Method for Optical Resolution of Racemic Homochlorcyclizine and Comparison of Optical Isomers in Antihistamine Activity and Pharmacokinetics

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A method was developed for semi-preparative scale enantioseparation of racemic homochlorcyclizine (HCZ) by high performance liquid chromatography (HPLC) on Chiralcel OD column. The best resolution was achieved using an eluent composed of *n*-hexane plus 0.2 M isopropylamine. By this method, about 5.0 mg of racemic HCZ could be resolved completely in one run. The optical purity of the enantiomers were both greater than 99.9%.

The studies of antihistamine activity on guinea pig ileum demonstrated that *l*-HCZ is significantly more potent than *d*- and racemic HCZ. The pharmacokinetics of *d*- and *l*-HCZ after oral administration to rats also differed.

The successful resolution of racemic HCZ permits comparison of the pharmacokinetics and antihistamine activity of the enantiomers.

Keywords homochlorcyclizine; optical resolution; HPLC; enantioselectivity; pharmacokinetics; rat; antihistamine activity; guinea pig

Introduction

Most drugs are used as racemates, *i.e.* mixtures of the enantiomers. However, it is gradually becoming clear that the enantiomers frequently differ in pharmacological action and/or pharmacokinetic properties.[1-3] For example, chlorpheniramine maleate, which is an antihistamine, is marketed both as a racemate and the *d*-isomer. This is because the *d*-isomer is more effective pharmacologically in clinical use than the *l*-isomer[4] and also because stereoselectivity has been observed in pharmacokinetic profiles of dogs[5] and humans.[5,6]

Homochlorcyclizine hydrochloride (HCZ), 1-[(4-chlorophenyl)phenylmethyl]hexahydro-4-methyl-1*H*-1,4-diazepine dihydrochloride, is a potent antihistamine which has been used for many years.[7] It is marketed as a racemate, since a method for separating the enantiomers is not yet available.

This paper describes the semi-preparative resolution of racemic HCZ by high performance liquid chromatography (HPLC) and the pharmacokinetic profiles of *d*- and *l*-HCZ after oral administration to rats. Also reported is a comparison of the antihistamine activity of *d*-, *l*- and racemic HCZ on isolated guinea pig ileum.

Materials and Methods

Materials Racemic HCZ and diphenhydramine hydrochloride (DPH) were purchased from Nippon Bulk Yakuhin Co., Ltd. (Osaka). Histamine dihydrochloride was obtained from Nacalai Tesque, Inc. (Kyoto). All other chemicals were of analytical reagent grade.

Resolution of Racemic HCZ The semi-preparative scale resolution of racemic HCZ with Chiralcel OD (25 cm × 1 cm i.d.) (Daicel, Tokyo) was carried out. The Shimadzu HPLC system (Kyoto) consisted of a LC-6A pump, SCL-6B system controller, SIL-6B autoinjector, FCV-100B fraction collector, CR-4A data processor and SPD-6A ultraviolet detector (265 nm). Chromatography was performed at 25 °C and a flow rate of 2.0 ml/min. The mobile phase was *n*-hexane containing 0.2 M isopropylamine. Free base of HCZ was extracted from alkaline aqueous solution with hexane and loaded on the HPLC. Two fractions with retention times (t_R) of 19 min and 23 min (Fig. 1) were collected separately and evaporated to dryness. The dihydrochloride salt was precipitated with hydrochloride gas in isopropyl alcohol. About 400 mg each of the two enantiomers, as the dihydrochlorides, were prepared from 1 g of racemic HCZ (salt). Their optical rotations were determined using a digital polarimeter (DIP-360, JASCO, Tokyo). The optical purity of the enantiomers was determined by HPLC resolution.

Determination of Antihistamine Activity on Isolated Guinea Pig Ileum Female Hartley strain guinea pigs (4 weeks of age) were used. The ileum was removed and cut preparations were suspended in a 10-ml tissue bath containing Tyrode solution aerated with 95% O_2 and 5% CO_2 and maintained at 37 °C. The ileal muscle preparations were fixed under a resting tension of 1 g and allowed to stabilize for 60 min. Tension changes were recorded isometrically with a force-displacement transducer on an ink-writing recorder (RM-6100, Nihon Koden, Tokyo). The concentration–response curves with histamine in the absence and presence of antagonists were obtained by the cumulative method. The antihistamine activity of each antagonist was expressed as a pA_2 value, calculated by the standard method.[8]

Pharmacokinetic Experiments Male Sprague-Dawley rats (275 ± 14 g) were used. The jugular vein was cannulated.[9,10] Rats were made to fast overnight after operation. They were then given 50 mg/kg of HCZ *via* a stomach tube and were not allowed to take water for 4 h after the administration. Blood samples (0.25 ml) were collected from the jugular vein at regular intervals after administration, and 1 ml of blood sample was collected at last sampling point (8 h). The total sampling volume was 2.5 ml.

Determination of HCZ The blood concentration of HCZ was determined by HPLC. The blood samples were prepared by combining 0.25—1 ml of blood with distilled water to bring the total volume to 1 ml. To this solution, 3 ml of distilled water, 0.2 ml of internal standard solution (DPH 3 μg/ml) and 0.2 ml of 4 N NaOH were added with vigorous mixing. The drug was extracted with 5 ml of *n*-hexane. The extract was evaporated to dryness under reduced pressure at 40 °C and then dissolved in the mobile phase (120 μl). Next 100 μl of the solution was injected into the HPLC. The apparatus employed was a Shimadzu pump LC-6A equipped with an ultraviolet detector (SPD-6A) set at 240 nm. Analyses were performed at room temperature on a Shim-pack CLC-CN column (15 cm × 60 mm i.d.) (Shimadzu). The mobile phase was pH 3 phosphate buffer–acetonitrile (67 : 33). The flow rate was 1.5 ml/min.

Results and Discussion

Resolution of Racemic HCZ Chiralcel OD, a chiral stationary phase of silica-based cellulose tris(3,5-dimethyl-phenylcarbamate), is used for the optical resolution of many racemic compounds.[11-13] Racemic HCZ could be completely resolved using a mixture of *n*-hexane with 0.2 M isopropylamine as an eluent, but not with *n*-hexane–isopropanol or *n*-hexane–ethanol. The chromatogram of racemic HCZ is shown in Fig. 1. The resolution factor[7] was 5.9. Using our method, about 5.0 mg of racemic HCZ could be resolved in one application. The column was

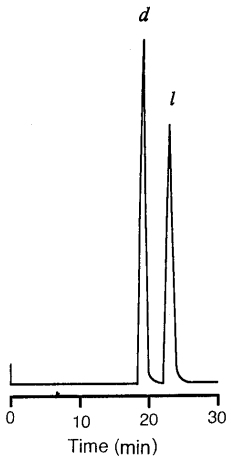

Fig. 1. Chromatographic Resolution of Racemic HCZ on a Chiralcel OD Column

Mobile phase, *n*-hexane containing 0.2 M isopropylamine; flow rate, 2 ml/min; wavelength, 265 nm; temperature, 25°C; sample amount, 2.5 mg as racemic HCZ.

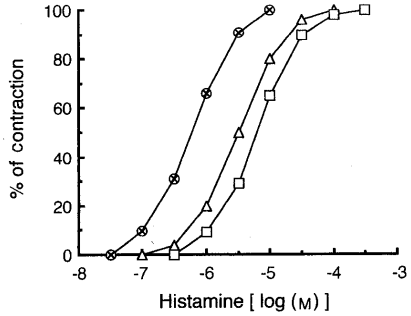

Fig. 2. Concentration–Response Curves to Histamine in the Absence (×) or Presence of *d*- (○), *l*- (□) or Racemic (△) HCZ (3×10^{-9} M Each) in Isolated Guinea Pig Ileum

sufficiently stable for the eluent used during the present work. The first fraction ($t_R = 19$ min) was the *d*-isomer: $[\alpha]_D^{25} = +1.10°$ ($c = 1\%$, H_2O), and the second fraction ($t_R = 23$ min) was the *l*-isomer: $[\alpha]_D^{25} = -1.10°$ ($c = 1\%$, H_2O). The optical purities of both *d*- and *l*-HCZ were greater than 99.9%. No racemization was observed during the preparation, and the enantiomers showed no significant alterations of the optical purity for periods exceeding 5 months. Thus, this method was very useful for the optical resolution of racemic HCZ.

Antihistamine Activity The antihistamine activities of *d*- and *l*- and racemic HCZ were evaluated by determining their inhibitory potencies against histamine-mediated contraction in isolated guinea pig ileum. Figure 2 illustrates the inhibitory effect of *d*-, *l*- and racemic HCZ (3×10^{-9} M each) on concentration–response curves to histamine. In the presence of *l*- and the racemic HCZ (3×10^{-9} M), the concentration–response to histamine indicated significant shifts to the right, respectively. On the other hand, *d*-HCZ showed no effects of shifting on the curve to histamine even in the same concentration as *l*- and racemic HCZ.

The concentration–response curves to histamine shifted in a parallel fashion to the right in the presence of each antagonist at concentrations of 10^{-9} to 10^{-7} M. The pA_2 values of *d*-, *l*- and racemic HCZ were 7.60 ± 0.05, 9.58 ± 0.08 and 9.20 ± 0.06 (mean ± S.E., $n = 5$), respectively. The antihistamine activity of *l*-HCZ was approximately 2.5 times as potent as the racemic HCZ. The activity of *d*-HCZ was about 100-fold less than that of *l*-HCZ. However, the potency of *d*-HCZ with the smallest pA_2 values of the three HCZ was about the same as that of diphenhydramine ($pA_2 = 7.75$[14]).

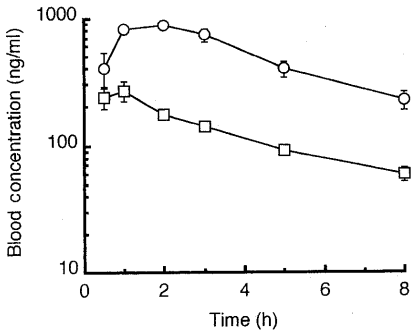

Fig. 3. Blood Concentrations of HCZ after Oral Administration of 50 mg/kg of *d*- (○) and *l*-HCZ (□) to Rats

Each point represents the mean ± S.E. of 3—5 rats.

Pharmacokinetics Figure 3 shows the blood concentration *versus* the time profile afrer oral administration of 50 mg/kg of *d*- and *l*-HCZ to rats. Clearly, the blood levels were higher for *d*-HCZ. The mean $AUC_{0-\infty}$ of *d*-HCZ (5.42 μg·h/ml) was approximately 4.5 times that of *l*-HCZ (1.24 μg·h/ml). The time required to reach the maximum blood concentration and the half life were almost the same for *d*- and *l*-HCZ.

Further studies on the pharmacokinetics of HCZ enantiomers are in progress.

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