Bi-Directional Chiral Inversion of Ketoprofen in CD-1 Mice

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ABSTRACT The R enantiomers of some of the 2-arylpropionic acid non-steroidal antiinflammatory drugs (NSAIDs) are known to undergo metabolic chiral inversion to their more pharmacologically active antipodes. This process is drug and species dependent and usually unidirectional. The S to R chiral inversion, on the other hand, is rare and has been observed, in substantial extents, only for ibuprofen in guinea pigs and 2-phenylpropionic acid in dogs. After i.p. administration of single doses of racemic ketoprofen or its optically pure enantiomers to male CD-1 mice and subsequent study of the concentration time-course of the enantiomers, we noticed substantial chiral inversion in both directions. Following racemic doses, no stereoselectivity in the plasma-concentration time courses was observed. After dosing with optically pure enantiomer, the concentration of the administered enantiomer predominated during the absorption phase. During the terminal elimination phase, however, the enantiomers had the same concentrations. Our observation is suggestive of a rapid and reversible chiral inversion for ketoprofen enantiomers in mice. *Chirality 9:29–31, 1997.*

KEY WORDS: 2-arylpropionate; reversible chiral inversion; NSAIDs

INTRODUCTION

Ketoprofen, a chiral nonsteroidal antiinflammatory drug (NSAID) of 2-arylpropionic acid class (profens), is commonly used in the treatment of rheumatoid arthritis and osteoarthritis, as well as other connective tissue disorders and pain.[1,2] Similar to other NSAIDs, the therapeutic activity of ketoprofen is due to its apparent inhibition of prostaglandin synthesis. The S enantiomer is considered to be the antiinflammatory enantiomer with the R enantiomer being less active, or inactive. A number of studies have indicated that some of the profens undergo in vivo chiral inversion where the R enantiomer is transformed to the S antipode.[3] Previous work from our group suggested 80% and 10% chiral inversion for ketoprofen in rats[4] and humans,[5] respectively. Further, Aberg et al.[6] found inversion of R-ketoprofen to its antipode in eight species. They classified the studied animals as either "extensive" (>50% inversion) or "limited" (<50%) inverters depending on their ability to invert R-ketoprofen. In general, the chiral inversion of NSAIDs is unidirectional (R → S) with some exceptions: Chen et al.[7] reported bi-directional chiral inversion for ibuprofen in guinea pigs, rabbits, and rats. The inversion was substantial in all three species after administration of sterechemically pure R enantiomer. The inversion of the S enantiomer, on the other hand, was substantial only in guinea pigs. In addition, Tanaka et al.[8] observed a significant extent of chiral inversion in both directions following administration of 2-phenylpropionic acid to dogs. They also refer to unpublished work indicating bi-directional ketoprofen inversion in mice.

The purpose of this paper is to report our observation of substantial bi-directional chiral inversion after i.p. administration of racemic, R- and S-ketoprofen to CD-1 mice. A parenteral route of administration was used to avoid potential complications of the gut involvement in the process of chiral inversion.

MATERIALS AND METHODS

Racemic ketoprofen and the individual enantiomers (optical purity >98.8%) were supplied by Sepracor (Marlborough, MA). Indomethacin was purchased from Sigma Chemical Company (St. Louis, MO). All chemicals and solvents used were of analytical grade.

Assay

A previously validated stereospecific assay was used.[9] To 0.1–0.4 ml of mouse plasma was added indomethacin as internal standard and the contents were acid extracted with isooctane-isopropanol (95:5). After evaporation of the organic layer, the residue was reconstituted in mobile phase and injected into an HPLC. Ketoprofen enantiomers were directly resolved on a Chiralpak AD chiral column (25 cm × 4.6 mm I.D., Chiral Technologies Inc. Exton, PA) at 254 nm. The minimum quantifiable concentration (MQC) of the assay was found to be 0.25 μg/ml based on 0.1 ml of mouse plasma samples with less than 8% inter or intra day

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coefficient of variation. Aliquots of 0.1 (0.5–2 h samples) or 0.4 (0 and 3–6 h samples) ml of plasma was analyzed. All samples, except those collected at time zero, contained ketoprofen enantiomers above the MQC. The assay exhibited no chiral conversion.

Animals

The study was carried out according to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences (CIOMS), University of Alberta. Male CD-1 mice (27–33 g) were obtained from Charles River laboratories (Willmington, MA), then housed in the vivarium in temperature and humidity controlled rooms maintained on a 12 h light/12 h dark cycle. Animals were allowed free access to food and water for the duration of the experiments unless specified.

Pharmacokinetic Studies

Pharmacokinetics and the possibility of inversion were evaluated in groups of mice dosed (i.p.) with R-, S-, or racemic ketoprofen (5, 5, and 10 mg/kg, respectively) which were dissolved in Sorensons' phosphate buffer (pH 8). Following administration of the drugs, the mice were anesthetized with ether and blood samples were collected by cardiac puncture into 1 ml heparinized syringes at 0.5, 1, 2, 4, and 6 h post dose (n = 3 for each time point). Immediately following blood collection animals were sacrificed in a CO_2 chamber. The samples were centrifuged, plasma obtained and frozen at –60°C until analyzed.

In Vitro Chiral Inversion

To evaluate the possibility of in vitro chiral inversion of the pure enantiomers, 0.1 ml of the stock solutions were incubated in water or mouse plasma (37°) for up to 24 h. Aliquots were taken at 0.33 and 24 h and assayed for ketoprofen enantiomers.

Data Analysis

The observed plasma concentrations were plotted versus time and the area under the curves (AUC_{0-6}) were calculated using the trapezoidal rule. The terminal t½ was calculated from the log-linear terminal phase of best-fitted lines estimated using linear regression.

Statistical Analysis

Each data point represents an individual mouse. Hence, the AUC of the mean plasma concentrations have been calculated and the values are presented with no variance. The individual plasma concentrations, on the other hand, are presented as mean ± standard deviation and the differences between enantiomers concentrations were assessed using the two sided paired Student's *t*-test at $\alpha = 0.05$.

RESULTS

Following administration of stereochemically optically pure S- or R-ketoprofen to the mice, substantial presence of the respective antipode was noticed in plasma (Fig. 1) indicating chiral inversion in both directions. The mean plasma concentration-time curves peaked (Tmax) in 0.5 to 1 h post-dose following all three treatments. Following the racemic doses, no significant stereoselectivity was observed in the peak plasma concentrations (7.53 ± 0.79 versus 6.07 ± 0.53 μg/ml for S and R, respectively, Tmax 1 h) which was consistent with similar total (S + R) AUC values (16.6 versus 15.19 μg/ml following S and R doses, respectively). After administration of stereochemically pure doses, the administered enantiomer presented significantly higher peak plasma concentration than the antipode (8.67 ± 1.47 versus 3.18 ± 0.39 μg/ml after S and 2.11 ± 0.34 versus 3.22 ± 0.32 μg/ml after R for S and R, respectively, Tmax, 0.5 h except 1 h for S after R). Following administration of S-ketoprofen, the first collected sample contained the maximum concentration (Fig. 1). It is, therefore, possible that the true peak concentration might have been attained before the first sample collection time of 0.5 h. The overall AUC values also appeared different following the optically pure doses (9.44 versus 6.44 after S and 6.45 versus 9.23 $\mu g \cdot ml^{-1} \cdot h$ after R, for the S and R enantiomers, respectively). Interestingly, after reaching a maximum, the plasma concentration of the enantiomers declined to the same levels (Fig. 1). The log linear terminal phase of the plasma concentration-time curves had t½ of 1.24, 1.52, and 2.25 h for the S enantiomer, and 1.40, 2.44, and 2.71 for the R enantiomer after administration of S-, R-, and racemic ketoprofen, respectively. The significance of the observed differences in AUCs and t½ values were not examined as the data points, each from individual mice, were pooled to construct a single plasma concentration-time curve for each treatment.

On incubation of the individual enantiomers in mouse plasma, we found <1% inversion to the respective antipode.

DISCUSSION

Chiral R to S inversion has been observed for many profens in various species.[3,10] The S to R inversion, on the other hand, is rare although suggested in 1986 by Fournel et al.[11] The extent of inversion of the R enantiomer varies from limited (e.g., flurbiprofen) or complete (e.g., fenoprofen).[3] It is interesting, however, that for profens that do undergo substantial S to R inversion, a state of equilibrium seems to exist. Such is not the case for R to S inversion. Chen et al.[7] reported equal extent of chiral inversion for both enantiomers of ibuprofen in guinea pigs. Tanaka et al.[8] also observed that following administration of optically pure 2-phenylpropionic acid to dogs, the enantiomers concentrations reach the same level during the post-absorptive phase. These observations are consistent with those of ours and suggest the presence of a reversible metabolic inversion.

Complete spontaneous chemical racemization has been observed for other chiral compounds such as ketorolac.[12] The observed chiral inversion of ketoprofen in mice, however, was likely enzymatically mediated, as incubation of optically pure enantiomers of ketoprofen with plasma did not result in production of significant amount of the antipode.

To delineate the kinetics of the observed inversion is difficult due to its very rapid rate. Nevertheless, the extent of the inversion should not exceed 50% since a portion of

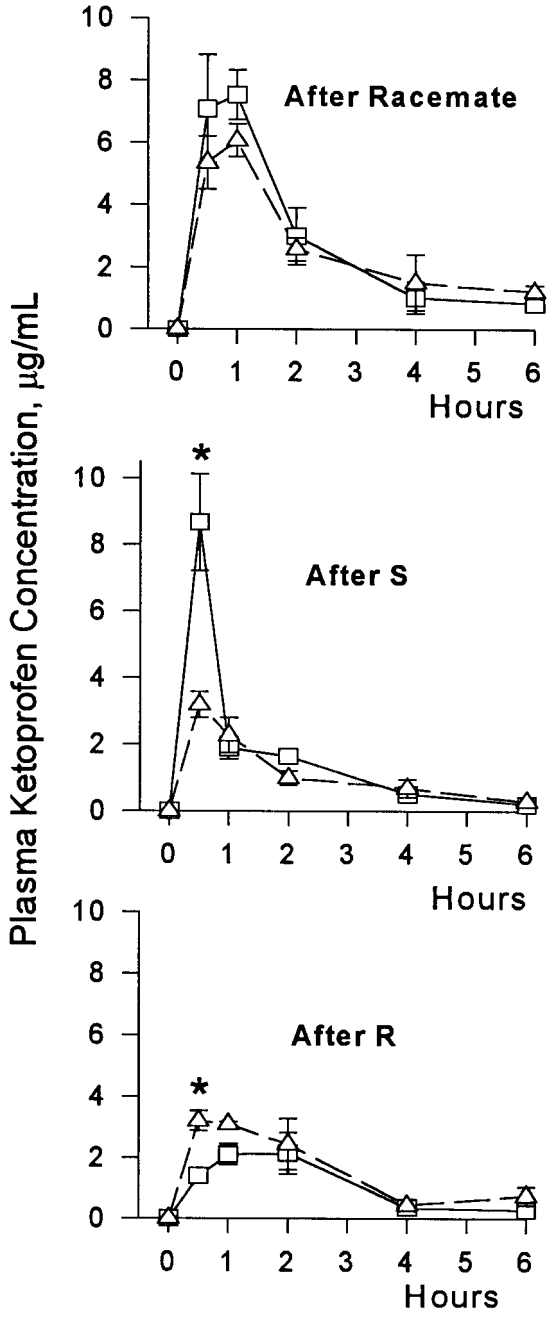

Fig. 1. Plasma concentration of S (□) and R (△) enantiomers of ketoprofen following *ip* administration of 10 mg racemate or 5 mg of each stereochemically pure enantiomers to mice. Error bars represent standard deviation of the mean. Each mean represent three mice. Significant stereoselectivity (*) was observed only 0.5 h after both doses of S- and R-ketoprofen.

the dose may be cleared before undergoing inversion and the remaining amount reaches an equilibrium with its antipode.

In a reversible process, the concentrations of the two enantiomers should decline with the same t½[13] which appears to be the case with the ketoprofen enantiomers. Due to our experimental design, however, we were not able to assess the statistical significance of the differences between the enantiomers' t½. Nevertheless, the fact that the concentration of the enantiomers were very close during the terminal phase (Fig. 1), suggests that the differences between the t½ of the enantiomers were not significant despite their numerical differences. In addition, superimposible plasma concentration time courses for the enantiomers suggest that the clearance rather than formation is the rate limiting step, i.e., very rapid interconversion of the two substrates. This appears to be the case for ketoprofen in the mouse (Fig. 1), ibuprofen in the guinea pig[7] and 2-phenylpropionic acid in dogs.[8]

The observation that ketoprofen undergoes bidirectional chiral inversion in the mouse has no apparent clinical relevance. Nevertheless, during pre-clinical stages, in choosing an animal model to study stereochemical aspects of drugs action and disposition, the information provided herein may prove useful.

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