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Trace analysis of haloperidol and its chiral metabolite in plasma by capillary electrophoresis

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Abstract

Capillary zone electrophoresis was developed for the simultaneous determination of haloperidol (HP) and its chiral metabolites [(+)- and (−)- reduced haloperidol, (+)- and (−)-RHP] in human plasma. The method involved the presence of an internal standard and liquid–liquid extraction from plasma. After concentration, the residue from the organic extract was dissolved in aqueous acid for capillary electrophoretic analysis. The background electrolyte was Tris–phosphate buffer with dimethyl-β-cyclodextrin and PEG 6000. In spiked plasma the quantitative ranges were 40–400 n*M* for HP and 50–500 n*M* for (+)-RHP or (−)-RHP. The intra-day and inter-day relative standard deviations ($n=3$) were all <20% for each substance. The detection limits were found to be 15 ng/ml for HP and 30 ng/ml for both enantiomers of RHP (S/N=3, injection 20 s). All recoveries were >70%. We investigated the in vivo metabolism of HP in Chinese schizophrenia patients. The results show that (−)-RHP seems to be the only chiral metabolite from these two HP-dosed patients.

Keywords: Enantiomer separation; Haloperidol

1. Introduction

Haloperidol (HP) is widely used in clinical medicine for the treatment of schizophrenia and related psychotic disorders. HP's main metabolite, reduced haloperidol (RHP) (see Fig. 1), probably contributes to the pharmacological activity of the parent drug. Monitoring of both HP and RHP plasma levels in patients undergoing HP therapy has been suggested as a better clinical indicator than that of HP level alone [1–4]. However, little is known about the enantiomeric composition of RHP [(+)-, (−)- or racemic] resulting from HP treatment. Therefore, in this work the enantioseparation of RHP in plasma is investigated.

A number of methods for the determination of HP and RHP in biological samples have been published. Some important techniques recently used are gas chromatography with surface ionization detection [5] or with mass spectrometry (MS) [6] and liquid chromatography with electrochemical detection [7,8] or with MS [9]. Also, non-selective methods such as radioimmunoassay [10] have been used. At present, the published methods offer sensitive detection but lack the ability for enantioseparation of RHP. Only one HPLC method using a chiral column was developed for commercial reagents, but not for biological samples [11]. Capillary electrophoresis (CE) is an analytical technique that has been shown to be useful for chiral separations because of its high separation efficiency, its ease of use, and its low cost (minimal use of reagents and cheap columns com-

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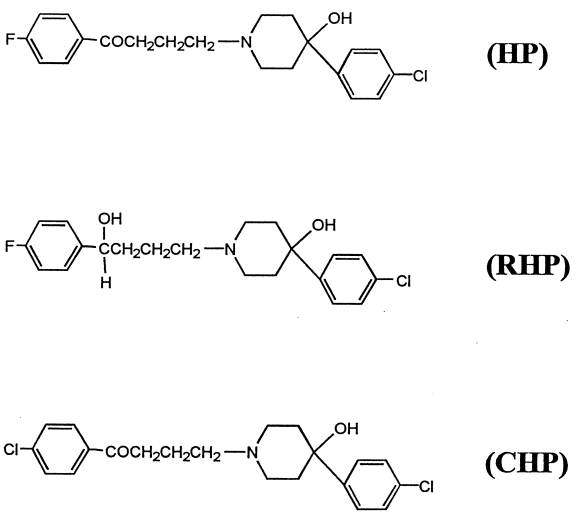

Fig. 1. Structures of haloperidol (HP), reduced haloperidol (RHP) and chlorohaloperidol (CHP).

pared to expensive and usually short-lived chiral HPLC columns). Tomlinson and co-workers investigated the metabolism of HP by CE with UV detection [12], with MS [13], or with on-line MS equipped with an array detection [14]. These methods still did not focus on the chiral resolution of RHP. In our previous reports [15,16] we have developed CE methods with cyclodextrin derivatives as chiral selectors which allow the enantioseparation of standard RHP. The purpose of this work was to extend our investigations to gain an understanding of the enantiomeric composition of RHP from HP dosing. Because plasma concentrations of HP and its metabolite in HP-treated patients are in the low nanogram per milliliter range, and given the poor sensitivity of the CE apparatus (due to a short optical path), a sample concentration after a liquid–liquid extraction process from the plasma samples was necessary. This study was established for the pretreatment and trace analysis of HP and chiral RHP in plasma. For practical approach, the enantiomeric profile of RHP from HP therapy in two Chinese schizophrenia patients is demonstrated.

For enhancement of sensitivity, we reviewed the relevant pretreatment methods including double stacking and supported liquid membrane techniques of Palmarsdottir et al. [17] and head-column field-amplified sample stacking by Zhang et al. [18]. Further application of this method coupling with pretreatment techniques to pharmacokinetic studies of HP-treated patients is in progress.

2. Experimental

2.1. Reagents and chemicals

Racemic RHP and chlorohaloperidol (Janssen, Olen, Belgium), (+)- and (−)-RHP (Parke-Davis, Ann Arbor, USA), HP (Aldrich, Milwaulkee, WI, USA), dimethyl-β-cyclodextrin (di-Me-β-CD) (Beckman) and phosphoric acid (H_3PO_4), tris-(hydroxymethyl)aminomethane (Tris), poly(ethylene glycol) (PEG) 6000, heptane, isoamyl alcohol and hydrochloric acid (Merck, Darmstadt, Germany) were used without further treatment. Milli-Q apparatus (Millipore, MA, USA) treated water was used for the preparation of buffer and related aqueous solutions. Solution of Tris (40 m*M*)–phosphate buffer at pH 2.5 was obtained by neutralizing Tris solution (40 m*M*) with H_3PO_4 (85%) and simply expressed as Tris–phosphate buffer (40 m*M*, pH 2.5). A solution of PEG 6000 (20 mg/ml) was prepared in Tris–phosphate buffer (40 m*M*, pH 2.5). Stock solutions of racemic RHP (1.0 m*M*), HP (1.0 m*M*) and chlorohaloperidol (CHP) (internal standard, I.S.) (1.0 m*M*) were prepared in 0.01 *M* HCl and suitably diluted as working solution.

2.2. Capillary electrophoresis

A Beckman P/ACE 2200 instrument with an UV-detector (Fullerton, CA, USA) was used with an uncoated fused-silica capillary tube [37 cm (effective length 30 cm)×50 μm I.D.]. The slit aperture in the capillary holder was 200×100 μm. The detector was set at 200 nm. Capillary conditioning between runs was effected by rinsing with 0.1 *M* NaOH (5 min), water (5 min) and run buffer (5 min), applied positive pressure at the injection end. At 25°C, a constant voltage of 20 kV (anode at injection end) was applied throughout the run. The current gradually increased to about 85 μA during the first 15 s after power application. All operations and electropherograms were computer controlled using GOLD software version 7.1.

2.3. Extraction and concentration of plasma sample

Using CHP (800 ng) as the I.S., a 5-ml volume of plasma was subjected to basic (NaOH) extraction, acid (HCl) back-extraction, acid wash and basic (NaOH) re-extraction. The extraction solvent was heptane–isoamyl alcohol (98:2, v/v) for the whole procedure. After extraction, the organic solvent was evaporated to dryness under a stream of nitrogen in a water bath (50°C). The dried residue was reconstituted in 100 μl of 0.1 *M* HCl and injected onto the CE system.

2.4. Calibration curve, sensitivity, and reproducibility

For calculation, corrected areas were used (area divided by migration times). Calibration curves were prepared by adding known amounts of HP and racemic RHP (40–400 n*M* for HP and 50–500 n*M* for each enantiomer of RHP) and CHP (800 ng) to a 5-ml drug-free plasma. The calibration graph was established with the corrected peak-area ratio of HP, (+)-RHP or (−)-RHP to CHP as ordinate (*y*) vs. the concentration of HP or each RHP in n*M* as abscissa (*x*). Intra-day and inter-day replicates were calculated with plasma containing 40, 80 or 200 n*M* of HP and 50, 100 or 250 n*M* of each enantiomer of RHP.

2.5. Application

We investigated the in vivo metabolism of two HP-dosed schizophrenia patients using this developed CE method. The patients were treated with an oral HP regimen at hospital. Doses of HP (50 and 60 mg) were given once a day at bedtime (21:00–22:00 p.m.). Venous blood samples were collected in heparinized tubes on the next day in the morning (06:00–09:00 a.m.) while the patient fasted overnight. All blood samples were centrifuged immediately, plasma separated and stored at −60°C until analysis.

3. Results and discussion

Optimizing the parameters for the chiral separation of RHP was studied in our previous report [16]. Furthermore we investigated the quantitation for the plasma samples. Separations were performed in a mixed background electrolyte of di-Me-β-CD (10 m*M*) in Tris–phosphate buffer (40 m*M*, pH 2.5) and PEG 6000 solution (20 mg/ml) (1:0.1, v/v). A composite electropherogram for a standard mixture of HP (400 n*M*), racemic RHP (1000 n*M*) and CHP (800 ng) spiked in plasma and plasma blank is shown in Fig. 2, indicating no significant interference from the plasma sample.

3.1. Validation of HP, (+)- and (−)-RHP determination in plasma

The linear regression equations are listed in Table 1. The mean correlation coefficients for the calibration curves obtained from three separate experiments ranged from 0.9922 to 0.9968. The data indicate good linearity of this method for the intra-day and inter-day assay.

The precision (relative standard deviation, R.S.D.) of the proposed method for spiked samples at 40–200 n*M* for HP and 50–250 n*M* for (+)-RHP or (−)-RHP was studied. The results, presented in Table 2, show that the intra-day and inter-day variances at the three concentrations were all below

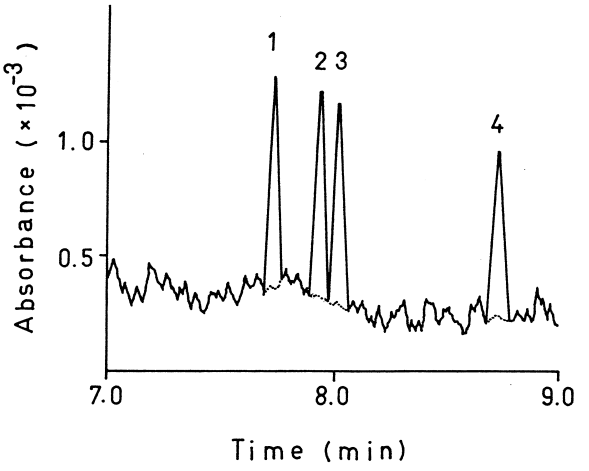

Fig. 2. Composite electropherogram for the determination of haloperidol (HP) (400 n*M*), racemic reduced haloperidol (RHP) (1000 n*M*) and chlorohaloperidol (CHP) (800 ng) in spiked plasma (solid line) and plasma blank (dotted line). Peaks: 1, HP; 2, (−)-RHP; 3, (+)-RHP; 4, CHP. See text for details.

Table 1
Calibration curves for the analysis of HP and racemic RHP spiked in plasma

Analyte	Calibration curve ($n=3$)
HP	Intra-day assay: $y=(0.0608\pm0.0377)+(0.0026\pm0.0001)x$, $r=0.9968$ Inter-day assay: $y=(0.01916\pm0.0366)+(0.0029\pm0.0003)x$, $r=0.9922$
(−)-RHP	Intra-day assay: $y=(0.0859\pm0.0303)+(0.0020\pm0.0001)x$, $r=0.9942$ Inter-day assay: $y=(0.0587\pm0.0300)+(0.0021\pm0.0001)x$, $r=0.9954$
(+)-RHP	Intra-day assay: $y=(0.0602\pm0.0049)+(0.0022\pm0.0001)x$, $r=0.9967$ Inter-day assay: $y=(0.0439\pm0.0140)+(0.0024\pm0.0002)x$, $r=0.9936$

20%. The relative recoveries of the method, as shown in Table 2, were all >70%, which was calculated from the calibration graph constructed from plasma spiked with different amounts of analytes over the range of 40–400 n*M* for HP and 50–500 n*M* for each RHP. The detection limits were found to be 15 ng/ml for HP and 30 ng/ml for each enantiomer of RHP (S/N=3).

Table 2
Precision and accuracy of the analysis of HP and racemic RHP spiked in plasma

Concentration spiked (n*M*)	Concentration found ($n=3$) (n*M*)	R.S.D.	Recovery (%)
HP	Intra-day		
40.00	30.02	11.24	75.05
80.00	90.15	10.31	112.68
200.00	201.74	9.28	100.87
	Inter-day		
40.00	28.01	12.31	70.02
80.00	92.45	10.82	115.56
200.00	199.29	11.19	99.64
(−)-RHP	Intra-day		
50.00	35.87	16.60	71.75
100.00	124.74	13.41	124.74
250.00	244.90	11.75	97.96
	Inter-day		
50.00	38.37	18.26	76.74
100.00	113.10	16.33	113.10
250.00	250.09	11.32	100.03
(+)-RHP	Intra-day		
50.00	37.65	16.99	75.30
100.00	118.62	12.64	118.62
250.00	242.37	10.53	96.94
	Inter-day		
50.00	42.63	18.19	85.26
100.00	108.83	14.54	108.83
250.00	222.70	13.22	89.08

During sample pretreatment, the stability of HP and RHP under 50°C and 0.1 *M* HCl was cursorily studied. Comparing the normalized peak area ratios of these drugs with those of standard solution, no significant difference exists. This indicates that the drugs through sample concentration are stable.

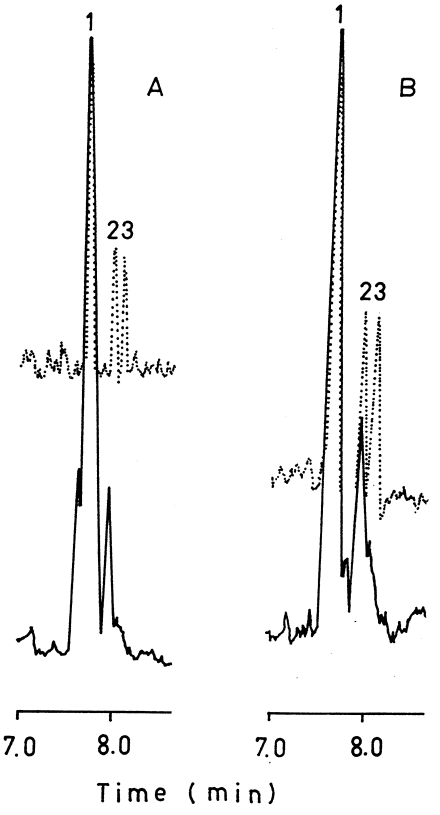

Fig. 3. Composite electropherogram for the analysis of plasma from HP-dosed patient A (dosing 65 mg), patient B (dosing 50 mg) (solid line) and spiked plasma (dotted line). Peaks: 1, 2 and 3, HP, (−)-RHP and (+)-RHP, respectively.

3.2. Plasma profile of RHP metabolite

We investigated the in vivo metabolism of two HP-dosed schizophrenia patients using this developed CE method. The electropherograms resulting from the analysis of the plasma sample are shown in Fig. 3. Comparing the relative migration times with a spiked plasma sample and the reference enantiomeric RHP, it is interesting that there is only one chiral metabolite, (−)-RHP, exhibited by these two patients. The measured concentrations of HP and (−)-RHP were located between 30–100 ng/ml. Further application of the method to pharmacokinetic studies of HP-treated patients is suggested.

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