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Short communication

Stereoselective determination of cetirizine and studies on pharmacokinetics in rat plasma

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Abstract

Enantiomers may confer benefits over racemates in therapeutic uses and we developed a chiral separation method of cetirizine enantiomers, a second generation H1 histamine receptor antagonist, in rat plasma. α_1-Acidglycoprotein based chiral stationary phase(AGP-CSP), monitored with UV at 230 nm was used to separate the enantiomers. Observed enantioselectivity (α) was 2.0. The AGP-CSP was also used at a preparative scale to isolate the enantiomers with an optical purity of greater than ee 99%. In addition, an analysis was carried out for the cetirizine enantiomers in rat plasma to study the differences of enantiomers in pharmacokinetics. Both (+)- and (−)-cetirizine were separated using a reversed-phase column of AGP, and were detected at the range of 2.5–200 $\mu g\,ml^{-1}$ in plasma. Although there was no recognizable differences in pharmacokinetics between the enantiomers in rat, the method appears to be useful for their pharmacokinetic studies.

Keywords: Cetirizine

1. Introduction

The great differences in pharmacological effects and pharmacokinetics between the two enantomeric forms of many drugs have led us to in recent years emphasize the need of methods for enantioselective separation and determination in biological samples [1]. Especially, it is known that some chiral antihistamine drugs show pronounced stereoselective activity. Fig. 1 is the chemical structures of chiral antihistamine drugs. For example, the (+)-forms of chlorpheniramine and neobenodine are much more potent (100 to 200 fold) than their (−)-enantiomer [2]. These results are due to the highly sensitive nature of antihistamine activity upon the precise stereochemistry between the drug and histamine receptor [3].

Racemic cetirizine is a non-sedating H1-receptor antagonist used in patients with urticaria and allergic rhinintis [4]. The compound is one of the second generation H1 histamine receptor antagonists which generally offer some significant advantages beyond the first generation compounds. The advantages include; (1) less sedation, (2) little anticholinergic activity and (3) longer duration which improves

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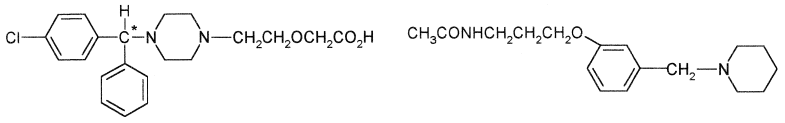

Cetirizine Roxatidine

Fig. 1. Structures of cetirizine and the internal standard (roxatidine). The asterisk (*) indicates the chiral center of cetirizine.

patient compliance [5,6]. Moreover optically pure cetirizines show some stereoselective pharmacological effects. (+)-Cetirizine is more useful for the treatment of urticaria and (−)-cetirizine is for the treatment of allergic disorders while avoiding the adverse effects associated with the racemic mixture of cetirizine [7–9].

However the reason for therapeutic difference upon each enantiomer is not known yet, and in this study we investigated the pharmacokinetic difference between both enantiomers. The pharmacokinetics of cetirizine have been reported [10,11], but no work has been reported about enantioselective pharmacokinetics in biological fluids.

2. Experimental

2.1. Materials and reagents

Racemic cetirizine was a gift from UCB (Seoul, Korea) and roxatidine (internal standard) was a gift from Han-Dok (Seoul, Korea). Both (+)- and (−)-cetirizine were obtained by preparative HPLC. All solvents were HPLC grade from Merck (Darmstadt, Germany). And all other chemicals were of analytical-reagent grade.

2.2. Apparatus and HPLC conditions

2.2.1. Analytical application

The HPLC system consisted of an Waters 600 pump and an UV detector set at 230 nm (Waters 490E, Milford, Massachusetts, USA). Injections were done using an injection valve (Waters U6K). Chromatograms were recorded and integrated with a Waters 746.

The column used was a AGP column (15 cm×4.0 mm I.D.; Regis, Morton Grove, IL, USA) with a AGP guard column (1 cm×3.0 mm I.D.).

2.2.2. Preparative application

The HPLC system consisted of an Waters Delta Prep 4000 pump and an UV detector set at 230 nm (Waters 486). Injections were done using an injection valve (Waters Delta Prep 4000). Chromatograms were recorded and integrated with a Waters 746.

The semi-preparative column used was a AGP column (15 cm×10.0 mm I.D.) (Regis).

2.3. Chromatography

2.3.1. Selection of mobile phase in reversed-phase analytical chromatography

Reversed phase HPLC was carried out with an AGP column (15 cm×4.0 mm I.D.) at a flow-rate of 0.9 ml min^{-1} and at ambient temperature. Racemic cetirizine solutions (100 μg ml^{-1} in mobile phase) were chromatographed (10 μl injection) to select a suitable mobile phase used in the system. From the chromatograms obtained, the chromatographic parameters, such as separation factor (α) and resolution factor (R_s), were calculated in the usual manner.

2.3.2. Preparative chromatography

The preparative isolation of cetirizine was accomplished with 10 m*M* aqueous phosphate buffer (pH 7.0)-acetonitrile (96:4, v/v) as the mobile phase, at a flow-rate of 4 ml min^{-1}. Racemic cetirizine (500 μg ml^{-1} in mobile phase) was injected onto the CSP and the eluted fractions containing the individual enantiomers were collected separately. The fractions from successive injections were pooled and the drug was extracted with ethylacetate by shaking gently for 20 min after acidified by the addition of citrate buffer (1 *M*, pH 5). Phases were separated by centrifugation

and the organic layer was transferred to another tube and evaporated to dryness at 40°C. The purity of the isolated isomers was checked by HPLC using the identical conditions of analytical chromatography.

2.3.3. Peak assignment

The chromatographic fractions corresponding to (+)- and (−)-cetirizine were identified by UV (Perkin–Elmer Lamda 12, Ülbergen, Germany), LC–MS (Hewlett–Packard 1100, Palo Alto, Califonia, USA), circular dichroism (CD) (Jobin-Yvon CD 6, Longjumeau, France) and polarimeter (Perkin-Elmer 243, Ülbergen, Germany).

2.4. Extraction procedure

The extraction procedure for racemic cetirizine in rat plasma was as follows. A 100 μl sample of plasma was added to 50 μl of 10 m*M* phosphate buffer (pH 7.0) in an ependorf tube and then the protein was precipitated with 50 μl of 25% perchloric acid. The mixture was stirred on a vortex mixer for 10 s and centrifuged at 2000 g for 3 min. The clear supernatant was separated then acidified by the addition of 2.0 ml of citrate buffer (1 *M*, pH 5.0), the drug was extracted with 8.0 ml ethylacetate by shaking gently for 20 min.

After centrifugation, the organic layer was transferred to another tube and evaporated to dryness at 40°C. The residue was dissolved in 100 μl of 10 m*M* phosphate buffer (pH 7.0) and 100 μl of internal standard solution (roxatidine 200 $\mu g\,ml^{-1}$) was added, and 10 μl of the solution were injected into HPLC apparatus.

2.5. Standard curves

Standard curves were constructed from plasma samples containing (+)-cetirizine and (−)-cetirizine with concentration ranging from 2.5 to 200 $\mu g\,ml^{-1}$ ($n=5$).

2.6. Application

Oral doses (80 $mg\,kg^{-1}$) of racemic cetirizine with 2 ml of water were administered to five male wistar rats (body weight: 240~260 g). Blood samples were taken with heparinized syringes at timed intervals, i.e. 10 min, 30 min, 1, 2, 4, 6, 8, 10, 23, 27 and 30 h after dosing. The plasma was separated by centrifugation (1500×*g*, 10 min) and stored at −20°C until use.

3. Results and discussion

Highly selective interactions between given enantiomers of drugs and proteins in biological systems form the basis of affinity chromatography and are well documented [12,13]. In particular, columns based on an immobilized protein, such as bovine serum albumin and α_1-acidglycoprotein appear to provide wide appliacability and resolving power [14,15].

Table 1 summarizes the effect of different buffers (pH and concentration) and organic solvents on the chromatographic parameters. Changing the organic solvents from isopropanol, methanol to acetonitrile gave higher values of both α and Rs for the cetirizine enantiomers.

As shown in Fig. 2, both α and R_s decreased with decreasing pH. The optimum buffer concentration was found to be 10 m*M*. Based on these results 10 m*M* phosphate buffer (pH 7.0)–acetonitrile (95: 5, v/v) was chosen as the mobile phase.

Circular dichroism spectra of cetirizine enantiomers derived from the peak fractions on the preparative chromatogram separation of the cetirizine racemate are shown in Fig. 2a. We assigned the (+)-cetirizine to the isomer corresponding to the first peak and the (−)-cetirizine to the second peak by measuring the optical rotation using a polarimeter.

To find out the extraction efficiencies for both enantiomers in rat plasma, cetirizine was spiked to be 10, 50 and 100 $\mu g\,ml^{-1}$ and extracted with either chloroform or ethylacetate in the range of pH 4~7. The optimum extraction efficiency was obtained at pH 5 with ethylacetate as the solvent of choice. Extraction recovery of (+)-cetirizine was 73.2 (1.3% and that of (−)-cetirizine was 71.2 (2.0% ($n=5$).

The linearity of the method was evaluated in the concentration range of 2.5 to 200 $\mu g\,ml^{-1}$ for cetirizine enantiomers. The coefficients of correlation were better than 0.997 ($n=5$).

Table 1
Effect of the different buffers (pH and concentration) and organic modifiers on the chromatographic parameters of cetirizine

Mobile phase				Resolution factor, R_s	Separation factor, α
PB[a] m*M* (A)	pH	organic solvent (B)	A:B		
10	7.0	isopropanol	98:2	0.88	1.17
10	7.0	methanol	93:7	1.84	1.64
10	7.0	acetonitrile	95:5	2.00	1.98
10	7.0	acetonitrile	94:6	1.12	1.26
10	7.0	acetonitrile	93:7	0.60	1.20
10	6.0	acetonitrile	95:5	0.89	1.28
10	5.0	acetonitrile	95:5	0.40	1.16
10	4.0	acetonitrile	95:5	0.25	1.14
20	7.0	acetonitrile	95:5	1.96	1.95
40	7.0	acetonitrile	95:5	1.97	1.97

[a] Phosphate buffer.

Fig. 3 shows typical chromatograms obtained from samples of blank rat plasma (A), plasma spiked with racemic cetirizine and the internal standard (B) and plasma sample obtained at 1 h (C) after administration of 80 mg kg^{-1} of racemic cetirizine to a rat. The detection limit for cetirizine enantiomers was 300 ng ml^{-1} of plasma and quantitation limit was 800 ng ml^{-1}. Also, cetirizine is known to be very stable under the in vivo conditions applied here [16].

There was no interference in the recovery of cetirizine enantiomers from endogenous compounds in rat plasma samples. Under these conditions, the retention times for (+)-cetirizine, (−)-cetirizine and the internal standard were 13 min, 17 min and 28 min, respectively.

The intra- and the inter-day precision of the this method were investigated Table 2. and coefficients of variation were below 6%.

Fig. 4 shows the plasma concentration–time profile of the cetirizine enantiomers after oral administration of racemic cetirizine. The plasma concentration of (+)-cetirizine are similar to that of (−)-

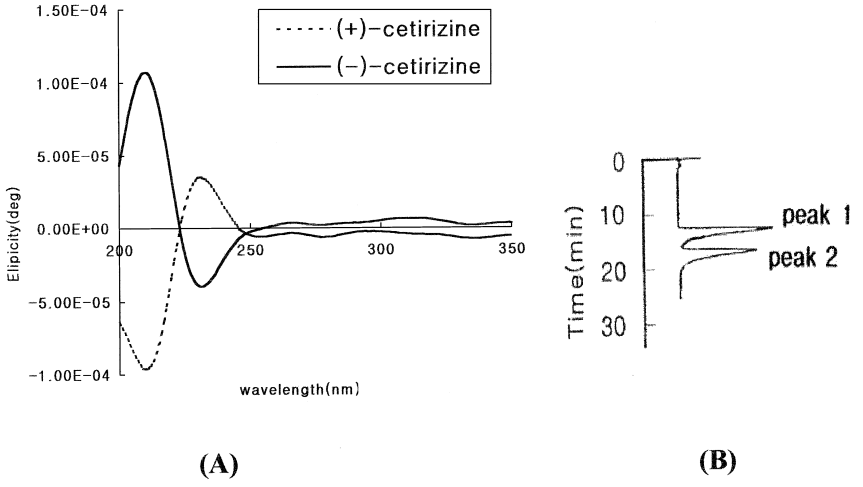

Fig. 2. (A) CD spectra of the peak fractions. The solid line represents the spectrum derived from peak 1 and the dotted line represents that from peak 2, (B) Typical chromatogram of racemic cetirizine dissolved in mobile phase.

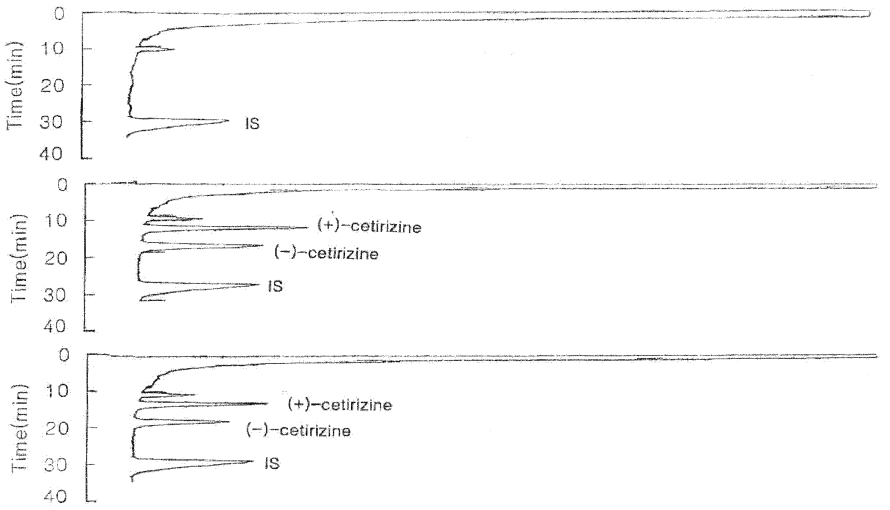

Fig. 3. Chromatograms of plasma samples. (A) Blank plasma; (B) plasma spiked with 20 μg of racemic cetirizine and 20 μg of roxatidine; (C) plasma sample obtained 1 h after oral administration of 80 mg kg^{-1} racemic cetirizine to a rat.

cetirizine, suggesting that the pharmacokinetics of cetirizine in the rat are not stereoselective.

4. Conclusion

The HPLC method reported is highly selective for the determination of cetirizine enantiomers in plasma. The method was useful for pharmacokinetic studies of cetirizine enantiomers. However there was no difference in pharmacokinetics between both enantiomers in rat.

Table 2
Intra- and inter-day precision for the assay of (+)-cetirizine and (−)-cetirizine

	Spiked concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})[a]	RSD (%)
Intra-day	10.0	9.8±0.5	5.1
(+)-cetirizine	50.0	49.3±1.6	3.2
	100.0	99.7±2.4	2.4
(−)-cetirizine	10.0	9.7±0.5	5.2
	50.0	49.0±1.6	3.3
	100.0	99.5±3.1	3.1
Inter-day	10.0	9.7±0.5	5.2
(+)-cetirizine	50.0	49.2±2.4	4.9
	100.0	99.6±4.7	4.7
(−)-cetirizine	10.0	9.7±0.5	5.2
	50.0	49.0±2.3	4.7
	100.0	99.4±4.1	4.1

[a] Mean±SD (n=5).

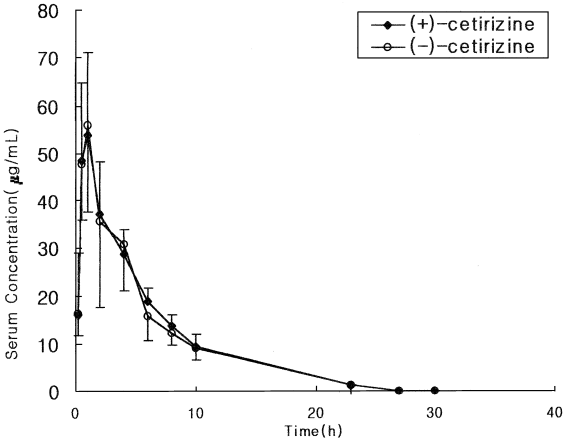

Fig. 4. Plasma concentration–time curves for (+)- and (−)-cetirizine after oral administration of 80 mg kg^{-1} of racemic cetirizine to male wistar rats. Mean values (±SD) are given (n=5).

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