J. Pharm. Pharmacol. 1992, 44: 44–47
Received March 6, 1991

The Optical Resolution of Racemic Chlorpheniramine and Its Stereoselective Pharmacokinetics in Rat Plasma

EIICHI SAKURAI, SEIJI YAMASAKI, YUKISUMI IIZUKA, NOBORU HIKICHI AND HIROSHI NIWA

Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-Chome, Aoba-ku, Sendai 981, Japan

Abstract—An ovomucoid-conjugated column has been developed for the chiral stationary-phase liquid chromatographic resolution of racemic chlorpheniramine with a quantitation limit of 0·05 μg mL^{-1}. The assay was used to study the stereoselective kinetics of chlorpheniramine enantiomers in rats. After bolus intravenous administration of racemic chlorpheniramine maleate (20 mg kg^{-1}), plasma concentration of the (−)-form was higher than that of the (+)-form. In the elimination phase, the concentrations of (+)- and (−)-chlorpheniramine in the plasma declined biexponentially with half-lives of 18·2 and 50·0 min, respectively. Although there was no significant difference in blood-to-plasma concentration ratio of both enantiomers, the apparent total blood clearance of (+)-chlorpheniramine was twice as large as that of the (−)-isomer. Binding of (−)-chlorpheniramine to rat plasma protein was stronger than that of (+)-chlorpheniramine suggesting stereoselective pharmacokinetics may be due to a difference in the plasma protein binding.

Chlorpheniramine (Chp) is a widely used potent histamine H_1-receptor blocker with two optical isomers (Nauta & Rekker 1978). (+)-Chp has been shown to be some 200 times more effective than its enantiomer in-vivo in protecting guinea-pig against histamine (Roth & Govier 1958). Moreover, in studies of inhibition of [^{3}H]mepyramine binding in-vitro and in-vivo in guinea-pig brain, (+)-Chp was 100-fold more active than the (−)-isomer (Hill et al 1978). On the other hand, this compound may be a potential antiparkinsonian agent besides the antihistamines, as Chp significantly decreased levels of 3,4-dihydroxyphenylacetic acid (dopac), although it had no effect on the levels of dopamine in mouse brain (Shishido et al 1991). However, it was previously reported that there is a lack of stereospecificity in reducing dopac levels, suggesting a lack of stereospecificity in inhibition of dopamine uptake in the regional parts of rats (Sakurai et al 1991). Thus, those studies suggested stereoselectivity in the disposition of the drug.

The intention of this study was to investigate stereoselective relations between plasma kinetics and plasma protein binding in rats.

Material and Methods

Materials

(±)-Chp maleate (Kowa, Nagoya, Japan), (+)-Chp maleate (Yoshitomi Pharmaceutical Industry, Osaka, Japan) and (−)-Chp maleate (Schering Corporation, New Jersey, USA) were kindly supplied from their respective companies. All other reagents were of the highest grade available.

Animal experiments

Male Wistar rats, 250–300 g (Japan SLC Inc., Hamamatsu, Japan) were starved for 18 h before the experiments and were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). (±)-Chp maleate in 0·9% NaCl (saline) was rapidly injected into the femoral vein at a dose of 20 mg kg^{-1} (14·1 mg kg^{-1} of free base equivalent). Blood samples were collected by cardiocentesis into heparinized-vacutainers at 2, 5, 15, 30, 60 and 90 min after drug administration. The plasma was promptly separated by centrifugation at 3000 rev min^{-1} for 10 min. The plasma was deproteinized in an ice bath in 2 vol of ice-cold 0·4 M perchloric acid. The deproteinized sample was centrifuged at 10 000 rev min^{-1} for 20 min at 4°C, and the supernatant stored at −20°C until assayed.

Correspondence: E. Sakurai, Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-Chome, Aoba-ku, Sendai 981, Japan.

Analytical methods

The concentrations of Chp enantiomers in the plasma were determined by HPLC as follows: supernatant (1 mL) was mixed with 1 mL of 5 M sodium hydroxide and 7 mL of benzene in a centrifuge tube. The mixture was vigorously shaken for 10 min and centrifuged for 10 min at 3000 rev min^{-1}. Five mL of the organic layer was evaporated to dryness under reduced pressure. The residue was redissolved in 100 μL of acetonitrile—0·05 M phosphate buffer (pH 5·6) (9:1, v/v) and a 20 μL sample was injected onto the HPLC-column. The HPLC was carried out with a L-5000 (Yanagimoto, Kyoto, Japan) apparatus equipped with a UV detector (UV-8 model II, Tosoh) and an ovomucoid-conjugated column (Ultron ES-OVM, 150 × 4·0 mm i.d., Shinwa Kako, Kyoto, Japan). Material was eluted with acetonitrile—0·05 M phosphate buffer (pH 5·6) (9:1 v/v) at a flow rate of 1·0 mL min^{-1} at 30°C, and the absorbance at 228 nm was measured. The retention times of (−)- and (+)-Chp were 11·9 and 17·8 min, respectively.

In-vitro plasma protein binding

Normal plasma (0·9 mL) was incubated with 0·1 mL of (±)-Chp solution in 0·01 M phosphate buffer (pH 7·4) at a final concentration of 20 to 400 μM for 20 min at 37°C (Ueda et al 1988). After ultrafiltration using an Ultracent-10 membrane (Tosoh, Tokyo, Japan), the unbound concentrations of (+)- and (−)-Chp were determined as described in the analytical methods. Protein concentrations were estimated by the method of Lowry et al (1951).

Blood-to-plasma concentration ratio

The whole blood was collected via a jugular artery 30 min after injection of heparin at a dose of 0·1 mL/100 g (100 units) (Terasaki et al 1982). Small samples (10 μL) of isotonic solutions containing various amounts of (±)-Chp (50 to 400 μM) were added to test tubes containing 2·0 mL of blood preincubated for 3 min at 37°C. The tubes were incubated with shaking (2 Hz) for 5 min at 37°C. The plasma was then separated by centrifugation at 3000 rev min^{-1} for 10 min and the concentrations of both enantiomers in the plasma were determined. Metabolism during incubation was negligible; no decrease was observed in the plasma concentrations of (+)- or (−)-Chp up to 30 min. There was also no chiral inversion.

Data analysis

Plasma concentration-time curves were analysed by a two-compartment model according to the non-linear least-squares regression analysis program MULTI for biexponential decline (Yamaoka et al 1981). The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were calculated by standard linear trapezoidal integration with extrapolation to infinite time. The binding data were also fitted using a non-linear least-squares analysis program MULTI (Yamaoka et al 1981). Values are presented as mean ± s.e. of means for n experiments and enantiomeric differences were analysed for their significance by Student's *t*-test.

Results

Simultaneous determination of Chp enantiomers in plasma

Fig. 1 shows chromatograms of benzene extracts of plasma obtained 30 min after rapid intravenous injection of saline (A) or racemic Chp (B). There are no interfering peaks in the region with a retention time corresponding to that of (−)-Chp (11·9 min) or (+)-Chp (17·8 min) in the chromatogram. Each enantiomer peak is symmetrical and well separated from other peaks. The calibration curve for each enantiomer was linear over a range from 0·05 to 10 μg mL^{-1} with a correlation coefficient of more than 0·99 (n = 8) (data not shown), and the lower limit for quantitation was 0·05 μg mL^{-1}.

Time course of decrease in plasma concentration of Chp

Fig. 2 shows the time course of changes in plasma concentration of each enantiomer after rapid intravenous injection of racemic Chp maleate (20 mg kg^{-1}). The pharmacokinetic parameters of Chp enantiomers are listed in Table 1.

In-vitro plasma protein binding

The unbound fractions (f_u) of (+)- and (−)-Chp in rat plasma were 0.15–0.29 and 0·11–0·25 at a concentration of 25 to 200 μM, respectively. The Scatchard plots (Fig. 3) of both enantiomers binding to rat plasma protein revealed a high- and a low-affinity component. The binding parameters are listed in Table 2. The dissociation constant of (+)-Chp in the high-affinity component was higher than that of (−)-Chp. The numbers of binding sites (n_2) of (+)- and (−)-Chp in the low-affinity component for binding to rat plasma protein,

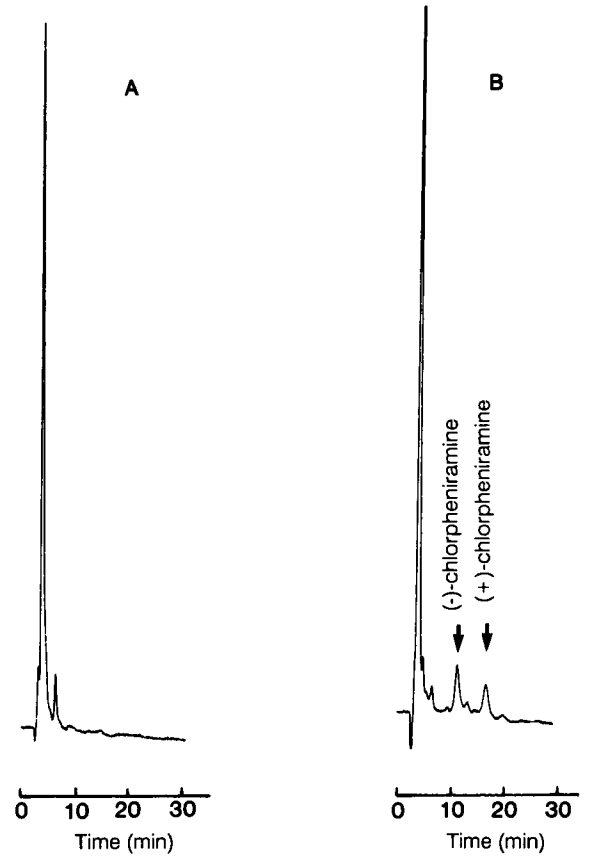

FIG. 1. Chiral stationary-phase liquid chromatograms of extracted samples of plasma of rats after i.v. injection of racemic chlorpheniramine. (±)-Chlorpheniramine (14·1 mg kg^{-1} as the free base) in saline (500 μL kg^{-1}) was injected i.v.; 30 min later the rats were killed and the blood was obtained. Control rats were treated with saline only. Plasma was deproteinized with 0·4 M perchloric acid, and chlorpheniramine enantiomers in the extracts were determined by HPLC as described in Materials and Methods. A, control samples; B, racemic chlorpheniramine-treated samples.

determined by ultrafiltration, were found to be 4·92 and 7·39 nmol (mg prot)$^{-1}$, respectively. No inhibition of (+)-Chp binding by (−)-Chp or inhibition of (−)-Chp binding by (+)-Chp to plasma protein was observed.

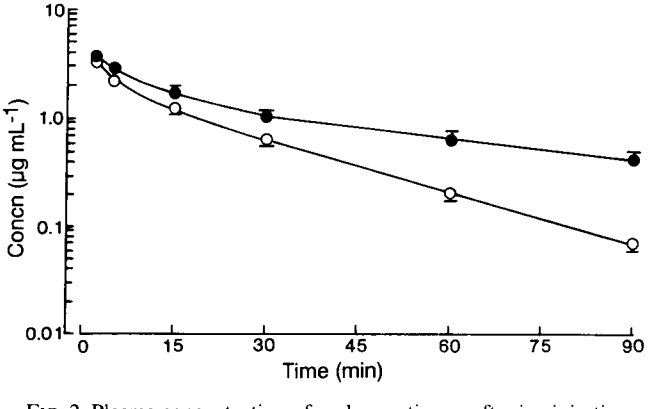

FIG. 2. Plasma concentration of each enantiomer after i.v. injection of racemic chlorpheniramine maleate (20 mg kg^{-1}) into rats. Conditions were as for Fig. 1 except that blood was obtained at the indicated times after injection of racemic chlorpheniramine. Each point is the mean ± s.e. of 5 experiments. ○, (+)-enantiomer; ●, (−)-enantiomer.

Table 1. Plasma pharmacokinetic parameters of each enantiomer after rapid intravenous administration of racemic chlorpheniramine maleate (14·1 mg kg^{-1} of free base equivalent) to rats.

Parameter	Meaning	Value (+)-enantiomer	Value (−)-enantiomer	Unit
A	Defined in the equation below	3·03 ± 0·41	2·74 ± 0·16	$\mu g\ mL^{-1}$
B	Defined in the equation below	2·14 ± 0·03	1·50 ± 0·18[a]	$\mu g\ mL^{-1}$
α	Defined in the equation below	0·411 ± 0·099	0·123 ± 0·024[a]	min^{-1}
β	Defined in the equation below	0·038 ± 0·001	0·014 ± 0·003[a]	min^{-1}
k_{12}	Transfer rate constant from C* to P*	0·178 ± 0·061	0·050 ± 0·009[a]	min^{-1}
k_{21}	Transfer rate constant from P* to C*	0·191 ± 0·030	0·053 ± 0·015[a]	min^{-1}
k_{e1}	Elimination rate constant from C*	0·080 ± 0·008	0·037 ± 0·004[a]	min^{-1}
V_1	Volume of distribution of C*	1369·2 ± 118·1	1658·9 ± 8·2[a]	$mL\ kg^{-1}$
V_2	Volume of distribution of P*	1221·6 ± 133·6	1611·7 ± 178·9[a]	$mL\ kg^{-1}$
V_{ss}	Steady state volume of distribution (V_1+V_2)	2590·8 ± 15·5	3270·6 ± 187·1[a]	$mL\ kg^{-1}$
$t_{\frac{1}{2}\alpha}$	Half-time of distribution phase	1·8 ± 0·5	5·9 ± 1·2[a]	min
$t_{\frac{1}{2}\beta}$	Half-time of elimination phase	18·2 ± 0·3	50·0 ± 9·4[a]	min
AUC	Area under the plasma concentration-time curve	61·2 ± 3·4	119·9 ± 5·4[a]	$\mu g\ min\ mL^{-1}$
MRT	Mean residence time	23·4 ± 1·6	52·0 ± 0·9[a]	min

A bolus dose of racemic Chp maleate was administered intravenously and the plasma Chp enantiomer concentrations were measured for 90 min at the times shown in Fig. 2. The data were fitted by the equation, $C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ for the plasma concentration, C_t, at time t, and pharmacokinetic parameters (average of 4–5 experiments) were calculated as described in the Method. C* and P* represent the central and peripheral compartment, respectively. [a] $P < 0.01$.

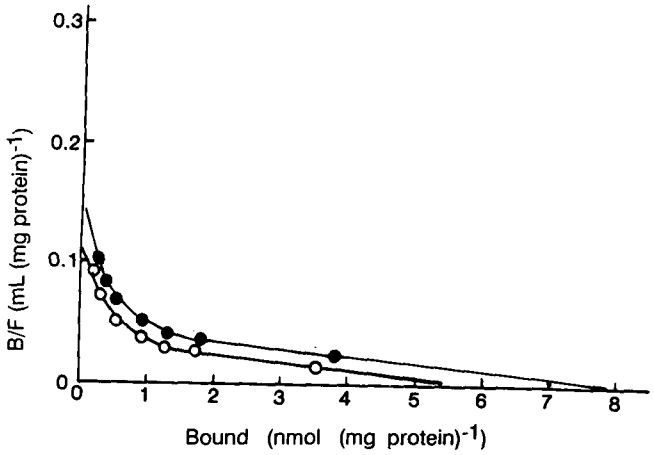

FIG. 3. Scatchard plots of racemic chlorpheniramine binding to rat plasma protein. Each point represents the mean for 4 experiments. ○, (+)-enantiomer; ●, (−)-enantiomer.

In-vitro blood-to-plasma concentration ratio

The values of 1·38 ± 0·30 and 1·50 ± 0·21 (n = 5) were obtained for the blood-to-plasma concentration ratios (R_B) of (+)- and (−)-Chp, respectively (Table 3), and no concentration dependency was observed in the blood concentration range from 69 to 550 ng mL^{-1} of each enantiomer.

The apparent total blood clearance ($CL_{tot.B.app}$) can be expressed as

$$CL_{tot.B.app} = \frac{Dose}{R_B AUC_p} \quad (1)$$

Table 3. Blood-to-plasma concentration ratio and apparent total blood clearance of racemic chlorpheniramine.

	R_B	$CL_{tot.B.app}$ (mL min^{-1} kg^{-1})
(+)-Enantiomer	1·38 ± 0·30	83·5 ± 4·7
(−)-Enantiomer	1·50 ± 0·21	39·2 ± 1·8*

Each result represents the mean ± s.e. of 5 experiments. R_B, blood-to-plasma concentration ratio; $CL_{tot.B.app.}$, apparent total blood clearance. * $P < 0.01$.

Using R_B and the parameters listed in Table 1 $CL_{tot.B.app}$ of (+)- and (−)-Chp were calculated to be 83·5 ± 4·7 and 39·2 ± 1·8 mL min^{-1} kg^{-1}, respectively.

Discussion

Several studies have demonstrated the disposition of Chp (Miyazaki & Abuki 1976; Vallner et al 1979; Huang & Chiou 1981; Thompson & Leffert 1980; Kotzan et al 1982; Rumore 1984). Miyazaki & Abuki (1976) reported the stereochemical differences in the disposition of this drug using mass fragmentography with the aid of the stable isotope technique in healthy subjects. However, this technique requires one enantiomer to be labelled previously with stable isotope. In this study, we have established a simple method for the chiral stationary-phase liquid chromatographic resolution of racemic Chp using an ovomucoid-conjugated column with a quantitation limit of 0·05 $\mu g\ mL^{-1}$; the stereoselective

Table 2. Binding parameters of racemic chlorpheniramine to rat plasma protein.

	Kd_1 (μM)	n_1 (nmol (mg protein)$^{-1}$)	n_1/Kd_1 (mL (mg protein)$^{-1}$)	Kd_2 (μM)	n_2 (nmol (mg protein)$^{-1}$)	n_2/Kd_2 (mL (mg protein)$^{-1}$)
(+)-Enantiomer	9·38 ± 0·36	0·54 ± 0·03	0·058 ± 0·006	143·9 ± 8·6	4·92 ± 0·11	0·035 ± 0·001
(−)-Enantiomer	7·20 ± 0·31*	0·70 ± 0·002*	0·098 ± 0·004*	162·6 ± 2·4	7·39 ± 0·06**	0·046 ± 0·001**

Each result represents the mean ± s.e. of 4 experiments. Kd_1, Kd_2: dissociation constants, n_1, n_2: number of binding sites, n_1/Kd_1, n_2/Kd_2: binding. * $P < 0.05$, ** $P < 0.01$.

pharmacokinetics of Chp in rat plasma could then be measured.

Our results indicate stereoselectivity in the disposition of Chp after intravenous injection of racemic Chp in rat and suggest that this stereospecificity may be due to a difference in rat plasma protein binding. The low affinity components of plasma protein binding of the enantiomers will be negligible under the conditions of the pharmacokinetic study as the peak plasma concentrations were only about 10 μM.

However, Fujiwara et al (1989) reported that in dogs there was no difference in the plasma levels of Chp when (+)- or (−)-Chp was injected intravenously, but plasma levels of (+)-Chp brought about by oral administration of (+)-Chp were evidently higher than those from (−)-Chp. Those authors considered that this discrepancy was due neither to a difference in the elimination rate nor a distribution volume, but rather due to a first-pass effect in the absorption process. It has also been shown that in man the concentration of (+)-Chp after simultaneous oral administration of hexadeuterium-labelled (+)-Chp and non-labelled (−)-Chp was higher than that of (−)-isomer and the biological half-lives were 24 and 15 h, respectively (Miyazaki & Abuki 1976). Although the discrepancy in pharmacokinetics of Chp enantiomers may be explained by interspecies differences, further investigation will be necessary on plasma protein binding and metabolism of both enantiomers to elucidate the stereoselective pharmacokinetics of Chp.

References

Fujiwara, K., Iwamoto, K., Kawai, S., Sakamoto, T. (1989) Behavior of chlorpheniramine in vivo after administration of d- and l-chlorpheniramine maleate. Yakugaku Zasshi 109: 59–64

Hill, S. J., Emson, P. C., Young, J. M. (1978) The binding of [^{3}H]mepyramine to histamine H1 receptors in guinea-pig brain. J. Neurochem. 31: 997–1004

Huang, S. M., Chiou, W. L. (1981) Pharmacokinetics and tissue distribution of chlorpheniramine in rabbits after intravenous administration. J. Pharmacokinet. Biopharm. 9: 711–723

Kotzan, J. A., Vallner, J. J., Stewart, J. T. (1982) Bioavailability of regular and controlled-release products. J. Pharm. Sci. 71: 919–923

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randal, R. J. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Miyazaki, H., Abuki, H. (1976) Mass fragmentographic determination of d- and l-chlorpheniramine with aid of the stable technique. Chem. Pharm. Bull. 24: 2572–2574

Nauta, W. T., Rekker, R. F. (1978) Structure-activity relationship of H1-receptor antagonists. In: Rocha, E., Silva, M. (eds) Handbook of Experimental Pharmacology; Histamine II and Antihistaminics. Springer-Verlag, Berlin, pp 234–239

Roth, F. E., Govier, W. M. (1958) Comparative pharmacology of chlorpheniramine (chlor-trimeton) and its optical isomers. J. Pharmacol. Exp. Ther. 124: 347–349

Rumore, M. M. (1984) Clinical pharmacokinetics of chlorpheniramine. Drug Intell. Clin. Pharm. 18: 701–707

Sakurai, E., Yamasaki, S., Hikichi, N., Onodera, K. (1991) Effects of d-, l- and dl-chlorpheniramine on dopamine and 3,4-dihydroxyphenylacetic acid levels in the regional parts of rat brain. Tohoku J. Exp. Med. 163: 239–244

Shishido, S., Oishi, R., Saeki, K. (1991) In vivo effects of some histamine H1-receptor antagonists on monoamine metabolism in the mouse brain. Naunyn Schmiedebergs Arch. Pharmacol. 343: 185–189

Terasaki, T., Iga, T., Sugiyama, Y., Hanano, M. (1982) Experimental evidence of characteristic tissue distribution of adriamycin. Tissue DNA concentration as a determinant. J. Pharm. Pharmacol. 34: 597–600

Thompson, J. A., Leffert, F. H. (1980) Sensitive, GLC-mass spectrometric determination of chlorpheniramine in serum. J. Pharm. Sci. 69: 707–710

Ueda, T., Uchida, E., Kobayashi, S., Oguchi, K., Yasuhara, H. (1988) Studies on the binding of quinidine, disopyramide and lidocaine to purified human α_1-acid glycoprotein. Xenobio. Metabol. Dispos. 3: 739–746

Vallner, J. J., Needham, W. C., Viswanathan, C. T. (1979) Intravenous administration of chlorpheniramine to seven subjects. Curr. Ther. Res. 26: 449–453

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885