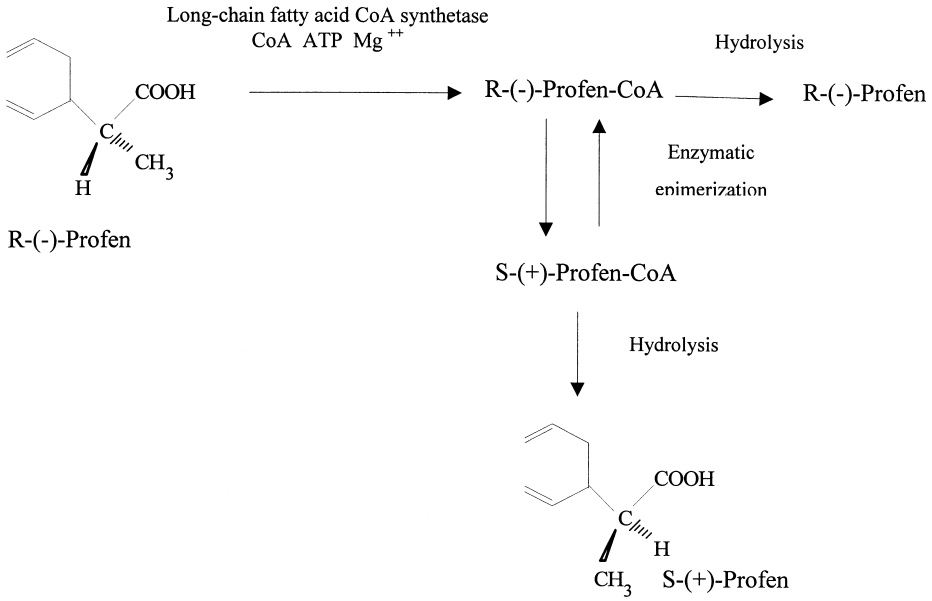

Figure 1. Chiral inversion process of aryl-2-propionic acids or profens

The chiral inversion may be of toxicological significance because the intermediate acyl-CoA thioester can modulate lipid metabolism. In addition, the acyl-CoA thioester can inhibit mitochondrial β-oxidation of fatty acids, favouring the development of microvesicular steatosis (Freneaux *et al.*, 1990; Zhao *et al.*, 1992), can be incorporated into triglycerides, altering plasma membranes and second messenger mechanisms (Williams *et al.*, 1986; Sallustio *et al.*, 1987), and can lower serum lipid levels (Fears *et al.*, 1978; Kemal and Casida, 1992). In rat liver homogenates, the formation of (*R*)-ibuprofen-CoA is dependent on the concentrations of both CoA and (*R*)-ibuprofen

(Tracy *et al.*, 1993). Consequently, the rate of chiral inversion of (*R*)-profens may be affected by compounds that affect the intracellular concentrations of CoA.

Treatment with clofibrate, a hypolipidaemic agent, induces a number of hepatic enzymes associated with fatty acid metabolism, including the microsomal long-chain acyl-CoA ligase (Kawashima *et al.*, 1984) and various acyl-CoA hydrolases (Kawashima *et al.*, 1983; Mentlein *et al.*, 1986). A significant increase in the rate of elimination of (*R*)-ibuprofen was observed in liver homogenates from rats treated with clofibric acid (Knights *et al.*, 1991).

The purpose of this work was to study *in vivo* the effects of clofibrate on the stereoconversion of (*R*)-(–)-FPF in guinea-pigs. Clofibrate is not widely used in veterinary medicine, but it has important effects on lipid metabolism and consequently on the process of chiral inversion. We have previously demonstrated that the microsomes of rats pre-treated with clofibrate thiosterified (*R*)-fenoprofen much more effectively than those of control rats (Soraci and Benoit, 1995). Clofibrate was therefore used as a representative of inductors capable of increasing the chiral inversion process.

MATERIALS AND METHODS

Chemicals

(*R*)-(–)- and (*S*)-(+)-FPF were obtained by stereospecific crystallization, using α-methylbenzylamine (Fluka Chemika-BioChemica, Buchs, Switzerland) as the chiral inducer (Hayball and Meffin, 1987). Final purities, determined by high-performance liquid chromatography (HPLC) (Sallustio *et al.*, 1987) were 98.0% for (*R*)-(–)- and 98.6% for the (*S*)-(+)-FPF. The derivatization reagents tryethylamine and ethyl chloroformate were purchased from Merck (Darmstadt, Germany) and L-leucinamide from ICN Pharmaceuticals (Costa Mesa, CA, USA). Clofibrate was obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents were obtained from usual commercial sources.

Animals, treatment and sampling

Twenty-four male albino guinea-pigs (*Cavia caviaei*), weighing 800–1000 g, were used. A heparinized polyethylene cannula was inserted into the jugular vein while the animals were under ketamine anaesthesia (Holliday-Scott SA, Buenos Aires, Argentina). After recovery from the anaesthesia, the animals were randomly assigned into two groups of 12 animals each. One group was pretreated by gavage with 280 mg/kg of clofibrate (5% w/v solution in methylethylcellulose) for three days and the other group was the control. Six guinea-pigs from each group received (*R*)-(–)-FPF, while the remaining animals were given the (*S*)-(+)-enantiomer, intravenously at doses of 5 mg/kg. Each compound was administered in saline and Tween 20 (90:10).

Blood samples (approximately 500 µl) were taken from the jugular vein at standardized intervals up to 2 h after administration of the (*S*)-(+)- or (*R*)-(–)-

enantiomer. All the samples were immediately centrifuged for 10 min at 900*g*, and then the plasma was separated and stored at –20°C until analysis.

Four animals from both the control and clofibrate-treated groups were killed and their livers were excised for quantification of free CoA.

Analytical method

FPF enantiomers were extracted from plasma by a solid phase extraction procedure (Sep-Pak cartridges C_{18}, Waters Associated, Milford, MA, USA) (San Martin *et al.*, 1996). The dry residue was derivatized with L-leucinamide by a method adapted from Foster and Jamali (1987) (Soraci *et al.*, 1995), and analysed by HPLC, using a reversed-phase column as previously described (San Martin *et al.*, 1996).

The concentrations of free CoA in the liver homogenates were determined by HPLC by the method of Xiaotao and Hall (1993). Briefly, the liver homogenate (500 µl) was deproteinized with perchloric acid and the supernatant was adjusted to pH 4 with 2 mol/L potassium hydroxide. The concentrations of free CoA were then analysed by reversed-phase HPLC as described by Ingebrestsen and Farstad (1980).

Data analysis

The (*R*)-(–)-FPF and (*S*)-(+)-FPF plasma concentration curves as functions of time were plotted and the areas under the curve (AUC) were measured by the linear trapezoidal rule (Baggot, 1977). The enantiomeric stereoconversion of (*R*)-(–)-FPF into (*S*)-(+)-FPF was calculated using the following formula (Pang and Kwan, 1983; Beck *et al.*, 1991):

$$\text{Inversion rate} = \frac{\text{AUC}_{(S)} \text{ after } (R) \times \text{dose } (S)}{\text{AUC}_{(S)} \text{ after } (S) \times \text{dose } (R)}$$

In this equation, AUC_S after (*R*) and AUC_S after (*S*) are the AUCs calculated following administration of (*S*)-(+)-FPF after (*R*)-(–)-FPF or after (*S*)-(+)-FPF administration, respectively. The data were analysed by ANOVA. Student's *t*-test was used for mean comparisons between treatments.

RESULTS

Figure 2 shows the mean plasma concentrations of (*R*)-(–)- and (*S*)-(+)-FPF after administration of (*R*)-(–)-FPF to six control guinea-pigs. There was a chiral inversion from the inactive enantiomer, (*R*)-(–)-FPF to the active isomer, (*S*)-(+)-FPF. The C_{max} (maximum plasma concentration) of the (*S*)-(+)-FPF enantiomer was reached 10 minutes after IV administration of (*R*)-(–)-FPF. The mean percentage of chiral

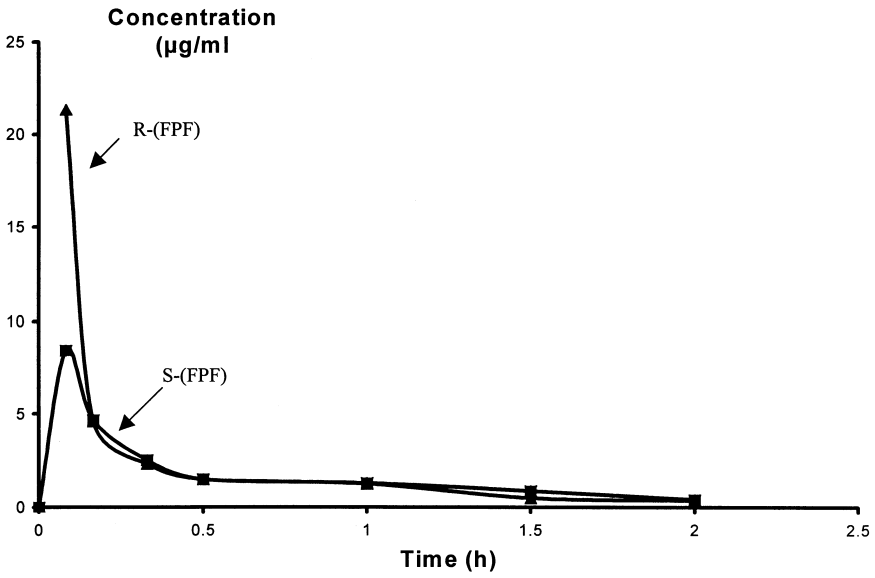

Figure 2. Plasma profiles of fenoprofen enantiomers obtained after IV administration of 5 mg/kg of (*R*)-(–)-FPF to guinea-pigs ($n = 6$)

inversion of (*R*)-(–)-FPF in the control animals was $62.1\% \pm 7.5\%$, while a significant increase was observed ($89.1\% \pm 10.1\%$) in the clofibrate-pretreated guinea-pigs (Figure 3).

On the other hand, (*R*)-(–)-FPF did not appear in the plasma after administration of (*S*)-(+)-FPF to the control (Figure 4) or clofibrate-pretreated animals (Figure 5). Figure 6 shows the levels of free CoA in the liver homogenates from the control and clofibrate-pretreated guinea-pigs.

DISCUSSION

Stereoselective conversion of arylpropionates has been documented in humans (60%) (Rubin *et al.*, 1985), rats (42%) (Berry and Jamali, 1991), sheep (80%) (Soraci *et al.*, 1995), dogs (90%), horses (38%) (Soraci *et al.*, 1996), and cats (93%) (Castro *et al.*, 1998).

In the present study, clofibrate pretreatment significantly increased the chiral conversion from the (*R*)-(–)-FPF enantiomer to the isomeric form (*S*)-(+)-FPF. This result is consistent with the increased chiral inversion of ibuprofen observed in isolated perfused rat livers and suspensions of rat hepatocytes that had been pretreated with clofibric acid (Roy-de Vos *et al.*, 1996).

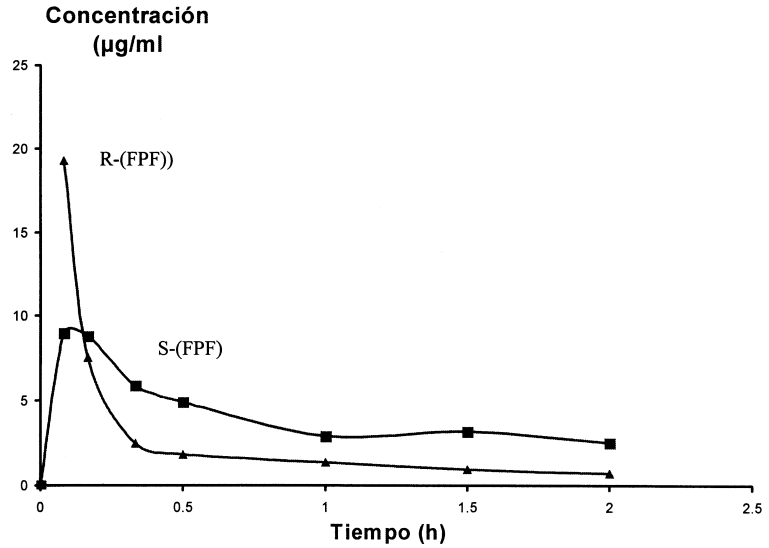

Figure 3. Plasma profiles of fenoprofen enantiomers obtained after IV administration of 5 mg/kg of (*R*)-(–)-FPF to guinea-pigs ($n = 6$) that had received clofibrate

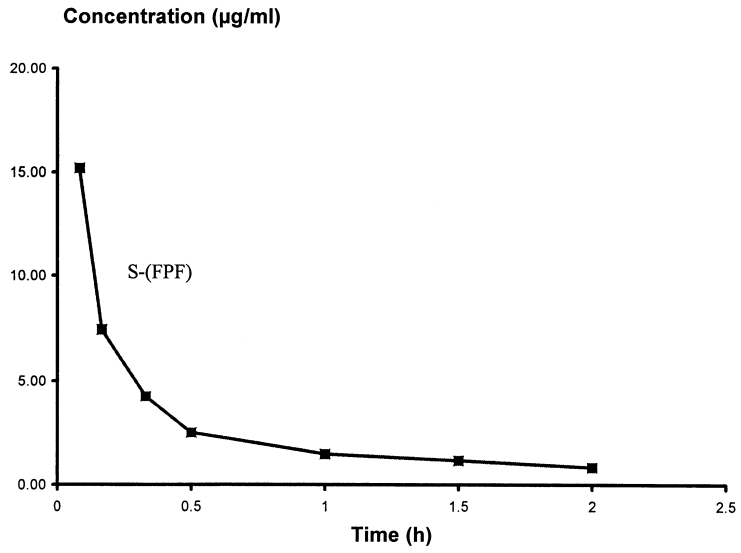

Figure 4. Plasma profiles of fenoprofen enantiomers obtained after IV administration of 5 mg/kg (*S*)-(+)-FPF in guinea-pigs ($n = 6$)

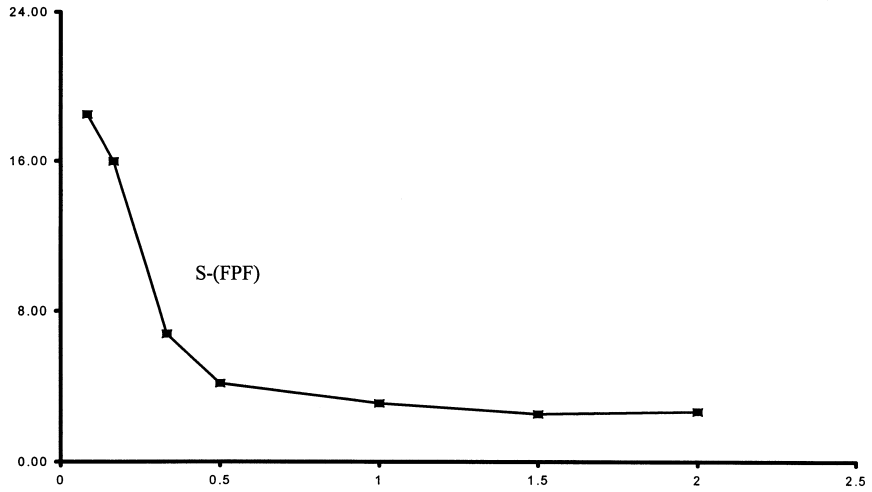

Figure 5. Plasma profiles of fenoprofen enantiomers obtained after IV administration of 5 mg/kg (*S*)-(+)-FPF in guinea-pigs that had received clofibrate

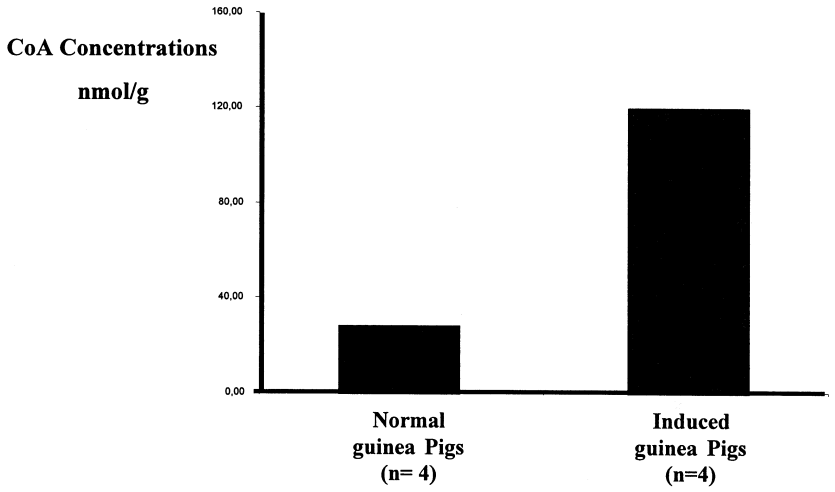

Figure 6. Liver concentrations of free coenzyme A in control and clofibrate-induced guinea-pigs

The increase in chiral inversion of (*R*)-(–)-FPF in guinea-pigs pretreated with clofibrate may be due to an increase in the induction of acyl-CoA ligases (Kawashima *et al.*, 1984). A significant increase in the *in vivo* thioesterification of (*R*)-(–)-FPF has been demonstrated in the hepatic microsomes of rats pretreated with clofibrate (Soraci and Benoit, 1995). However, *in vitro* studies of chiral inversion (Roy-de Vos *et al.*, 1996) have demonstrated that the addition of clofibric acid to the perfusate produced an acceleration of stereoconversion of ibuprofen, but this phenomenon cannot be due to an increase in acyl-CoA synthetase, as this enzymatic induction is a slow process (Mayer, 1996). In consequence, the reason for an increase in the chiral inversion of ibuprofen under the above-mentioned conditions may be a shift in the disposition of the intracellular pools of CoA (Roy-de Vos *et al.*, 1996).

The concentrations of free CoA in the liver of animals pretreated with clofibrate were significantly higher than those in control guinea-pigs. These results are in agreement with earlier findings by Skrede and Halvorsen (1979), who reported that the administration of clofibrate to rats caused a 2–3-fold increase in the total amount hepatic CoA. Therefore, it is possible that the pretreatment with clofibrate might modulate the chiral inversion of (*R*)-(–)-FPF by two distinct mechanisms: (i) by inducing both ligase and hydrolase activities capable of releasing CoA from other acyl-CoA thioesters (Kawashima *et al.*, 1983, 1984; Berge *et al.*, 1984) and/or by inducing synthesis of CoA; (ii) by inducing a shift in the intracellular pools of CoA by an unknown mechanism (Roy-de Vos *et al.*, 1996).

In conclusion, the results obtained in the present experiment indicated that pretreatment with clofibrate significantly increased the chiral conversion from the (*R*)-(–)-FPF enantiomer to the pharmacologically active (*S*)-(+)-FPF isomer. Toxicity may occur due to a higher concentration in the plasma of the active (*S*)-enantiomer (responsible for gastrointestinal and renal toxicity). Furthermore, a higher concentration of CoA activated (*R*)-profen formed during the chiral inversion process may (a) enter the pathways of lipid metabolism, modifying membrane function by the formation of hybrid triglycerides, (b) inhibit the β-oxidation of fatty acids and (c) interfere with the metabolism of cholesterol. Further toxicological studies are needed before considering the therapeutic use of this kind of drug association.

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