Pharmacokinetic difference between S-(+)- and R-(-)-etodolac in rats

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ABSTRACT

AIM: To study whether etodolac enantiomers have pharmacokinetic difference after oral administration . **METHODS:** Fourteen rats, divided into two groups randomly, were orally given S-(+)- or R-(-)-etodolac at a single dose of 20 mg/kg, respectively. Blood samples were collected before and at 5, 10, 20, 30 min and 1, 3, 6, 12, 24, 48, 72 h after treatment. The plasma samples were analyzed with a high-performance liquid chromatographic method. **RESULTS:** The calibration curves were linear in the range of 0.5−50.0 mg/L (*r*=0.9999) to S-(+)-etodolac and 2.0−200.0 mg/L (*r*=0.9999) to R-(-)-etodolac, respectively. The main pharmacokinetic parameters of S-(+)- and R-(-)-etodolac were as follows: $t_{1/2(\lambda z)}$ 18±4 h *vs* 19.4±2.2 h, t_{max} 3.3±2.6 h *vs* 4±4 h; C_{max} 29±6 mg/L *vs* 97±14 mg/L, AUC_{0-t} 706±100 h·mg·L^{-1} *vs* 2940±400 h·mg·L^{-1}, CL(s) 0.030±0.006 L·kg^{-1}·h^{-1} *vs* 0.0065±0.0010 L·kg^{-1}·h^{-1} and *V/F* 0.25±0.22 L·kg^{-1} *vs* 0.03±0.05 L·kg^{-1}. There was no significant difference in $t_{1/2(\lambda z)}$, and t_{max} between S-(+)- and R-(-)-etodolac (*P*>0.05). The $C_{max,}$ and AUC_{0-t} of R-(-)-etodolac were markedly higher (*P*<0.05), while the CL(s) and *V/F* were markedly lower than those of S-etolodac (*P*<0.05). **CONCLUSION:** There is pharmacokinetic difference between S-(+)- and R-(-)- etodolac enantiomers in rats after oral administration.

INTRODUCTION

Many drugs have been developed as a racemic mixture (50:50) of the S- and R-enantiomers. Nonsteroidal anti-inflammatory drugs (NSAIDs) of 2-arylpropionate class is an important group of racemic medication. The S-isomer of NSAIDs is generally thought to express pharmacological activity and/or be associated with clinical efficacy[1]. Etodolac (Ultradol® Lodine®) is a chiral NSAID that has been marketed as a racemate.

S-(+)-Etodolac shows almost all the pharmacological activity, while R-(-)-etodolac shows little[2]. It is indicated for the management of pain, and for the management of the signs and symptoms of rheumatoid arthritis and osteoarthritis. As a COX-2 selective inhibitor, etodolac can not only enhance its anti-inflammatory and analgesic activity but also improve patient compliance[3]. Especially etodolac could prevent the development of
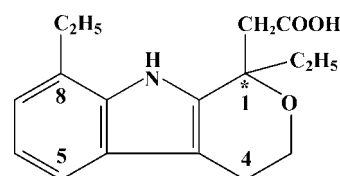
Structure of etodolac.

articular damage and actually cause a regression of lesion[4].

Etodolac has been found to show stereoselective pharmacokinetics in human[5] and in the rat[2]. Brocks *et al*[6] have confirmed this by studying pharmacokinetic difference of etodolac enantiomers after giving racemic etodolac intravenously. However, etodolac are usually used orally. Moreover, etodolac enantiomers may show different pharmacokinetic characters when given individually. So our aim was to study the difference between enantiomers after each enantiomer was given orally.

MATERIALS AND METHODS

Chemicals Etodolac enantiomers [*S*-(+)-etodolac, *R*-(-)-etodolac] were the gifts from Wyeth-Ayerst Research Division (Princeton, NJ). Internal standard (IS) oxaprozin was provided by Shanghai Sanxin Pharmaceutical Co, Ltd. High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were used. Ethyl acetate, hexane, triethylamine, and hydrochloric acid were of analytical grade.

Animals Male Sprague-Dawley rats (Grade II) were provided by the Experimental Animal Center, Fudan University.

Assay Concentrations of *S*-(+)- and *R*-(-)-etodolac were determined in plasma using a RP-HPLC method[7]. The HPLC instrument (Waters) is consisted of a model 510 pump, U6K injector, a variable 486 UV detector (set at 280 nm), and a reverse-phase analytical column (300 mm×3.9 mm ID, 5 µm). The mobile phase was consisted of acetonitrile: water: triethylamine (16:84: 0.002) and was pumped at a flow rate of 1.0 mL/min. Hangzhou Empire Chromatographic Station was used.

Sample preparation Following addition of 50 µL internal standard (100 mg/L) and acidification with 0.08 mL of hydrochloric acid 1.0 mol/L. The constituents of 200 µL of plasma were extracted with 4.0 mL of 5 % ethyl acetate in hexane. The sample was vortex mixed and centrifuged for 10 min. The organic layer was transferred to clean tubes and evaporated to dryness under nitrogen. The residue was redissolved in 50 µL of methanol; aliquots of 10 µL of the solution were injected into the HPLC system.

Dosing and sample collection Fourteen rats were divided into two groups randomly and given etodolac enantiomers orally at a single dose of 20 mg/kg after fast overnight. Venous blood was collected into heparinized tubes before and at 5, 10, 20, 30 min and 1, 3,

6, 12, 24, 48, and 72 h after drug administration. Plasma was separated by centrifugation for 10 min. The specimens were stored at refrigerator until analysis.

Data analysis C_{max} and t_{max} were noted directly from the data. AUC_{0-t} were calculated by the trapezoidal rule up to the last sampling time(t) and the total area ($AUC_{0-\infty}$) was determined by formula: $AUC_{0-\infty}=AUC_{0-t}+C_t/k_{el}$. The k_{el} was calculated by least-squares regression from the data for the last points of the plasma concentration-time curves. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2}=0.693/k_{el}$. The main parameters of *S*-(+)- and *R*-(-)-etodolac were compared by analysis of variance.

RESULTS

Calibration curves Blank plasma samples were spiked with standard solutions of the *S*-(+)- and *R*-(-)-etodolac to reach final concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, and 50.0 mg/L or 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg/L in plasma, respectively. The calibration curve was determined according to the procedure mentioned above. The ratio of area (*Y*) of etodolac enantiomers and IS were linear over their concentration, respectively. Linear equation were $Y=0.0276C-0.0115$ [*S*-(+)-etodolac, 0.5-50.0 mg/L, $r=0.9999$, $n=7$] and $Y=0.0261C-0.0126$ [*R*-(-)-etodolac, 2.0-200.0 mg/L , $r=0.9999$, $n=7$].

The detectable limit of etodolac enantiomers was 0.1 mg/L (S/N>3).

Absolute recovery and relative recovery Plasma samples ($n=5$) with the final concentrations of 1.0, 5.0, 25.0 mg/L for *S*-isomer and 5.0, 25.0, 100.0 mg/L for *R*-isomer were extracted according to the procedure mentioned above. The absolute recovery of *S*-(+)- or *R*-(-)-etodolac was measured by comparison of the areas of etodolac after injection of the extracted sample with those obtained after injection of the standard solution containing equivalent concentrations of the drug. The relative recovery of *S*-(+)- or *R*-(-)-etodolac was determined by comparing the concentrations calculated by calibration curve with those of the standard solution (Tab 1).

Intra-day and inter-day precision Various concentrations of etodolac enantiomers were added into blank plasma ($n=5$). Inter-day and intra-day ($n=5$) statistics including mean and RSD were generated for each standard concentration (Tab 2).

Drug concentration-time curves Plasma concentration-time profiles indicated that marked qualita-

Tab 1.　The absolute recovery and relative recovery of etodolac enantiomers in rat plasma by HPLC determination. *n*=5.

	C/ mg·L^{-1}	Absolute recovery/%	RSD/ %	Relative recovery/%	RSD/ %
S-(+)- etodolac	1.0	71.91	3.06	119.0	2.14
	5.0	74.45	3.11	96.6	1.07
	25.0	75.15	1.88	94.1	1.73
R-(-)- etodolac	5.0	71.18	2.92	106.9	2.99
	25.0	73.47	2.16	94.0	1.93
	100.0	75.42	1.98	100.4	1.71

Tab 2.　The intra-day and inter-day precision of etodolac enantiomers in rat plasma by HPLC determination.　*n*=5.

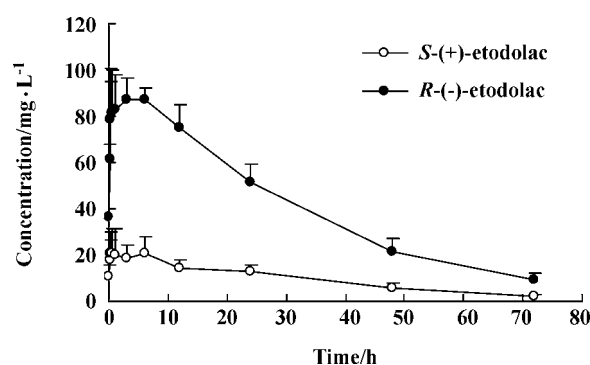|		Intra-day precision		Inter-day precision	
	C/mg·L^{-1}	Observed concentra- tion/mg·L^{-1}	RSD %	Observed concentra- tion/mg·L^{-1}	RSD %
S-(+)-etodolac	1.0	1.19	3.11	1.17	7.08
	5.0	4.83	2.16	4.80	2.92
	25.0	23.53	1.13	23.72	2.33
R-(-)-etodolac	5.0	5.34	2.99	5.08	4.66
	25.0	23.50	1.93	24.18	2.08
	100.0	100.42	1.71	100.16	1.08

Fig 1.　Mean concentration-time curve of etodolac enantiomers in rats following oral administration at a single dose of etodolac 20 mg/kg, respectively.　*n*=7.　Mean±SD.

Tab 3.　Main pharmacokinetic parameters of etodolac enantiomers after ig at a single dose of 20 mg/kg in rats, respectively.　*n*=7.　Mean±SD.　^{a}*P*>0.05, ^{b}*P*<0.05, ^{c}*P*<0.01 *vs* S-(+)-etodolac.

Parameters	*S*-(+)-etodolac	*R*-(-)-etodolac	*S:R*
λ_z/h^{-1}	0.040±0.008	0.036±0.004^a	-
$T_{1/2(\lambda z)}$/h	18±4	19.4±2.2^a	-
t_{max}/h	3.3±2.6	4±4^a	-
V/F(c)/h·kg^{-1}	0.25±0.22	0.03±0.05^b	9.63
CL(s)/L·h^{-1}·kg^{-1}	0.030±0.006	0.0065±0.0010^c	4.63
C_{max}/mg·L^{-1}	29±6	97±14^c	0.30
AUC$_{0-t}$/h·mg·L^{-1}	706±100	2940±400^c	0.24
AUC$_{0-\infty}$/h·mg·L^{-1}	767±119	3203±504^c	0.24
MRT	29±5	28±4	-

tive and quantitative differences existed between *S*-(+)- and *R*-(-)-etodolac after a single dose of 20 mg/kg. Concentrations of inactive *R*-(-)-etodolac were much greater than those of active *S*-(+)-enantiomer.　A second peak developed between 3 and 6 h post-dose on the plasma concentration-time curve of *S*-(+)-etodolac (Fig 1).

Pharmacokinetic parameters　The calculated pharmacokinetic parameters further reflected the marked differences between the enantiomers.　The C_{max} and AUC$_{0-t}$ of *R*-(-)-etodolac were greatly higher (*P*<0.05), while the CL(s) and *V/F* were lower than that of *S*-(+)-enantiomer (*P*<0.05).　There was no significant difference in λ_z, $t_{1/2(\lambda z)}$, and t_{max} between *S*-(+)- and *R*-(-)-etodolac (*P*>0.05, Tab 3).

DISCUSSION

　　Etodolac individual enantiomers had not been developed into a new drug.　So we could not study their stereoselective pharmacokinetics in human.　The rat was the appropriate animal for the pharmacokinetic study of etodolac according to the reference[5].　Due to the actually clinical administration route of etodolac, we chosen intragastric administration that was different from the study of Brocks *et al*[6].　We thought oral administration was more close to the clinical situation in human.

　　Most of pharmacokinetic parameters for etodolac individual enantiomer showed great and interesting difference as compared with those of racemic etodolac[7]. C_{max} (69 mg/L), AUC$_{0-t}$ (2034 mg·h·L^{-1}), and *CL/F* (0.0088 L·h^{-1}·kg^{-1}) of rac-etodolac were close to those of *R*-(-)-etodolac, while *V/F* (0.21 L/kg) was close to that of *S*-(+)-enantiomer.　Moreover, the shape of plasma concentration-time curve of rac-etodolac was similar to that of *S*-(+)-etodolac in the second peak[7].

　　The enantioselective pharmacokinetics of oral administration of individual enantiomers were similar to

those of iv racemic mixture[6,8,9]. The mean S/R ratio for $AUC_{0-72\,h}$ in these rats was 0.24, which was close to 0.31[8] and 0.26[6] in the literatures. The mean S/R ratio for C_{max} was 0.30. These results confirmed that the difference existed in concentration of etodolac enantiomers. Etodolac was unique among the NSAIDs administered as racemate, because the concentrations of active enantiomer following a single dose were much lower than those of inactive enantiomer[10].

The V/F and CL/F of S-(+)-etodolac were 9.6- and 4.6-fold as much as those of R-(-)-etodolac, respectively. Brocks *et al* found those were 12.0 and 6.6 fold in human[9]. S-Enantiomer was extensively distributed *in vivo* and was also easier to be cleaned. That was the reason why S-enantiomer had lower plasma concentration *in vivo*. Several reasons could explain the high V/F and CL(s) of S-(+)-etodolac. Firstly, the stereoselectivity of the pharmacokinetic profile of etodolac found in plasma was different in other tissues. Brocks *et al*[11] found an example in the synovial fluid. After a single dose of etodolac 200 mg, the S-(+)- to R-(-)-concentration ratio in 6 patients with rheumatoid arthritis was 0.074 in plasma, and 0.17 in synovial fluid. The concentrations of the R-etodolac did not differ between synovial fluid and plasma. But, there was a 1.7-fold higher concentration of S-enantiomer in the synovial fluid than in the plasma. In addition, the ratios of S-etodolac concentration in heart, liver, kidney, fat, and brain to that in plasma were higher than R-enantiomer[8]. Secondly, the plasma protein binding of enantiomers was different. Etodolac was mainly binding to albumin[12], and the binding rate was more than 90 %. Etodolac binding to albumin also had stereoselectivity. The R-enantiomer bound to a significantly greater extend than the S-enantiomer to plasma albumin. Thirdly, there was difference in metabolism and biliary clearance. Although nearly 100 % of the administered S-etodolac was recovered in rat bile, only 30 % of R-enantiomer was recovered following administration of racemate. S-(+)-etodolac not only had a greater biliary clearance, but also exhibited extensive entero-hepatic recycling[5]. This attributed to the second peak developed in the plasma of both S-(+)- and racemic etodolac.

Etodolac is a unique and interesting agent due to the stereoselectivity present in its pharmacokinetics.

Because the concentrations of the inactive R-(-)-etodolac were much higher than those of active S-enantiomer, purified S-enantiomer was suggested to be used clinically. Hence, further study including pharmacokinetics of individual S-enantiomer should be done.

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