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Stereospecific Pharmacokinetics and Toxicodynamics of Ketorolac After Oral Administration of the Racemate and Optically Pure Enantiomers to the Rat

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ABSTRACT To determine the stereospecific pharmacokinetics and gastrointestinal permeability (GI) changes (surrogate measures of toxicity) in the rat following oral administration of S, R, and racemic ketorolac (KT),optically pure enantiomers (S and R 2.5 mg/kg), and racemic KT (5 mg/kg) were administered orally to male Sprague-Dawley rats and plasma samples were collected for 6 h post-dose for pharmacokinetic assessments. KT-induced changes in GI permeability were assessed using sucrose and ^{51}Cr-EDTA as markers of gastroduodenal and distal intestinal permeability, respectively. After the racemate, R-KT was predominant in plasma (AUC S/R, 0.45). No significant differences in pharmacokinetic indices were evident following administration of the racemate as compared with individual enantiomers. In plasma, there was only negligible S-KT after administration of R-KT. After S-KT, on the other hand, AUC of R-KT was found to be 6.7% of that of S-KT. Both permeability markers showed considerable interanimal variability. Gastroduodenal permeability was significantly increased from baseline by the racemate but not by either of the two enantiomers administered alone. Permeability to ^{51}Cr-EDTA was not significantly increased above baseline for any of the treatments. The plasma concentration of R-KT found after administration of S-KT may be from the <2% chiral impurity which appears magnified due to its slower clearance as compared with its antipode. There is no evidence of a pharmacokinetic interaction between the enantiomers. Since 2.5 mg/kg S-KT is somewhat less toxic on the gastroduodenum than 5 mg/kg racemate, it may be a safer alternative to the latter, at least in the rat model. *Chirality 11:201–205, 1999.*

Ketorolac (KT) (Fig. 1) is a potent and effective chiral NSAID. Similar to other NSAIDs, the limiting factor for KT use is development of gastrointestinal (GI) side effects, possibly due to its potent cyclooxygenase inhibitory effect.[1,2] However, in epidemiologic and case studies, KT has been singled out from other NSAIDs as having a distinctly higher risk of gastroduodenal toxicity.[3,4]

An inherent problem with examining adverse GI effects caused by KT and other NSAIDs is that detection is difficult and invasive, and is usually only demonstrated after one of the complications (e.g., bleeding, perforation, or hemorrhage) becomes clinically apparent.[5] This is complicated by a poor correlation between upper GI symptoms of distress and endoscopically proven gastropathy.[6]

Recently, NSAID-induced GI permeability increases which precede ulceration have been shown to be a prerequisite for the mucosal inflammation and the gross toxicological manifestations seen with NSAID use.[7–9] The suitability of the rat as a model using urinary excretion of sucrose for upper GI[10] and ^{51}Cr-EDTA for distal intestinal inflammation[11] has previously been reported. There is a significant positive correlation between GI permeability and ulceration.[12] These markers of permeability offer a simple noninvasive surrogate measurement of GI abnormalities.

Since the antinflammatory effect of chiral NSAIDs is usually atributed to the S enantiomers, considerations have been given to the development of stereochemically pure formulations of these drugs. For some NSAIDs, however, such an attempt is complicated by metabolic[13,14] or spontaneous[15,16] chiral inversion. In addition, pharmacokinetic[17] and/or pharmacodynamic[18] interactions between enantiomers should also be considered in the rationale for developing stereochemically pure formulations.

The purpose of this study was to examine whether a rationale exists for developing stereochemically pure formulations of KT. We therefore studied the pharmacokinet-

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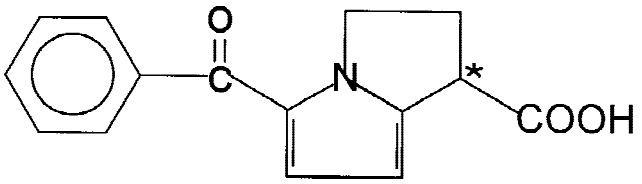

Fig. 1. Ketorolac. The asterisk denotes the position of the chiral center.

ics and the effect on the GI permeability of KT and its stereochemically pure enantiomers in the rat.

MATERIALS AND METHODS

Chemicals

Racemic KT was supplied by Syntex Inc. (Palo Also, CA). Individual enantiomers of KT (optical purity >98%) were supplied by Sepracor Inc. (Marlbrough, MA). Naproxen was purchased from Sigma Chemical Company (St. Louis, MO). Sucrose and Trinder's reagent (glucose oxidase, peroxidase, 4-a,inoantipyrine, and *p*-hydroxybenzend in pH 7 buffer) were purchased from Sigma (St. Louis, MO). D-Glucose and methylcellulose were purchased from BDH chemicals (Toronto, Canada). ^{51}Cr-EDTA (specific activity 570 mCi/mg) was purchased from Dupont (Wilmington, DE). ELISA assay plates were purchased from Fisher Scientific (Edmonton, Canada). All other chemicals and solvents used were of analytical grade.

Assay

Plasma concentration of KT enantiomers was determined using a previously reported direct chiral HPLC method.[15] Briefly, the assay involved organic solvent extraction of the enantiomers from 100 µl acidified sample and their resolution using a Chiralpak AD column (Daicel Chemical Industries, Exton, PA) followed by UV detection at 313 nm. The minimal quantifiable concentration was 250 ng/ml based on 0.1 ml plasma.

Animals

Male Sprague-Dawley rats (250–300 g) housed at ambient temperature and humidity in individual metabolic cages with wire mesh floors (allowing for selective quantitative collection of urine and feces) were used in all of the studies. All studies were carried out according to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences.

Pharmacokinetic Studies

Pharmacokinetics of KT enantiomers in the rat involved catheterization at the right jugular vein using silastic tubing (0.58 mm i.d. × 0.965 mm o.d.; Clay Adams, Parsippany, NJ). Animals were allowed to recover overnight and had access to water ad libitum. They were fasted overnight until 3 h post dose. Racemic (5 mg/kg), S (2.5 mg/kg), or R (2.5 mg/kg) KT dissolved in polyethylene glycol 400 was administered orally to each of the animals at 9 a.m. on the day following surgery using a gastric gavage (n = 8 for each group). Blood (0.2 ml) was collected from the jugular vein cannula at 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h after KT administration. The catheter was flushed with 0.2 ml of 100 U/ml heparin following each blood sample collection. Immediately following collection, plasma was separated from blood by centrifugation at 1800 *g* for 3 min using a Fisher model 235A microcentrifuge (Fisher Scientific, Edmonton, Canada). All samples were transferred to 1 ml polypropylene vials (Fisher Scientific, Edmonton, Canada), and were stored at –20°C until analyzed.

Sucrose Permeability

Sucrose permeability changes were measured using a previously reported method.[10] Rats (n = 6 for each treatment) were allowed free access to food and water for the duration of the experiment. Rats received either racemate (5 mg/kg), R (2.5 mg/kg), S (2.5 mg/kg) KT or vehicle (1% methylcellulose) at the same time of day (9 a.m.). One hour post-dosing, 0.5 ml of a solution containing 1.0 g/ml of sucrose was orally administered to each rat. Urine was collected 0 to 8 h following the administration of the sucrose solution. Relative permeability was determined by calculating the sucrose present in each urine sample as a percent of the administered dose after correcting for baseline levels of glucose and sucrose present in urine for each individual rat. Maximum increased sucrose permeability has been observed 1 h post NSAID administration.[10]

^{51}Cr-EDTA Permeability

Permeability of ^{51}Cr-EDTA was measured using a previously reported method.[11] Briefly, rat (n = 6/group) received racemate (5 mg/kg), R (2.5 mg/kg), S (2.5 mg/kg) KT and vehicle (1% methylcellulose) at the same time of day (9 a.m.). Three hours post-dosing, 0.5 ml of an aqueous solution containing 10 µCi/ml of ^{51}Cr-EDTA was orally administered to the rats using an 18-gauge 5-cm curved feeding gavage attached to a 1 ml syringe. Urine was collected for the 0–8 interval following the oral dose of ^{51}Cr-EDTA. After each collection period, 10 ml of tap water was used to rinse the metabolic cages. The samples were collected in the urine cups and transferred to scintillation vials. The scintillation vials were then counted directly. Maximally increased ^{51}Cr-EDTA permeability has been observed 3 h post-dose for many NSAIDs except indomethacin.[11,18]

Data Analysis

$AUC_{0\text{-}t}$ was measured by the linear trapezoidal method. For each enantiomer t_{max} was determined at the time of maximum concentration during its time course in plasma.

Statistical Analysis

All values are reported as mean ± standard deviation. Differences between pharmacokinetic parameters were as-

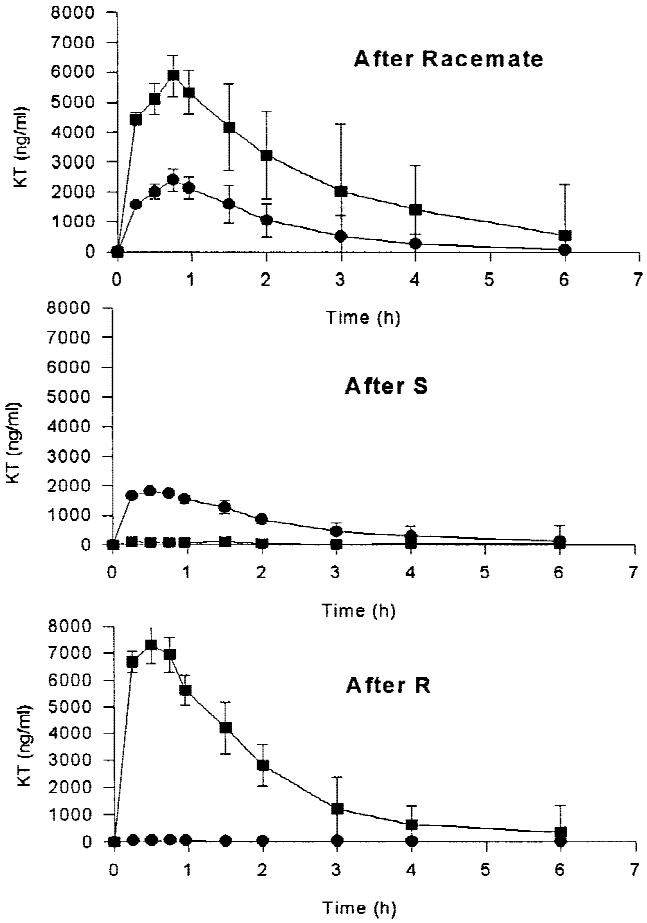

Fig. 2. Concentration (S, ●; R, ■)-time profiles following administration of S, R (2.5 mg/kg), and racemic KT (5 mg/kg) to male Sprague-Dawley rats; mean ± SD, n = 8/group.

sessed using Student's two-tailed t-test at $\alpha = 0.05$. Multiple group study designs (toxicity studies) were assessed using one-way ANOVA at an $\alpha = 0.05$ level of significance followed by Tukey's post-hoc analysis.

RESULTS

After administration of both racemic and stereochemically pure KT, plasma concentrations of R-KT were significantly higher than those of S-KT (Fig. 2, Table 1). Racemic and stereochemically pure doses yielded similar pharmacokinetic indices.

When KT was administered as either of the stereochemically pure enantiomers, antipode was present to some extent. The AUC of S-KT after administration of R-KT was 1.3% that of the R-KT, while after administration of S-KT the AUC of R-KT was 6.7% of the administered enantiomer (Fig. 2, Table 1).

Toxicodynamic data (Fig. 3) for both sucrose and ^{51}Cr-EDTA showed considerable interanimal variability. While sucrose permeability increased following all of the treatments, only the group that received racemate KT was significantly different from the controls. Permeability of ^{51}Cr-EDTA did not increase above the control levels following either enantiomers or the racemate.

DISCUSSION

Plasma concentration profiles of KT enantiomers in the rat were qualitatively similar to those previously observed in humans, with plasma concentrations of R enantiomer being predominant; AUC S/R: humans, 0.26,[15,19–23] rats, 0.45.[15] It is interesting to note that, similar to etodalac,[24] but in contrast to the majority of other chiral NSAIDs,[14] the predominant enantiomer in plasma is the less active R-KT. The observed stereoselectivity of KT plasma concentrations has been explained, in part, by the higher unbound fraction of S-KT in plasma.[15,20]

Since no significant differences were observed in the pharmacokinetic indices of KT enantiomers following administration of the racemate and individual enantiomers, it can be concluded that there is no interaction between the enantiomers. Interactions between enantiomers of the same drug have been reported for several drugs including propoxyphene[18] and flurbiprofen.[17]

The observation that administration of both stereochemically pure enantiomers results in small but significant concentration of the antipode is interesting. This could be attributed to a bi-directional spontaneous or chiral inversion and/or stereochemical impurity. KT has been shown to undergo racemization under specific conditions.[15] Under the conditions used in this study, spontaneous interconversion is unlikely.[15] The enantiomers used in this study both contained approximately 1–2% of the anipode as stereochemical impurity. Considering a three-fold faster clearance for S- as compared with R-KT, a 1–2% chiral impurity should result in an AUC for S-KT equal to 0.3–0.6% of R-KT. The observed 1.3% of R-KT as S-KT, therefore, may suggest a small R to S inversion. Chiral R to S inversion has been observed for many profens in various speces.[25] The S to R inversion, on the other hand, is rare and is only demonstrated for ibuprofen in guinea pigs,[26] 2-phenylpropionic acid in dogs,[27] and ketoprofen in mice.[28,29] The observed 6.7% of R-KT following administration of S-KT is also >1–2% of chiral impurity of the samples used. However, since the R enantiomer is cleared three-fold slower than S-KT, the AUC of R after administration of S may be explained by stereochemical impurity.

Following all treatments a trend toward increased sucrose permeability was noted (Fig. 3). However, only the racemate yielded results that were statistically significant. The lack of statistical significance following KT enantiomers may be due to the high interanimal variability in the results. The racemic KT-induced increased sucrose permeability correlates well with observations in humans that KT can cause gastroduodenal damage.[3,4] Since sucrose is readily cleaved in intestine, the appearance of intact sucrose in urine indicates permeability from the gastroduodenal segment and before reaching the distal intesting.[8–10] The rat permeability model, therefore, appears to be suitable for studies involving upper GI damage.

Intestinal permeability, measured by increased urinary excretion of ^{51}Cr-EDTA, was not elevated by either racemic KT or any of its enantiomers. An increase in ^{51}Cr-EDTA urinary excretion is suggested to reflect elevated permeability of the distal intesting. The probe seems to have

TABLE 1. Pharmacokinetic indices following oral administration of ketorolac

	After S		After R		After racemate	
	S	*R*	*S*	*R*	*S*	*R*
C_{max} (μg/ml)	2.18 ± 0.60	nd	nd	7.90 ± 2.50	2.50 ± 1.40	6.60 ± 2.60*
t_{max} (min)	32 ± 13	nd	nd	35 ± 17	43 ± 23	43 ± 23
$t_{1/2}$ (h)	1.23 ± 0.67	nd	nd	1.56 ± 0.81	1.07 ± 0.49	0.89 ± 0.38
AUC ($\mu g \cdot ml \cdot h^{-1}$)	4.17 ± 1.37	0.28 ± 0.17	0.19 ± 0.12	15.1 ± 6.0	4.88 ± 3.04	15.1 ± 9.30*

Mean ± sd; nd, not determined due to data fluctuations; *, significantly different from both S after racemate and S alone.

negligible permeability from the upper GI tract due, perhaps, to the limited surface area and residence time therein.[7,11] Hence, ^{51}Cr-EDTA is used as a surrogate marker of pathophysiological alterations of the intestine.[7,12] Many NSAIDs (e.g., diclofenac, indomethacin) cause significant intestinal toxicity demonstrated by both increased ^{51}Cr-EDTA[30] permeability and ulceration.[31] Our animal data suggest that KT is devoid of significant intestinal effects.

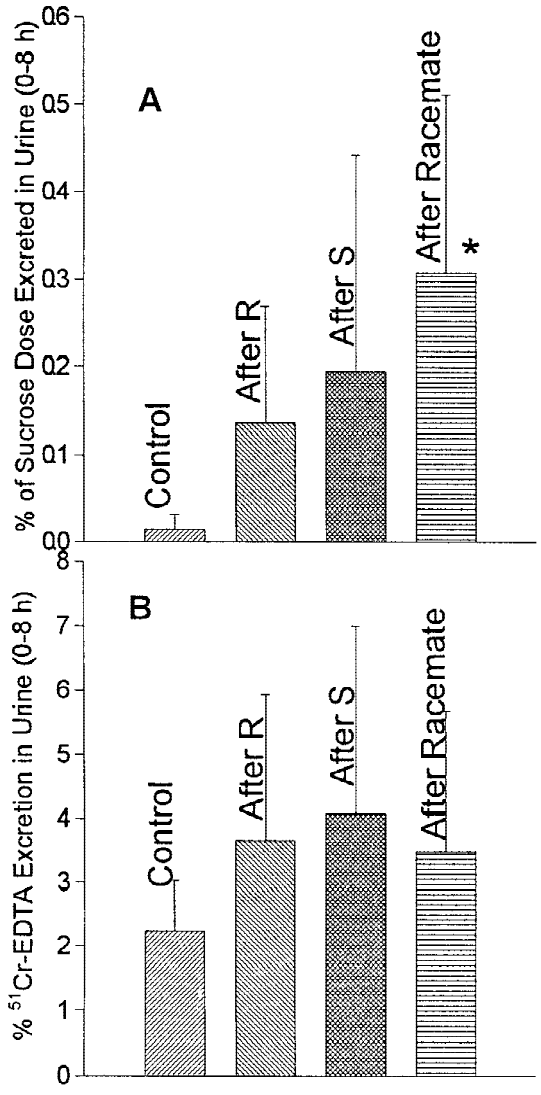

Fig. 3. Changes to GI permeability to sucrose **(A)** and ^{51}Cr-EDTA **(B)** in animals administered R and S KT (2.5 mg/kg) racemic KT (5 mg/kg) and controls; mean ± SD, n = 6/group; *, significantly different from control.

By using ^{51}Cr-EDTA as a surrogate marker of NSAID intestinal toxicity, we have demonstrated that, at least in the rat, stereochemically pure S enantiomers of ibuprofen, flurbiprofen, and ketoprofen are not safer than their respective racemate.[32] Using ulceration as an end point, similar observations have been made for flurbiprofen.[33] There is, however, another report that suggests a safer profile for S-flurbiprofen as compared with the racemate.[34] This report[34] has later been contradicted by data from the same laboratory.[33] Similar safety profiles for racemate and S enantiomer are not unexpected since the cyclooxygenase inhibitory activity of chiral NSAIDs, which is respponsible for both antiinflammatory and GI toxicity of these drugs, is ascribed mainly to the S enantiomer. The observation that with KT only the racemate causes elevation of gastroduodenal permeability to a statistically significant level may motivate investigators to examine the use of stereochemically pure S-KT more closely.

In conclusion, the pharmacokinetics of KT is stereoselective. Plasma concentration of the enantiomer with no antiinflammatory effect is significantly higher than its antipode. KT undergoes little or no chiral inversion. The use of surrogate markers of gastroinestinal toxicity provided preliminary evidence that, as compared with 5 mg/kg of the racemate, a 2.5 mg/kg dose of the stereochemically pure S-ketorolac 1) has no advantage with regard to the intestinal toxicity, and 2) it might be somewhat less toxic on the upper GI tract. This has therapeutic significance only if one assumes that R-KT does not contribute to the pharmacological effects of ketorolac. The clinical significance of these findings remains to be tested.

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