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Enantioselective Determination of Cetirizine in Human Urine by HPLC

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In order to study the simultaneous determination of (+)- and (-)-cetirizine in human urine we have developed a chiral separation method by HPLC. A chiral stationary phase of α_1-acidglycoprotein, the AGP-CSP, was used to separate the enantiomers. The pH of the phosphate buffer, as well as the content of the organic modifier in the mobile phase, markedly affected the chromatographic separation of (+)- and (-)-cetirizine. A mobile phase of 10 mmol/l phosphate buffer (pH 7.0)-acetonitrile (95 : 5, v/v) was used for the urine assays. Ultraviolet absorption was monitored at 230nm and roxatidine was employed as the internal standard for quantification.

(+)-Cetirizine, (-)-cetirizine and the internal standard were eluted at retention times of 12, 16, and 32 mins, respectively. The detection limit for cetirizine enantiomers was 400 ng/ml of urine. A pharmacokinetic study was conducted with the help of 5 healthy female volunteers who were administered with a single oral dose of racemic cetirizine (20 mg). The peak area ratios provided by the cetirizine enantiomers were linear($r>0.997$) over a concentration range of 2.5-200 μg/ml. The peak of the excreted cetirizine enantiomers appeared in the urine sample during the period of 1-2 hrs following the administration of the oral dose. The excreted level of (+)-cetirizine was slightly higher than (-)-cetirizine but the difference was not statistically significant. However, this method appears to have applications for enantioselective pharmacokinetic studies of racemic drugs.

Key words: Cetirizine, Chiral separation, Pharmacokinetics

INTRODUCTION

It is known that each enantiomer of the racemic mixtures of some chiral antihistaminic drugs such as chlorpheniramine, neobenodine etc. (Sakurai *et al.*, 1992) shows characteristic stereoselective activities and pharmacokinetics. The chemical structures of some chiral antihistaminic drugs are shown in Fig. 1. Cetirizine is a second generation antihistaminic drug; a non-sedating H_1-receptor antagonist used in patients with urticaria and allergic rhinitis (Pagliara *et al.*, 1998). It is known that optically pure cetirizine, sold as the racemic mixture, exhibits a distinct stereoselectivity in the pharmacological effects of its two optically pure enantiomers. For example, (+)-cetirizine is useful in the treatment of urticaria while (-)-cetirizine is effective in the treatment of allergic disorders. The separate use of each enantiomer has the advantage of avoiding the adverse effects associated with the use of racemic mixtures of cetirizine (Gray *et al.*, 1997; Corey *et al.*, 1996).

Chlorpheniramine   Cetirizine

Fig. 1. Structures of antihistamine drugs

In recent years, the large differences in pharmacokinetics between the two enantiomeric forms of many drugs has resulted in a great emphasis on the need for enantioselective separations and the subsequent deter-

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minations of them from biological fluids (Persson *et al.*, 1990). Cetirizine has a similar structure to homochlorcyclizine and the antihistamine potency of (-)-homochlorcyclizine is 100 times greater than that of (+)-homochlorcyclizine (Nishikata *et al.*, 1992). It is known, from oral administration studies to rats and humans, that there are significant differences in the pharmacokinetic behaviors between the two enantiomers of homochlorcyclizine (Nishikata *et al.*, 1993). Meanwhile no work has yet been reported on the pharmacokinetic profiles after the administration of racemic cetirizine.

Several chiral stationary phases have been developed for the direct resolution of racemic compounds by HPLC (Mehta, 1988). In particular, columns based on an immobilized protein, such as bovine serum albumin and α_1-acidglycoprotein (AGP) (Schill *et al.*, 1986), appear to provide wide applicability and resolving power.

This paper describes a simple enantioselective HPLC method for the simultaneous determination of cetirizine enantiomers in human urine using a chiral AGP column. Such a column has been found to be very useful for the chiral resolution of pharmaceutically active compounds in general (Miwa *et al.*, 1987; Monica, 1998). The method was applied to the analysis of urine samples from healthy volunteers who had received oral administration of racemic cetirizine.

MATERIALS AND METHODS

Chemicals

Racemic cetirizine was a gift from UCB Company (Seoul, Korea) and roxatidine was a gift from Han-Dok Pharmaceutical (Seoul, Korea). Both (+)- and (-)-cetirizine were obtained by preparative HPLC as described elsewhere (under review). Ambelite PAD-2 resin was purchased from Serva (Westbury, NJ, U.S.A.). All solvents were of HPLC grade from Merck (Darmstadt, Germany) and all other chemicals were of analytical reagent grade.

Chromatography

The HPLC system consisted of Waters 600 pumps with U6K injector (Milford, Massachusetts, U.S.A.) and a Waters 490E UV detector set at 230 nm (Milford, Massachusetts, U.S.A.). Chromatograms were recorded and integrated with a Waters 746 integrator (Milford, Massachusetts, U.S.A.). The separation of cetirizine enantiomers was conducted using an analytical AGP column (15 cm × 4.0 mm I.D.; Regis, Morton Grove, IL U.S.A.) with a guard column (1 cm × 3.0 mm I.D.) attached. Ten mmol/l phosphate buffer (pH 7.0)-acetonitrile (95:5) was used as a mobile phase at a flow rate of 0.9 ml/min.

Administration of cetirizine to humans

Oral doses (20 mg) of racemic cetirizine were administered to five volunteers (female, aged 30±2.4 years, body weight 55.4±3.2 kg, mean S.D.). The volunteers were asked to fast for 13 hrs prior to, and for 4 hrs after the administration of oral doses, with free access to drinking water during this period. Urine samples were collected, and their volumes measured, at the following time intervals : prior to dosing(time 0), and 0-1, 1-2, 2-6, 6-10, 10-14, 14-24, 24-36 and 36-48 hrs post-dosing. All samples were stored at -20°C until analysis.

Sample preparation

A 5 ml aliquot of a urine sample was added to a pasteur pipette which was filled with 2.5 cm of PAD-2 slurry. The pasteur pipette was washed with 5 ml of purified water, then 3 ml of methanol was added to the slurry. This methanolic solution was evaporated and after acidification by the addition of 8 mL of citrate buffer (pH 5.0), the drug was extracted with 10 mL of ethyl acetate by shaking gently for 20 min. After centrifugation (1500 × g, 10 min), the organic layer was transferred to another tube and evaporated until dry at 40°C. The residue was dissolved in 500 µl of 10 mmol/l phosphate buffer (pH 7.0), and 10 µl of the sample solution was loaded into the HPLC, together with 500 µl of internal standard solution (roxatidine 200 µg/ml).

RESULTS AND DISCUSSION

Mechanism of interactions between cetirizine enantiomers and AGP Highly selective interactions between given enantiomers of drugs and proteins in biological systems are well documented and form the basis of affinity chromatography (Schill *et al.*, 1986). It is generally assumed that the two dominating contributions to the strength of the binding of a ligand to a protein are electrostatic (ionic) and hydrophobic interactions. Especially, it is known that hydrophobicity, along with molecular size and electron excess charge on aliphatic nitrogen, is related to the retention of antihistamine on AGP (Nasal *et al.*, 1994). And the topography of the antihistamine-binding site on AGP was suggested to be a conical pocket with lipophilic regions at the mouth of the receptor and an anionic region close to the cone (Kaliszan *et al.*, 1996). In other words, the aromatic rings of cetirizine interact with the receptor at its pocket and the carboxyl group of cetirizine is positioned close to the cone.

Selection of optimum separation conditions of cetirizine enantiomers

It is also documented that the degree of retention on protein chiral stationary phases changes relative to the

mobile phase composition. Our results show that the pH of the phosphate buffer and the content of the organic modifier in the mobile phase markedly affect the chromatographic separation of (+)- and (-)-cetirizine. In a previous study, we investigated the optimum conditions for the separation of cetirizine enantiomers by changing the pH, the content of the organic modifier and the buffer strength of the mobile phases. By analyzing the chromatographic parameters (α, Rs), we determined the optimum conditions for separation (under review). A mobile phase of 10 mmol/l phosphate buffer (pH 7.0)-acetonitrile (95 : 5, v/v) was used as the mobile phase of choice for the urine assays. The ultraviolet absorption was monitored at 230nm, and roxatidine was employed as the internal standard for quantification.

Extraction efficiency

We also looked into the extraction efficiencies for both enantiomers in human urine at the level of 10, 50, and 100 μg/ml. We used chloroform and ethyl acetate as the extraction solvents in the range of pH 4.0-7.0. The optimum extraction efficiency was obtained at pH 5.0 with ethyl acetate as the extraction solvent.

Chromatography

We obtained typical chromatograms from the samples of blank human urine (A), the urine spiked with racemic cetirizine and internal standard (B), and the urine sample obtained 1-2 hrs after oral administration of 20 mg of racemic cetirizine to the volunteers (C) (Fig. 2). No interference from endogenous compounds was found when the human urine samples were analyzed. Under these conditions, the retention times for (+)-cetirizine, (-)-cetirizne and the internal standard were 12 min, 16 min, and 32 min, respectively.

Assay characteristics

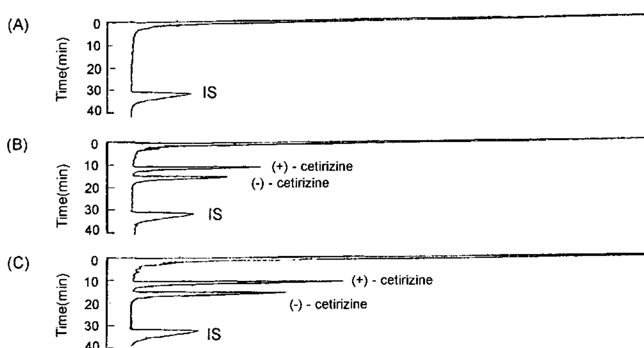

Fig. 2. Chromatograms of urine samples. (A) Blank urine; (B) urine spiked with 100 μg of racemic cetrizine and 100 μg of roxatidine; (C) urine sample obtained 2 hr after oral administration of 20 mg racemic cetirizine to a human volunteer.

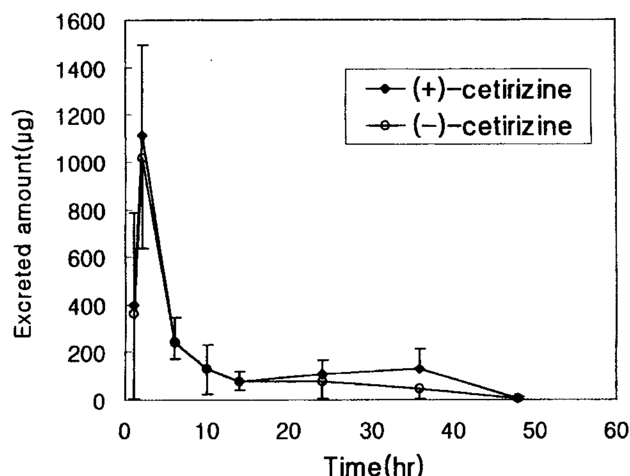

Fig. 3. Urine time-excreted amount/time profiles of cetirizine enantiomers after an oral administration of racemic cetirizine to 5 humans. (A) (+)-cetirizine ; (B) (-)-cetirizine

We investigated the time-excreted amount/time profiles of cetirizine enantiomers after an oral administration of racemic cetirizine to 5 human subjects (Fig. 3). The peak excreted amount/time was obtained from the urine samples collected during the 1-2 hr period after dosing. The peak area ratios of the cetirizine enantiomers were linear (r>0.997) over a concentration range of 2.5-200 μg/ml. The minimum detectable concentration of the cetirizine enantiomers was 400 ng/ml in urine (signal-to-noise ratio=3). The intra-day precision and the inter-day precision of the present method, as illustrated in Table I, show relative standard deviations of below 6%.

Table I. Intra- and inter-day precision for the assay of (+)-cetirizine and (-)-cetirizine

	Spiked Concentration (μg/mL)	[a] Measured Concentration (μg/mL)	[b] R.S.D (%)
Intra-day (+)-cetirizine	10.0	9.7±0.5	5.2
	50.0	49.5±1.5	3.0
	100.0	99.5±2.7	2.7
(-)-cetirizine	10.0	9.7±0.5	5.2
	50.0	49.0±1.6	3.3
	100.0	99.1±3.0	3.0
Inter-day (+)-cetirizine	10.0	9.5±0.5	5.3
	50.0	49.3±2.2	4.5
	100.0	99.2±4.2	4.2
(-)-cetirizine			
	10.0	9.6±0.5	5.2
	50.0	49.0±2.4	4.9
	100.0	99.0±4.0	4.0

[a] Mean±S.D.(n=5)
[b] Relative standard deviation

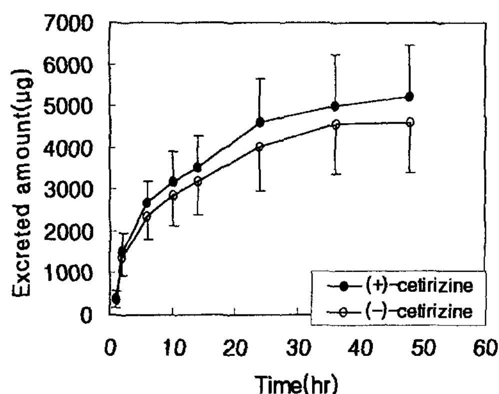

Fig. 4. Cumulative urinary excretion of the (+)- and (-)-isomers of cetirizine within 48 h after oral administration of 20 mg of racemic cetirizine

We also looked into the cumulative urinary excretion of 20 mg of orally given racemic cetirizine (Fig. 4). The amount of (+)-cetirizine recovered from the urine sample, was slightly higher than that of (-)-cetirizine, although not regarded as statistically significantly higher. The excretion tendencies of racemic cetirizine obtained from the current study are consistent with previous work (M. T. Roussel *et al.*, 1991). The present method provides a means of accurate, reproducible measurement of cetirizine in urine, suitable for either therapeutic drug monitoring or research applications. Due to the apparent lack of enantioselectivity in the excretion of cetirizine in humans, the non-enantiospecific assay would be considered sufficient for therapeutic drug monitoring purposes. Moreover, the procedure described here appears to be relatively simple so that it can be applied to practical analytical situations.

In conclusion, not only is the liquid chromatographic method described here applicable to direct simultaneous determination of (+)- and (-)-cetirizine in urine but also to for pharmacokinetic studies.

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