Determination of Tolperisone Enantiomers in Plasma and Their Disposition in Rats

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A stereoselective assay for the determination of tolperisone enantiomers in plasma by high performance liquid chromatography was developed. Calibration curves obtained for the enantiomers were linear over plasma concentrations of 0.1—3.0 μg/ml with a detection limit of 20 ng/ml. Following intravenous bolus administration of 10 mg/kg of racemic tolperisone to rats, stereoselective disposition of tolperisone enantiomers was observed, and plasma concentrations were significantly higher for *l*-tolperisone than for *d*-tolperisone at 5, 15 and 30 min after administration. When either enantiomer was administered alone to rats, both enantiomers were found in plasma, indicating that a mutual chiral inversion occurs in the body.

Keywords tolperisone; enantiomer; stereoselective disposition; chiral inversion; HPLC

Tolperisone (2,4′-dimethyl-3-piperidinopropiophenone) is used in the treatment of various spastic paralyses. Tolperisone contains a chiral center existing as a racemate, and is marketed as such. It is reported that the tolperisone enantiomers have different pharmacodynamic properties, *i.e.*, the *d*-tolperisone induces central skeletal muscle relaxation, while the *l*-tolperisone causes either peripheral vasodilatation or bronchodilation.[1] However, there are no reports available on the disposition of these tolperisone enantiomers. This is perhaps due to the lack of a rapid and sensitive assay. While resolution of tolperisone enantiomers by high performance liquid chromatography (HPLC) was reported, these analyses were done in the absence of plasma.[2] It is necessary to determine the enantiomer concentrations in biological fluid in order to evaluate their pharmacokinetic profiles. The present paper describes the determination of tolperisone enantiomers in plasma by HPLC and drug disposition in rats.

Experimental

Materials Racemic tolperisone was supplied by Nippon Kayaku Co., Ltd. Standard tolperisone enantiomers were prepared by fractional crystallization of diasteroisomeric salts with optically active acetyl phenylglycine according to the method of Furuta *et al.*[1] The purity of both *d*- and *l*-enantiomers was over 99%, as measured in our laboratory using the method described below. Oxprenolol hydrochloride (internal standard for HPLC) was extracted from the commercially available preparation (Trasacor®, Ciba-Geigy Co., Ltd.). All other chemicals were of analytical reagent grade.

Instrumentation and Chromatographic Conditions An HPLC (Shimadzu, LC-6A) equipped with a spectrophotometric detector (Shimadzu, SPD-6A) and a data processor (Shimadzu, C-R5A) was used with a SUMIPAX OA-4500 column (5 μm, 4 mm i.d. × 25 cm, Sumika Chemical Analysis Service, Ltd.). The HPLC mobile phase was *n*-hexane–chloroform–methanol–acetic acid (200 : 15 : 5 : 2; v/v/v/v) at a flow rate of 1 ml/min and the wavelength for detection was 254 nm. The mobile phase was deaerated ultrasonically before use and the analysis was carried out at room temperature.

Drug Administration Male Wistar strain rats (Japan SLC Inc., Shizuoka), weighing 170—230 g each, were used. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, the left carotid artery was cannulated with polyethylene tubing (PE 50, Becton Dickenson Co., Ltd.) which was passed subcutaneously to the back of neck to obtain blood samples. Rats were fasted overnight before drug administration but allowed free access to water. Racemic tolperisone or individual enantiomers dissolved in saline were administered to rats at a dose of 10 or 5 mg/kg, respectively, by intravenous bolus injection through the tail vein. Blood samples were drawn from the carotid artery cannula at the appropriate times and the plasma was immediately separated by centrifugation at 14000 rpm for 1 min at room temperature. The plasma fractions (200 μl) were transferred to a 10 ml glass-stoppered centrifuge tube, 0.1 ml of methanol was immediately added, and then these were stored at −80 °C until the time of assay.

Extraction Procedure To each 10 ml centrifuge tube containing plasma samples, 0.3 ml distilled water, 0.1 ml of methanol solution containing 50 μg/mg of oxprenolol hydrochloride (internal standard), 0.2 ml of 0.1 M phosphate buffer (pH 7.4) and 4.5 ml of diethyl ether were added and mixed. The tube was mechanically shaken for 5 min and then centrifuged at 3000 rpm for 5 min at room temperature. A 4.0 ml volume of organic phase was transferred into another centrifuge tube and evaporated to dryness under a stream of nitrogen at 0 °C. The residue was reconstituted with 150 μl of chloroform and 50 μl of the solution was injected into the HPLC column.

***In Vitro* Chiral Inversion Studies** *d*- or *l*-tolperisone was dissolved to a concentration of 3 μg/mg in saline, rat plasma and blood samples freshly collected, and these were incubated at 37 °C. Samples (200 μl) were drawn at the appropriate times and transferred to a 10 ml glass-stoppered tube containing 0.1 ml methanol. Extraction of tolperisone was carried out in a similar manner as above.

Results and Discussion

Resolution of Enantiomers by HPLC Figure 1 shows the chromatograms of drug-free plasma and plasma samples after administration of racemic tolperisone. There were no interfering peaks due to endogenous plasma components and each peak was well separated (separation factor: $\alpha = 1.66$). Retention times under these conditions

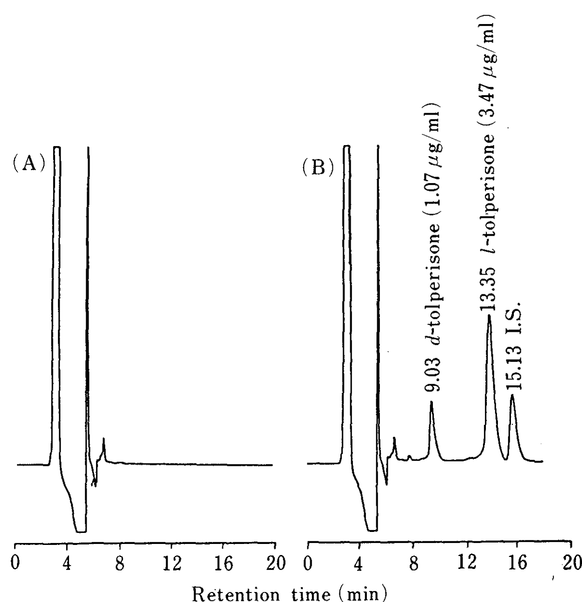

Fig. 1. Chromatograms of Tolperisone Enantiomers in Rat Plasma

(A) Blank. (B) After administration of racemic tolperisone.

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were 9.0 and 13.3 min for *d*- and *l*-tolperisone, respectively, and 15.1 min for the internal standard. Calibration curves were generated by least-squares linear regression analysis. The ratios of the peak area of the enantiomers to that of the internal standard showed excellent linearity in the concentration range of 0.1—3.0 μg/ml. The regression equations of the calibration curves were $Y=1.63X+0.021$ ($r=0.999$, $p<0.01$), $Y=1.42X+0.003$ ($r=0.999$, $p<0.01$) for *d*- and *l*-tolperisone in plasma, rspectively. The detection limit in plasma was 20 ng/ml for each enantiomer.

Reproducibility of the tolperisone enantiomer assay was determined at four plasma drug concentrations (0.1, 0.5, 1.0 and 3.0 μg/ml). The coefficient of variation for the enantiomers ranged from 0.66 to 3.99%. The extraction efficiency was determined in a spiked plasma sample by comparing the peak area ratios of th enantiomer before and after extraction, which were about 80%. No chiral inversion was observed during these extraction and resolution procedures. From these results, the present HPLC method is suitable for the determination of tolperisone enantiomers in plasma.

Disposition of Tolperisone Enantiomers in Rats Figure 2 shows the plasma concentration–time curves of tolperisone enantiomers after intravenous bolus injection of 10 mg/kg racemic tolperisone to rats. The time courses of *d*- and *l*-tolperisone in plasma showed stereoselectivity. Significantly higher concentrations of *l*-tolperisone were detected at 5, 15 and 30 min after administration as compared with those of *d*-tolperisone ($p<0.001$, $p<0.001$ and $p<0.01$, respectively). These plasma levels then decreased in a pattern approximately similar to that of *d*-tolperisone.

The mean pharmacokinetic parameters are presented in Table I. Area under the concentration–time curve (*AUC*) from 0 to 6 h was calculated by the trapezoidal rule for each enantiomer. The *AUC* from 6 h to infinity was calculated using the slope obtained from least-squares regression of the terminal phase. Clearance (*Cl*) was calculated by dividing the dose (5 mg/kg) by $AUC^{0\to\infty}$. The *l*-enantiomer *AUC* was about 1.8 fold higher than that of the *d*-enantiomer. The mean Cl was 4.83 l/h/kg for the *d*-enantiomer and 3.04 l/h/kg for the *l*-enantiomer. Pharma-

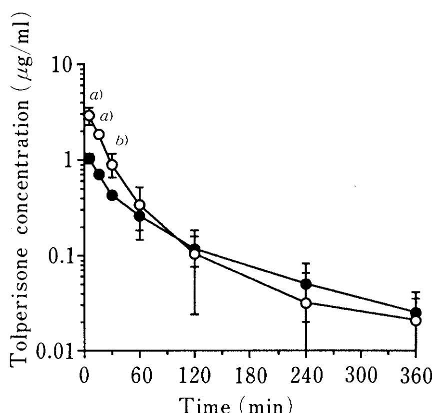

Fig. 2. Time Courses of Tolperisone Enantiomers after Intravenous Administration of Racemic Tolperisone (10 mg/kg) in Rats

—○—, *l*-tolperisone; —●—, *d*-tolperisone. Each point represents the mean ± S.D. of five rats. *a*) and *b*) $p<0.001$ and $p<0.01$ significantly different from *d*-tolperisone, respectively.

Table I. Pharmacokinetic Parameters after Intravenous Administration of *dl*-, *d*- or *l*-Tolperisone in Rats

	Parameters		*d*-Tol	*l*-Tol
dl-Tol	$AUC^{0\to6}$	(μg·h/ml)	0.96±0.137	1.60±0.453[a]
	$AUC^{0\to\infty}$	(μg·h/ml)	1.07±0.200	1.77±0.467[a]
	Cl	(l/h/kg)	4.83±0.778	3.04±0.853[a]
d-Tol	$AUC^{0\to6}$	(μg·h/ml)	1.11±0.186	0.33±0.146
	$AUC^{0\to\infty}$	(μg·h/ml)	1.35±0.322	—
	Cl	(l/h/kg)	3.92±0.852	—
l-Tol	$AUC^{0\to6}$	(μg·h/ml)	0.26±0.053	1.95±0.635
	$AUC^{0\to\infty}$	(μg·h/ml)	—	2.01±0.616
	Cl	(l/h/kg)	—	2.74±0.881

Each value represents the mean ± S.D. of five rats. *d*-Tol, *d*-tolperisone; *l*-Tol, *l*-tolperisone; —, not calculated. *a*) $p<0.05$ significantly different from *d*-Tol.

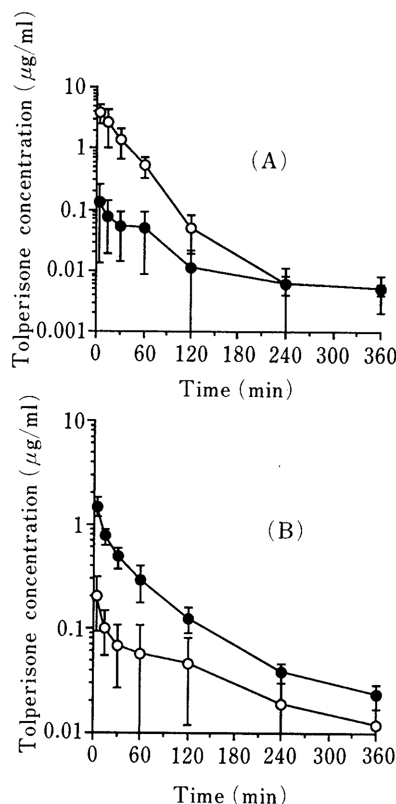

Fig. 3. Time Courses of Tolperisone Enantiomers after Intravenous Administration of *d*- or *l*-Tolperisone

(A) After administration of *l*-tolperisone (5 mg/kg). (B) After administration of *d*-tolperisone (5 mg/kg). —○—, *l*-tolperisone; —●—, *d*-tolperisone. Each point represents the mean ± S.D. of five rats.

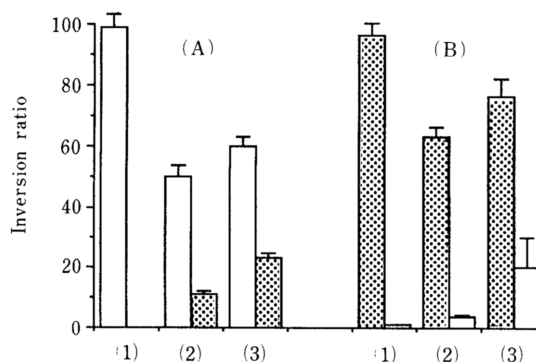

Fig. 4. Inversion Ratios of Tolperisone Enantiomer *in Vitro*

(A) *d*- to *l*-tolperisone. (B) *l*- to *d*-tolperisone. ▩, *l*-tolperisone; □, *d*-tolperisone. (1), in saline; (2), in plamsa; (3), in blood. Each bar represents the mean ± S.D. of three experiments.

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cokinetic differences between drug enantiomers are generally believed to be caused by stereoselective differences in liver metabolism and/or plasma protein binding.[3] We are now studying the stereoselective characteristics of tolperisone enantiomers.

It is also well known that several chiral 2-arylpropionic acid nonsteroidal anti-inflammatory drugs undergo chiral inversion in the body, *i.e.*, *R*-enantiomers are inverted to the respective *S*-enantiomers.[4]

Figure 3 shows the plasma concentration–time curves of tolperisone enantiomers after intravenous bolus administration of 5 ml/kg. When *d*-tolperisone was administered alone, its plasma profile was similar to that after bolus injection of 10 mg/kg racemic tolperisone, while its antipode was detected in plasma immediately after dosing. After administration of *l*-tolperisone alone, a similar trend was also observed. The appearance of the antipode in plasma was not due to enantiomeric impurity of the samples administered, since the purity of both enantiomers used in this study was over 99%.

In addition, Fig. 4 shows the ratio of chiral inversion *in vitro* at 6 h after incubation. Although the chiral inversion did not occur until after 6 h in saline solution, its inversion was observed in plasma and blood. In particular, about 20% of each enantiomer inverted to its antipode in blood.

From these results, the enantiomers appear to undergo a mutual chiral inversion in the body, but the bioinversion mechanisms involved are not yet clear.

In conclusion, the present assay which we have developed for the determination of tolperisone enantiomers is rapid and sensitive, providing evidence that tolperisone disposition is stereoselective in rats.

Acknowldgements The authors are grateful to Nippon Kayaku Co., Ltd. for the gift of racemic tolperisone and to Mr. Y. Matumoto of Sumika Chemical Analysis Service, Ltd. for helpful suggestions about resolution of tolperisone enantiomers.

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