Stereoselective Inversion of (*R*)-Fenoprofen to (*S*)-Fenoprofen in Humans

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Abstract □ The concentrations of the (*R*)- and (*S*)-enantiomers of fenoprofen (α-methyl-3-phenoxy-benzeneacetic acid) were measured in plasma and urine of volunteers after oral administration of the (*R,S*)-racemate. In addition, urinary concentrations of the (*R*)- and (*S*)-4′-hydroxy metabolite of fenoprofen, the major metabolite, were measured. The (*R*)-enantiomer of fenoprofen was stereoselectively inverted to (*S*)-fenoprofen, which was the major isomeric form found in plasma and urine. A potency comparison of the enantiomers in vitro showed the (*S*)-isomer to be 35 times more active than the (*R*)-isomer in inhibiting the fatty acid cyclo-oxygenase pathway from human platelets. In vivo, the similar pharmacological potency of the two enantiomers previously observed in experimental animals may have been due to the rapid inversion of the (*R*)- to (*S*)-isomer.

In recent years there have been an increasing number of reports on the stereoselective biotransformation of enantiomers of drugs having asymmetric centers. For example in humans, (*R*)-(−)-ibuprofen is preferentially metabolized to (*S*)-(+)-ibuprofen,[1] and (*R*)-(−)-benoxaprofen is converted to the (*S*)-(+)-enantiomer.[2] Animal studies revealed the stereoselective glucuronidation of oxazepam,[3] the inversion of (−)-cicloprofen to (+)-cicloprofen,[4] and the isomerization of (*R*)-(−)- to (*S*)-(+)-clidanac.[5] These inversions have been shown to affect pharmacological activity. In vitro, only the (*S*)-(+)-enantiomer of the anti-inflammatory drug, clidanac, inhibits prostaglandin synthesis; in vivo the (*S*)-(+)-isomer is more potent than the (*R*)-(−)-isomer in rats and mice, but the isomers are equipotent in guinea pigs.[5]

The present study examines the biotransformation of the anti-inflammatory analgesic agent (*R,S*)-fenoprofen (α-methyl-3-phenoxy-benzeneacetic acid) with emphasis on the stereoselective inversion of the (*R*)- to the (*S*)-enantiomer.[6]

Experimental Section

Reagents—Glusulase (Endo) from *Helix pomatia* contained 139,214 units β-glucuronidase/mL and 15,000 units of sulfatase/mL. 1,1′-carbonyldiimidazole and (*S*)-(−)-α-methylbenzylamine were purchased from Aldrich Chemical Company, Inc. Hexane, dimethylformamide, and methylene chloride were spectroquality solvents. Walpole's acetate buffer (pH 5), consisted of 0.2 M sodium acetate and 0.2 M acetic acid (7:3).

Drug Administration and Sample Collection—The four male volunteers in this study were fasted overnight (except for water) and for an additional 2 h after the first dose of (*R,S*)-fenoprofen. Either 600 or 1200 mg (acid eq.) of (*R,S*)-fenoprofen was administered orally every 8 h for a total of 7 doses as tablets of the calcium salt (Nalfon, Lilly). After the first and last dose, venous blood samples (10 mL each) were drawn into heparinized tubes. The plasma was collected by centrifugation and was stored at −20°C until analyzed. Aliquots of urine samples were similarly stored until analyzed.

Determination of (*S*)- and (*R*)-fenoprofen in Plasma and Urine—The levels of total fenoprofen were measured by HPLC.[7] This method, carried out in a separate laboratory, was used to assure that technical problems in measuring individual isomers did not provide flawed data. Briefly, fenoprofen was extracted into butyl chloride from acidified plasma and was then back-extracted into aqueous base. The aqueous phase was analyzed on a μ-Bondapak alkyl phenyl column (Waters Associates) eluted with acetonitrile:H_2O:acetic acid (50:50:2) with monitoring at 272 nm.

The individual isomers of (derivatized) fenoprofen were measured by GC. To 1 mL of plasma were added 150 μg of an aqueous solution of *d,l*-2-(4-phenoxyphenyl)valeric acid, as internal standard, and 2 mL of 10% trichloroacetic acid. The samples were mixed, allowed to stand for 10 min, and were extracted twice with 5 mL of hexane. The combined extracts were evaporated to dryness. To the residue was added 100 μL of carbonyldiimidazole solution (32.5 mg/mL in CH_2Cl_2), followed 5 min later by 10 μL of glacial acetic acid and 50 μL of (*S*)-(−)-α-methylbenzylamine. After 30 min, 0.5 mL of water was added and the diastereomeric amide derivatives of fenoprofen were extracted into hexane (2 mL). The residue remaining after evaporation of the hexane was reconstituted in 50 μL dimethylformamide and 1 μL was injected into the GC column. A Hewlett-Packard model 5830A gas chromatograph equipped with a flame ionization detector was fitted with a conditioned column (1 m × 6 mm o.d. × 4 mm i.d.) packed with 3% OV-17 (60–80 mesh) on Gas Chrom Q (Ohio Valley Specialty Chemical, Inc.). The respective operating conditions for analyses of fenoprofen and the 4′-hydroxy metabolite of fenoprofen were: injector at 310°C and 320°C; column at 265°C and 295°C; detector at 320°C and 350°C; gas flow rates at 40 mL/min N_2; 40 mL/min H_2, and 60 mL/min air. Retention times (of the derivatives) were: 7.5 min for (*S*)-fenoprofen, 8.2 min for (*R*)-fenoprofen, and 12.7 min for internal standard; 6.2 min for the (*S*)-4′-hydroxy metabolite of fenoprofen, 6.7 min for (*R*)-4′-hydroxy metabolite of fenoprofen, and 3.6 min for the internal standard. Quantitation was based on standard curves derived from the ratios of peak heights between each compound of interest and the internal standard.

Because most of a dose of fenoprofen is excreted in urine as glucuronide conjugates of fenoprofen and the 4′-hydroxy metabolite of fenoprofen,[8] all urine samples were hydrolyzed with Glusulase overnight at 37°C. To urine samples (0.1–3 mL) were added Walpole's buffer (0.6–1.4 mL) and Glusulase (10 μL/mL of urine). After hydrolysis, 1 mL of 1 M HCl was added prior to extraction with two volumes of hexane; the extracts were derivatized and analyzed as described above.

Urinary Determination of the (*S*)- and (*R*)-4′-Hydroxy Metabolites of Fenoprofen—The procedure for determining (*R*)-4′-hydroxy metabolite of fenoprofen in urine was modified slightly from that used for fenoprofen: 80 μg of internal standard was used, and samples were extracted at pH

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3.8–4.1 with methylene chloride, which also served as the solvent after derivatization.

Because the back side of the peak for the (*S*)-enantiomer of the 4′-hydroxy metabolite of fenoprofen amide tailed slightly into the peak for the later-eluting (*R*)-isomer, it was necessary to confirm whether some samples actually contained the (*R*)-isomer or merely interfering contaminants in the tail. These samples were analyzed by GC–MS; a 1.3-m column of 3% OV-17 was operated at 255°C; the detector was programmed to scan only *m/z* 363 (derivatized 4′-hydroxy metabolite of fenoprofen) and *m/z* 374 (derivatized internal standard).

Results

Because results are similar among individuals, group means are plotted. Likewise, only seventh dose data are presented because they did not differ from first dose data.

Plasma—The predominant isomer found in plasma at all time periods after administration of (*R,S*)-fenoprofen was (*S*)-fenoprofen (Fig. 1). Small amounts of (*R*)-isomer were often found in plasma at early time periods, but the *S:R* ratio never approached the 1:1 ratio of the racemate. At the 600-mg dose level, the *S:R* ratio approximated 3:1 at 0.5 h, but by 1 h the ratio had almost doubled, and by 4 h no (*R*)-fenoprofen was detectable. At the 1200-mg dose level, the (*R*)-enantiomer was detected at low concentrations in the plasma of only one volunteer for 6 h after the first dose, but it was not detected 2 h after the seventh dose.

Urine—When 600 mg of the racemate of fenoprofen was administered, no (*R*)-enantiomer was detected in hydrolyzed urine (Fig. 2). After the 1200-mg regimen, <4% of the total excreted fenoprofen was the (*R*)-enantiomer. Urinary excretion, as total enantiomers of unchanged fenoprofen, accounted for 37–50% of the combined dosages; most of the remainder was excreted as the (*S*)-enantiomer of the 4′-hydroxy metabolite of fenoprofen. The combined amounts of (*S*)-fenoprofen and the (*S*)-4′-hydroxy metabolite of fenoprofen in the urine accounted for 78–86% of the total amount of fenoprofen given in the 7 doses; this finding indicates a stereoselective biotransformation of (*R*)- to (*S*)-fenoprofen, although it does not exclude a contribution from preferential absorption and/or excretion of the (*S*)-isomer over the (*R*)-isomer as well.

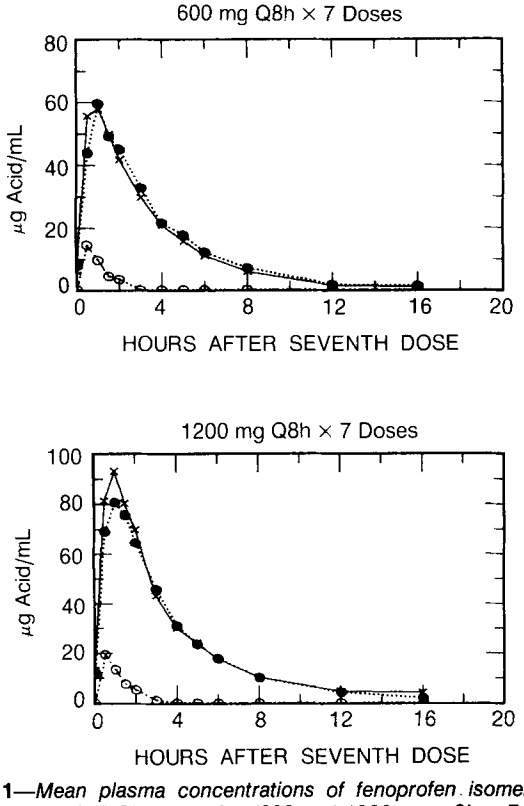

Figure 1—*Mean plasma concentrations of fenoprofen isomers after administration of (R,S)-fenoprofen (600 and 1200 mg, q8h × 7 doses). Key: (●) (S)-fenoprofen; (○) (R)-fenoprofen; (×) total fenoprofen.*

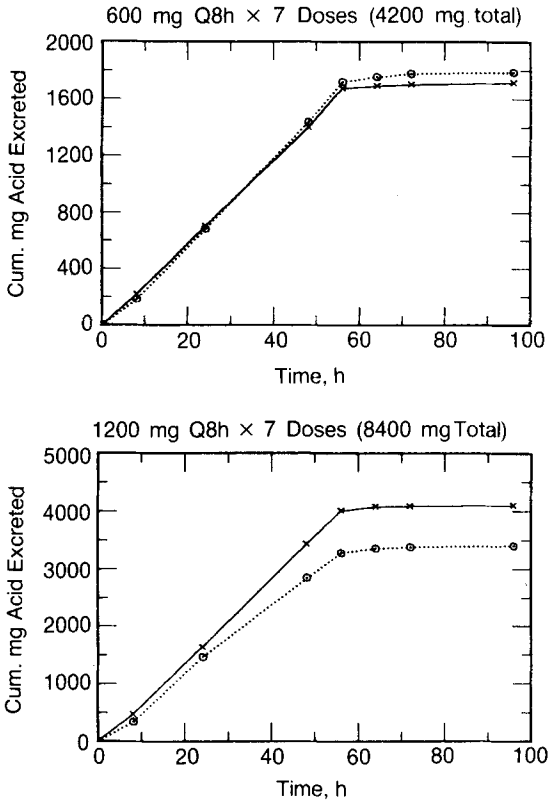

Figure 2—*Mean urinary levels of (S)-fenoprofen (×) and the (S)-4′-hydroxy metabolite of fenoprofen (○) after administration of (R,S)-fenoprofen (600 and 1200 mg q8h × 7 doses).*

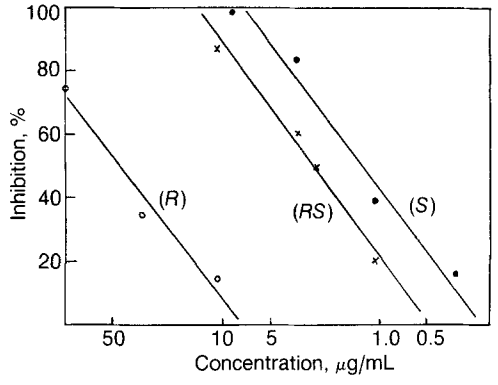

Figure 3—*Inhibition of the cyclo-oxygenase pathway from human platelets by (R,S)- (×), (R)-(○), and (S)-fenoprofen (●).*

(*R*)-, (*S*)-, and (*R,S*)-fenoprofen were compared as inhibitors in vitro of the fatty acid cyclo-oxygenase system for human platelets. This system is often used to detect drugs that have anti-inflammatory activity that may be associated with inhibition of prostaglandin synthesis.[9] On the basis of the concentration effecting 50% inhibition of the system, (*S*)-fenoprofen was two times more active than the racemate and ~35 times more active than the (*R*)-enantiomer (Fig. 3). Previous studies in the rat, mouse, and guinea pig showed no differences, in

vivo, in pharmacological and toxicological anti-inflammatory activities between enantiomers of fenoprofen.[10-12] Those findings may simply mean that in animals, as in humans, the (*R*)-fenoprofen was inverted quickly to the (*S*)-isomer. Unfortunately, in those studies neither plasma nor urine samples were analyzed for the presence of the individual isomers. In this connection, we recently analyzed (qualitatively) the urine specimens of rats, mice, and guinea pigs given (*R,S*)-fenoprofen orally and found the drug to be almost completely excreted as the (*S*)-enantiomers of conjugated fenoprofen and/or the 4′-hydroxy metabolite of fenoprofen.

Discussion

The individual (*R*)- and (*S*)-isomers of fenoprofen and the 4′-hydroxy metabolite of fenoprofen were studied in plasma and urine after administration of the (*R,S*)-racemate to four volunteers. Plasma and urine contained predominantly (*S*)-enantiomers of fenoprofen and the 4′-hydroxy metabolite. Small amounts of (*R*)-fenoprofen were present in plasma in samples drawn shortly after dosing; in only one case was (*R*)-fenoprofen detected in plasma beyond 4 h. The enzyme system(s) involved in the (*R*)- to (*S*)-inversion did not appear to be saturated at either dosage regimen used. Although less likely, a contribution from a selective absorption and/or excretion of one isomer over the other was not ruled out; nevertheless, the observation that 78–86% of the administered dose of fenoprofen was recovered as (*S*)-fenoprofen plus the (*S*)-4′-hydroxy metabolite after administration of the fenoprofen racemate indicated a significant stereoselective conversion of the (*R*)-fenoprofen to the (*S*)-enantiomer.

No studies have been done to determine if the glucuronide conjugation of fenoprofen or the 4′-hydroxy metabolite is stereoselective; studies of propranolol[13] have suggested that one enantiomer could be preferentially conjugated in humans. The presence of both the (*R*)- and (*S*)-4′-hydroxy metabolite of fenoprofen in urine suggests that both may be hydroxylated, but provides no information on the relative rates of reaction.

Several mechanisms involving the enzymes of lipid metabolism have been proposed for the inversion of (*R*)-isomers of arylpropionic acids to corresponding (*S*)-isomers.[14,15] An essential component of the proposal appears to be the loss of the chiral center by dehydrogenation, and subsequent reduction of this intermediate by an enoyl reductase that stereospecifically converts to the (*S*)-enantiomer. As emphasized by Hutt and Caldwell,[15] the significance of the chiral inversion of drugs is not restricted to its biochemical/metabolic implications, but is also important for assessment of a drug during its preclinical and clinical development.

References and Notes

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