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Enantioselective pharmacokinetics of homochlorcyclizine

III. Simultaneous determination of (+)- and (−)-homochlorcyclizine in human urine by high-performance liquid chromatography

Mayumi Nishikata, Aki Nakai, Hitomi Fushida, Keishiro Miyake and Takaichi Arita

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya 663 (Japan)

Ken Iseki and Katsumi Miyazaki

Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Sapporo 060 (Japan)

Akikazu Nomura

Department of Cardiovascular Medicine, School of Medicine, Hokkaido University, Sapporo 060 (Japan)

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ABSTRACT

A method is described for the simultaneous determination of (+)- and (−)-homochlorcyclizine (HCZ) in human urine by high-performance liquid chromatography on a chiral stationary phase of ovomucoid-bonded silica. The pH of the buffer and organic modifier in the mobile phase markedly affected the chromatographic separation. A mobile phase of methanol–0.02 *M* acetate buffer (pH 4.7) (25:75, v/v) at a flow-rate of 1.0 ml/min was used for the urine assays. The ultraviolet absorption was monitored at 240 nm, and diphenhydramine was employed as the internal standard for the quantitation. (+)-HCZ, (−)-HCZ and the internal standard were eluted at retention times of 15, 25 and 8 min, respectively. The limit of determination for HCZ enantiomers was *ca.* 50 ng/ml of urine. One of the metabolites in human urine, which was a quaternary ammonium-linked glucuronide, could also be determined in a manner similar to unchanged HCZ after β-glucuronidase hydrolysis. A pharmacokinetic study was conducted with three healthy volunteers, who each received a single oral dose of racemic HCZ (20 mg). Distinct differences were found between the two enantiomers, particularly in the metabolic process, that is, the urinary excretion as (−)-HCZ-glucuronide within 48 h was *ca.* four times higher than that of the (+)-isomer. This method should be very useful for enantioselective pharmacokinetic studies of HCZ.

Correspondence to: Katsumi Miyazaki, Ph. D., Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

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INTRODUCTION

Homochlorcyclizine (HCZ), a potent antihistamine, is administered as a racemic mixture. However, the antihistaminic activity of (−)-HCZ is *ca.* 100 times greater than that of (+)-HCZ in isolated guinea-pig ileum tests [1], and there are significant differences in the pharmacokinetics after oral administration of (+)- and (−)-HCZ to rats [1] and humans [2]. These studies were carried out using pre-separated HCZ enantiomers, and no work has yet been reported on enantioselective pharmacokinetic profiles after administration of racemic HCZ, because there has been no simultaneous analytical method for HCZ enantiomers.

Recently, several chiral stationary phases have been developed for the direct resolution of racemic compounds by high-performance liquid chromatography (HPLC) [3,4]. In particular, columns based on an immobilized protein, such as bovine serum albumin [5], α_1-acid glycoprotein [6] and ovomucoid (OVM) [7], enable wide applicability and resolving power.

This paper describes a simple enantiospecific HPLC assay for the simultaneous determination of HCZ enantiomers in human urine using a chiral OVM column, which is relatively stable to pH variation, heat and organic solvents and has been found to be very useful for the chiral resolution of pharmaceutically important compounds [8–11]. Additionally, the method was used to analyse urine samples from healthy volunteers after oral administration of racemic HCZ. The enantioselective pharmacokinetics are also discussed.

EXPERIMENTAL

Chemicals

Racemic HCZ and diphenhydramine hydrochloride (DPH) were purchased from Nippon Bulk Yakuhin (Osaka, Japan). Both (+)- and (−)-HCZ were obtained as described previously [1]. β-Glucuronidase (bovine liver, G-0251) was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade. The molecular structure of HCZ is shown in Fig. 1.

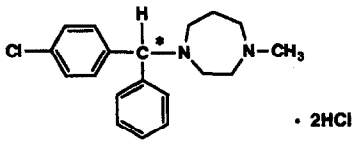

Fig. 1. Molecular structure of homochlorcyclizine (HCZ). The asterisk indicates the asymmetric carbon atom.

HPLC apparatus

The HPLC system consisted of an LC-6A pump and an SPD-6A UV detector set at 240 nm (Shimadzu, Kyoto, Japan). Injections were done using a Rheodyne 7125 injection valve (Cotati, CA, USA). Chromatograms were recorded and integrated with a Chromatopac C-R6A (Shimadzu).

Selection of mobile phase

An OVM column (Ultron ES-OVM, 150 mm × 4.6 mm I.D.) with a guard column (Ultron ES-OVMG, 10 mm × 4.0 mm I.D.) (Shinwa Chemical Industries, Kyoto, Japan) was used. Racemic HCZ solutions (100 μg/ml in water) were chromatographed (10-μl injection) to select a suitable mobile phase. From the chromatograms obtained, the chromatographic parameters, such as capacity factor (k'), selectivity factor (α) and resolution factor (R_s), were calculated in the usual manner [12].

Enantioselective determination of HCZ (assay I)

The mobile phase was acetate buffer (pH 4.7)–methanol (75:25, v/v). Chromatography was carried out at 25°C at a flow-rate of 1.0 ml/min.

Determination of total HCZ (assay II)

A Shim-pack CLC-CN column (150 mm × 60 mm I.D.) (Shimadzu) was used for the assay of total HCZ. The mobile phase was phosphate buffer (pH 3.0)–acetonitrile (67:33, v/v). The flow-rate was 1.5 ml/min.

Sample preparation

Determination of HCZ in human urine. A 200-μl volume of internal standard solution (DPH, 3 μg/ml) and 3 ml of purified water were added to 1 ml of urine sample in a glass cen-

trifuge tube. After alkalization by the addition of 0.2 ml of 4 *M* sodium hydroxide, the drug was extracted with 5 ml of *n*-hexane 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 reconstituted with 120 μl of 0.01 *M* hydrochloride and 100 μl of *n*-hexane. After vortex-mixing and centrifugation, the organic layer was discarded. The aqueous layer was made alkaline and extracted with *n*-hexane (0.5 ml) as described above. The organic solvent was evaporated, the residue was dissolved in 60 μl of the mobile phase, and 50 μl of the solution were injected into the HPLC apparatus.

Determination of HCZ glucuronide in human urine. Urine (0.5 ml) was incubated with 0.1 ml of β-glucuronidase (15 000 U/ml) in 4.5 ml of citrate buffer (pH 5) for 24 h at 37°C. The sample was analysed for HCZ as described above; the HCZ glucuronide was determined by subtracting the unaltered HCZ amounts from the total amounts.

Administration of HCZ to humans

Oral doses (20 mg) of racemic HCZ with 100 ml of water were administered to three volunteers (aged 24 ± 1.6 years, body weight 61.3 ± 1.9 kg, mean ± S.D.) who had consented to participate in this study. The volunteers were made to fast for 13 h piror to the administration and for 4 h after dosing but with free access to tap water. Urine samples were collected, and their volumes measured, at the following times: prior to dosing (time 0), and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 24, 36 and 48 h post-dose. All samples were stored at −80°C until analysis.

RESULTS AND DISCUSSION

The k', α, R_s and column efficiency (N) values for the OVM column have been reported to depend on the amount loaded because the binding capacity is low [10]. A 500-ng load of HCZ enantiomers in the mobile phase given below produced symmetrical peaks, and the optimal conditions were determined.

Table I summarizes the effect of different buffers and organic modifiers on the chromatographic parameters. Changing the organic modifier from acetonitrile to methanol gave higher values of both α and R_s for the HCZ enantiomers. Subsequently, the effects of the pH and the methanol content on the chromatographic parameters of HCZ were examined. As shown

TABLE I

EFFECT OF THE COMPOSITION OF THE MOBILE PHASE ON ENANTIOSELECTIVE PARAMETERS OF HCZ

Mobile phase	Capacity factor[a]		Selectivity factor, α	Resolution factor, R_s
	k'_1	k'_2		
Acetonitrile–pH 4.7 p.b.[b] (15:85)	2.86	3.08	1.08	1.43
Acetonitrile–pH 4.7 a.b.[c] (15:85)	2.73	3.08	1.13	1.46
Acetonitrile–pH 5.3 a.b.[c] (15:85)	6.73	7.88	1.17	1.83
Acetonitrile–pH 6.0 a.b.[c] (15:85)	24.44	30.00	1.23	1.94
Methanol–pH 4.7 a.b.[c] (30:70)	2.11	3.55	1.68	2.75

[a] Capacity factor subscripts 1 and 2 for (+)- and (−)-HCZ, respectively.
[b] 20 m*M* sodium phosphate buffer.
[c] 20 m*M* sodium acetate buffer.

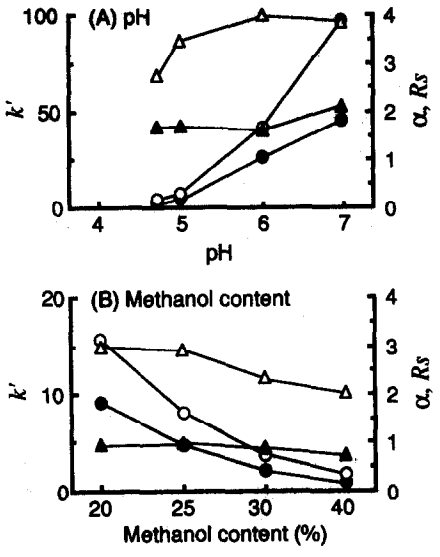

Fig. 2. Effect of (A) pH and (B) methanol content on the chromatographic parameters of HCZ. Conditions: column, Ultron ES-OVM (150 mm × 4.6 mm I.D.); flow-rate, 1.0 ml/min; detection wavelength, 240 nm; mobile phase, (A) 20 m*M* sodium acetate buffer–methanol (70:30, v/v), (B) 20 m*M* sodium acetate buffer–methanol. (●) k'_1; (○) k'_2; (▲) α; (△) R_s.

in Fig. 2, the capacity factors (k'_1 and k'_2) of HCZ significantly decreased with decreasing pH and with the addition of methanol to the eluent. In contrast, the α values were only slightly affected by decreasing pH or by changing methanol content. The k' value gradually decreased with increasing column temperature, but the enantioselectivity (α) was almost constant in the temperature range 15–30°C (data not shown). Based on these results, the optimum mobile phase was 20 m*M* acetate buffer (pH 4.7)–methanol (75:25, v/v). HPLC of biological samples was carried out at 25°C.

Fig. 3 shows typical chromatograms obtained from samples of blank human urine (A), urine spiked with racemic HCZ and internal standard (B) and β-glucuronidase-treated and untreated samples of urine obtained 6 h after oral administration of 20 mg of racemic HCZ to one volunteer (C and D, respectively). In a preliminary examination, we confirmed that deglucuronidation was completed within 12 h of β-glucuronidase treatment. There was no interference from endogenous compounds in human urine or in the β-glucuronidase-treated urine sample. Under these conditions, the retention times for (+)-HCZ, (−)-HCZ and the internal standard were 15, 25 and 8 min, respectively.

The extraction efficiencies for both enantiomers in human urine at the concentration of 500 ng/ml were *ca.* 98%, and the overall coefficients of variation for the (+)- and (−)-isomers at 500 ng/ml in urine were 2.90 and 4.85%, respectively. The (+)-HCZ to (−)-HCZ ratio [(+)/(−) ratio] was close to 1, which suggested that this sample procedure led to the extraction

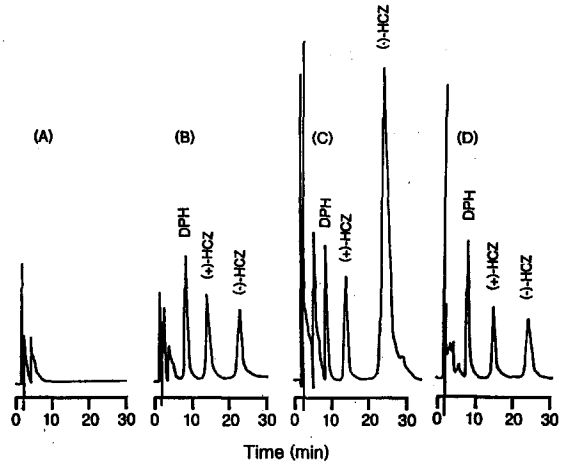

Fig. 3. Chromatograms of human urine samples. (A) Blank urine; (B) urine spiked with 1000 ng of racemic HCZ and 600 ng of DPH; (C) β-glucuronidase-treated and (D) untreated samples of urine collected 6 h after oral administration of 20 mg/kg racemic HCZ to a volunteer.

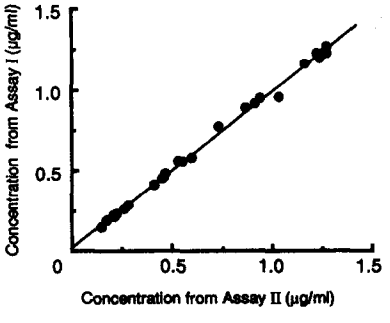

Fig. 4. Correlation between HCZ concentrations obtained from HPLC on OVM (assay I) and those from HPLC on CLC-CN (assay II): $y = 0.97x + 0.02$ ($r = 0.998$).

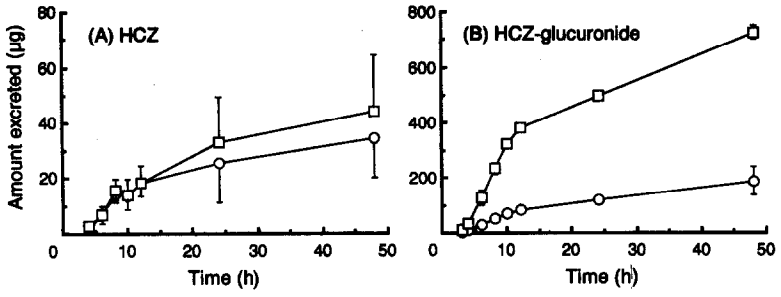

Fig. 5. Cumulative urinary excretion of (+)- and (−)-isomers as (A) HCZ and (B) HCZ-glucuronide, after oral administration of 20 mg of racemic HCZ to humans. Each point represents the mean ± S.D. of three volunteers: (○) (+)-isomer of HCZ or its glucuronide; (□) (−)-isomer of HCZ or its glucuronide.

of approximately equal amounts of both enantiomers. The calibration curves were linear ($r > 0.998$) in the concentration range 50–1500 ng/ml for HCZ enantiomer in urine. The limits of determination were 50 ng of (−)-HCZ and 10 ng of (+)-isomer per millilitre of urine.

Moreover, there was a good correlation ($r = 0.998$) between concentrations found in biological samples by HPLC on OVM and CLC-CN columns (assays I and II, Fig. 4). These results indicate that the present HPLC method is suitable for the determination of HCZ enantiomers in human urine, with satisfactory accuracy and precision.

The cumulative urinary excretion of the (+)- and (−)-isomers as HCZ and HCZ-glucuronide within 48 h after oral administration of 20 mg of racemic HCZ are shown in Fig. 5A and B, respectively. The amount of the glucuronide excreted was more than ten times that of the unchanged drug. The urinary excretion of (−)-HCZ-glucuronide isomer was significantly higher than that of the (+)-isomer, and the (+)/(−) ratio in urine was *ca.* 0.25 (Fig. 6). We have previously confirmed that the formation of a quaternary ammonium-linked glucuronide is an important metabolic pathway for HCZ in humans, and that the metabolic process was enantioselective, as shown by different urinary excretion rates for the (+)- en (−)-glucuronides after oral administration of each enantiomer of HCZ [2]. Furthermore, no optical antipode could be detected in urine after the administration of either the (+)- or (−)-HCZ alone, *i.e.* there was no indication that chiral inversion occurred in humans. These results suggest that the enantioselective formation of HCZ-glucuronide occurred after oral administration of racemic HCZ and agreed with the results observed after oral administration of either (+)- or (−)-HCZ alone [2].

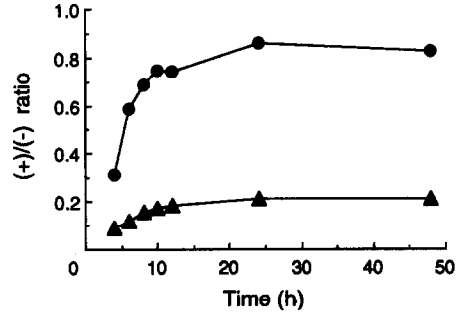

Fig. 6. Ratio of (+)-isomer to (−)-isomer of (●) HCZ and (▲) HCZ-glucuronide in urine collected up to 48 h after oral administration of 20 mg of racemic HCZ to humans. Each point was calculated from the data of Fig. 5A and B.

CONCLUSION

A method for the simultaneous HPLC determination of (+)- and (−)-HCZ in human urine has been developed. A pharmacokinetic study of HCZ by this enantioselective method indicated a clear difference between the rates of glucuronidation of the (−)- and (+)-isomers of HCZ. This method was used to investigate the enantioselective pharmacokinetics in urine after oral administration of racemic HCZ to humans.

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