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Stereoselective high-performance liquid chromatographic determination of ketamine and its active metabolite, norketamine, in human plasma

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Abstract

A stereoselective high-performance liquid chromatographic method for the determination of the enantiomers of ketamine and its active metabolite, norketamine, in human plasma is described. The compounds were extracted from plasma by liquid–liquid extraction three times in a combination of cyclohexane with 2.5 *M* NaOH, 1 m*M* HCl and 1 *M* carbonate buffer. Stereoselective separation was achieved on a Chiralcel OD column with a mobile phase of *n*-hexane–2-propanol (98:2, v/v). The detection wavelength was 215 nm. The lower limits of the determination of the method were 5 ng/ml for ketamine and 10 ng/ml for norketamine. The intra- and inter-day coefficients of variation ranged from 2.9 to 9.8% and from 3.4 to 10.7% for all compounds, respectively. The method was sensitive and sufficiently reproducible for stereoselective monitoring of ketamine and norketamine in human plasma during pharmacokinetic studies after the administration of ketamine for analgesia. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Ketamine (K) is a dissociative anesthetic agent that has been widely used in clinical practice [1,2]. It was reported that K produced analgesic effects for neuropathic pain of tolerance to antinociceptive effects of morphine when administered in a low dose without anesthetic [3–5]. K and its active metabolites, norketamine (NK), are optically active compounds. *S*-K is four times more potent in the analgesia than *R*-K [6]. NK may contribute to the analgesic effects following K administration [7]. We have prepared K tablets for patients with neuropathic pain of tolerance to antinociceptive effects of morphine [8]. Measurements of the concentration of the enantiomers of K and NK concentration in plasma are required for the detailed pharmacokinetic studies of K tablets in man. Several analytical methods for the determination of K in plasma by gas chromatographic (GC) methods have been reported [9–13]. However, these methods suffer from the lack of stereoselectivity and require derivatization and so on. High-performance liquid chromatographic (HPLC) methods for the determination of K and NK in

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plasma have also been reported [14–17]. However, there are few stereoselective and sensitive HPLC methods for K and NK. Bolze et al. [17] have reported a method with a lack of stereoselectivity, and Geisslinger et al. [16] have reported a method which suffers from a lack of sensitivity for investigating the pharmacokinetics after an administration of K for analgesia. Therefore, these HPLC methods are not applicable to the measurement of the enantiomers of K and NK concentrations in plasma after an administration of K for analgesia. To investigate an appropriate dose design of K for analgesia, we developed a simple, sensitive and stereoselective HPLC method for the determination of K and NK in human plasma after an administration of K for analgesia.

2. Experimental

2.1. Materials

The racemic K was used a commercially available chemical (Sigma, St. Louis, MO, USA). Pure enantiomers of K, NK and bromoketamine (BrK), used as the internal standard (I.S.) in the assay were obtained from Parke-Davis (Morris Palins, NJ, USA). All other chemicals were used as HPLC-grade or as guaranteed reagents.

2.2. Apparatus

The chromatographic system consisted of an LC-6A pump (Shimadzu, Kyoto, Japan), a 7125 injector (Rheodyne, Berkely, CA, USA) equipped with a 100-μl loop, a CTO-6A column oven and an SPD-6A UV detector (Shimadzu, Kyoto, Japan). Stereoselective separation was achieved on a Chiralcel OD column consisting of 3,5-dimethyl carbamoylated cellulose adsorbed on silica (25×0.46 cm I.D., Daicel, Tokyo, Japan).

2.3. Sample purification

To 0.5 ml of plasma were added 0.1 ml of 2.5 *M* NaOH and 25 ng of BrK (I.S.) in 5 ml of cyclohexane. The mixture was mixed using a mechanical mixer (TME-21, Toei Medical Instruments, Tokyo, Japan) for 1 min. After centrifugation in a KN-70 centrifuge (Kubota Seisakusho, Tokyo, Japan) at 3000 rpm for 10 min, 4.5 ml of the organic layer was transferred into another glass tube and was back-extracted into 1 ml of 1 m*M* HCl by mixing for 1 min. Removal of the organic layers was done following centrifugation at 3000 rpm for 10 min, and the aqueous layer of 0.95 ml was transferred to another glass tube. The aqueous layer alkalized with 0.5 ml of 1 *M* carbonate buffer (pH 10.5) was reextracted into 5 ml of cyclohexane by mixing for 1 min. After centrifugation at 3000 rpm for 10 min, 4.8 ml of the organic layer was transferred to another glass tube and was evaporated to dryness. The dry residue was dissolved in 100 μl of the mobile phase, and 50 μl of it was injected into the HPLC column.

2.4. Chromatographic conditions

The mobile phase consisted of *n*-hexane–2-propanol (98:2, v/v). The flow-rate of the mobile phase was 0.8 ml/min, and the column temperature was 35°C. The detection wavelength was 215 nm.

2.5. Calibration curves recoveries and precision of the assay

Solutions containing 1 μg/ml of the enantiomers of K and NK were prepared by dissolving the enantiomers of K and NK in methanol. After 5, 12.5, 25, 50, 125 and 250 μl of solution was placed into a centrifuge tube, the solvent was evaporated, and then 0.5 ml of drug-free human plasma was added to each residue. These samples were assayed according to the method described above. Recoveries were evaluated by comparing the extract and plasma samples containing 100 ng/ml of the enantiomers of K and NK with unextracted standard solutions. The intra- and inter-day variabilities were assessed for the coefficients of variation of five determinations in a plasma sample containing 20, 200 and 400 ng/ml of the enantiomers of K and NK.

2.6. Human study

The study was carried out in accordance with a protocol approved by the Tokyo Posts and Telecommunications Hospital Ethics Committee. A heal-

thy male volunteer (age 28 years and weight 65 kg), who gave informed consent for the study, received 50 mg of racemic K as two 25-mg tablets. Blood samples (6 ml) were collected via an indwelling venous cannula using a disposable syringe before dosing and at 10, 20, 30, 40, 50, 60, 90, 180, 240 and 360 min after dosing. Blood samples were immediately transferred into heparinized glass tubes. Plasma was immediately separated by centrifugation at 3000 rpm for 10 min and stored at −20°C until analysis.

3. Results and discussion

Several HPLC methods for the determination of K and NK in plasma have been reported; e.g. Bolze et al. [17] reported that the detection limit of racemic K and NK is 5 ng/ml. However, there are few stereoselective HPLC methods for K and NK [15,16]. In the preliminary study, chromatograms showed an interfering plasma peak in a single extraction with 2.5 *M* NaOH or 1 *M* carbonate buffer (pH 10.5). However, the present method produced the smallest interfering plasma peak on chromatograms because of the liquid–liquid extraction three times in the combination of cyclohexane with 2.5 *M* NaOH, 1 m*M* HCl and 1 *M* carbonate buffer. In the present method, the enantiomers of K and NK did not undergo inversion during the extraction. Typical chromatograms of standard solution, extract from drug-free human plasma, extract from human plasma supplemented with each enantiomers of 25 ng K, NK and 25 ng of BrK (I.S.) and an extract from plasma samples obtained from a healthy volunteer after oral administration of 50 mg of racemic K are shown in Fig. 1. No interfering peaks were observed when blank plasma samples were analyzed, and each peak was sharp. The retention times of *R*-K and *S*-K were 11.6 and 13.5 min and those of *S*-NK, *R*-NK and BrK were 25, 27 and 15.5 min, respectively (Fig. 1). In the pilot study, endogenous substances extracted with *n*-hexane and methylene chloride from plasma did not interfere with the analysis of K and NK; however, the extraction recovery of NK was about 60 and about 40%, respectively. In the present method, the extraction recoveries of K and NK from

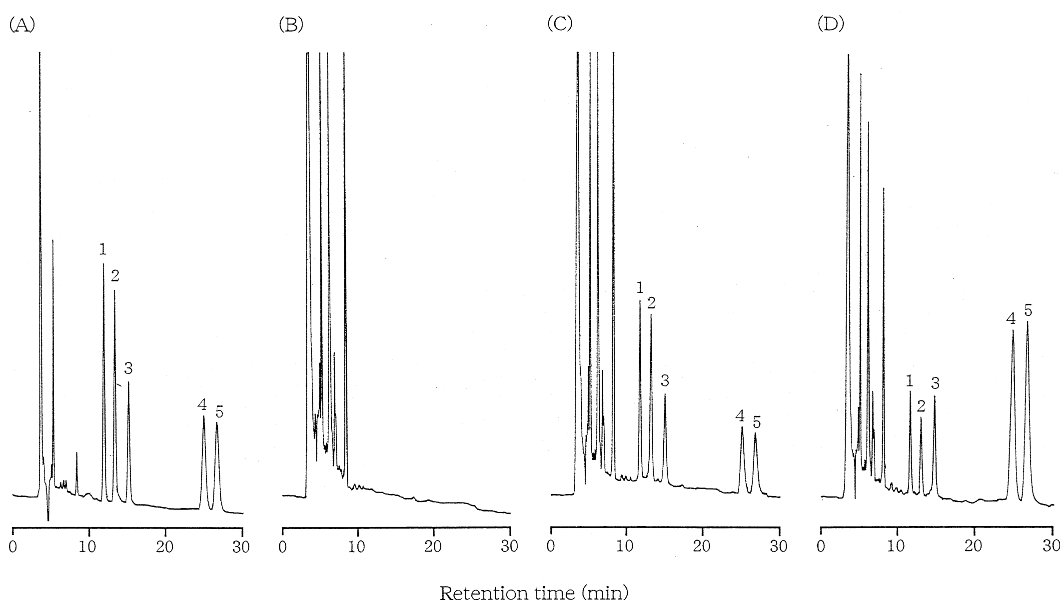

Fig. 1. Typical chromatograms obtained from (A) standard solution containing 25 ng of enantiomers of K, NK and 25 ng BrK; (B) extract from blank plasma; (C) extract from blank plasma spiked with 25 ng of enantiomers of K, NK and 25 ng BrK; (D) extract from healthy volunteer's plasma after oral administration of 50 mg of racemic K (*R*-K, 26 ng/ml; *S*-K, 22 ng/ml; *S*-NK, 117 ng/ml; *R*-NK, 130 ng/ml). 0.002 A.U.F.S.. Peaks: 1=*R*-K; 2=*S*-K; 3=BrK (I.S.), 4=*S*-NK; 5=*R*-NK. Abbreviations: K: ketamine, NK: norketamine, BrK: bromoketamine.

Table 1
Recoveries of each enantiomer of ketamine and norketamine[a]

	R-Ketamine	*S*-Ketamine	*R*-Norketamine	*S*-Norketamine
Recovery (%)	88.1±8.0	87.4±8.6	91.3±8.6	86.1±8.6
C.V (%)	9.1	9.8	10.8	10.2

[a] Each value represents the mean±SD of five determinations; C.V., coefficient of variation.

plasma using cyclohexane for extraction was >85%, and the coefficients of the variation were <11% as shown in Table 1. The calibration curves exhibited good linearity over the enantiomers of K and NK concentration range of 10–500 ng/ml, and the lines passed through the origin. Correlation coefficients of better than 0.998 were obtained for each enantiomer of K and NK. Although lower limits of the determination were <5 ng/ml for the enantiomers of K and NK with a signal-to-noise ratio of about 10:1, the present method was 5 ng/ml for the enantiomers of K and 10 ng/ml for the enantiomers of NK with coefficients of variation (C.V.) of less than 15% because of the precision of the assay. The intra- and inter-day precision of the method ranged from 2.9 to 9.8% (C.V.) and from 3.4 to 10.7% (C.V.) at 20, 200 and 400 ng/ml of the enantiomers of K and NK, respectively (Tables 2 and 3). Geisslinger et al. [16] reported the determination of the enantiomers of K and NK in plasma using liquid–liquid extraction. However this method, with lower limits of determination of about 40 ng/ml, suffers from the lack of sensitivity for investigating the pharmacokinetics after an administration of K for analgesia. Svensson et al. [15] reported the determination of the enantiomers of K and NK in plasma using solid-phase extraction with a Sep-Pak light C_{18} cartridge, because liquid–liquid extraction gave insufficient purification and sensitivity. However, we demonstrated that our method using liquid–liquid extraction has recoveries and sensitivities equal to those of Svensson.

To confirm whether the present method will be applicable to clinical studies, the time courses of the concentration of the enantiomers of K and NK in plasma after s single administration of 50 mg of racemic K to a healthy volunteer are shown in Fig. 2. *R*-K and *S*-K were found in the plasma 30 min after

Table 2
Intra-day variabilities in each enantiomer of ketamine and norketamine[a]

Conc. added (ng/ml)	*R*-Ketamine		*S*-Ketamine		*R*-Norketamine		*S*-Norketamine	
	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)
20	21.5±2.1	9.8	22.8±0.9	4.0	17.7±1.7	9.7	18.6±1.4	7.4
200	217.3±13.2	6.1	204.5±8.6	4.2	194.5±5.2	2.6	194.2±5.7	2.9
400	408.5±19.0	4.6	399.5±24.5	6.1	398.5±25.9	6.5	398.7±23.9	6.0

[a] Each value represents the mean±SD of five determinations; C.V., coefficient of variation.

Table 3
Inter-day variabilities of each enantiomer of ketamine and norketamine[a]

Conc. added (ng/ml)	*R*-Ketamine		*S*-Ketamine		*R*-Norketamine		*S*-Norketamine	
	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)	Conc. found (ng/ml)	C.V. (%)
20	20.8±1.5	7.4	20.6±2.2	10.7	20.2±1.9	9.2	19.9±1.9	9.5
200	200.5±11.8	5.8	199.8±14.1	7.0	193.7±8.1	4.1	194.2±6.7	3.4
400	401.7±22.2	5.5	406.1±32.1	7.9	406.0±19.4	4.7	406.1±17.7	4.3

[a] Each value represents the mean±SD of five determinations; C.V., coefficient of variation.

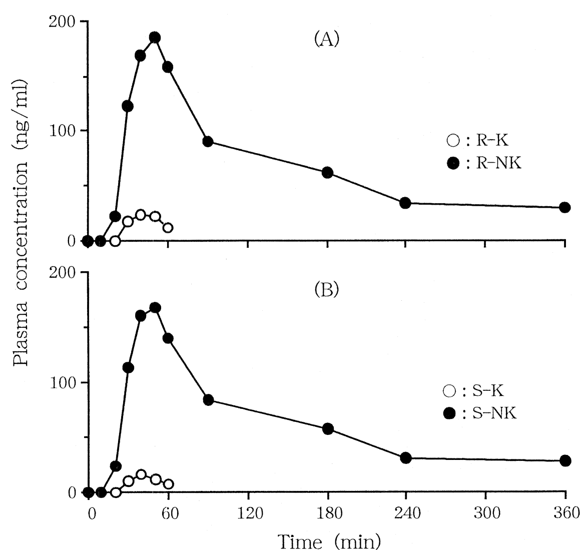

Fig. 2. Stereoselective plasma concentration–time profile of (A) *R*-enantiomers, (B) *S*-enantiomer of K and NK after oral administration of 50 mg of racemic K to a healthy volunteer. K: ketamine, NK: norketamine.

administration, and the maximum plasma concentrations of 24 and 16 ng/ml, respectively, were observed 40 min after administration. Thereafter, the enantiomers of K were less than the lower limits of the determination after 90 min. *R*-NK and *S*-NK were found in plasma 30 min after administration, and the maximum plasma concentrations of 185 and 168 ng/ml, respectively, were observed 50 min after administration. Thereafter, the concentrations of *R*-NK, *S*-NK decreased to 30 and 28 ng/ml, respectively, after 360 min. In the present method, 10 ng/ml, the lower limit of the determination for NK, is less sensitive than 5 ng/ml for K. However, it is useful for the investigation of the pharmacokinetics of K because the measurement of NK is possible up to 360 min after oral administration of 50 mg of K.

In conclusion, a simple, sensitive and reproducible method for the stereoselective determination of K and NK in human plasma by HPLC was established. The method will be particularly useful for pharmacokinetic study following administration of K for analgesia and will be applicable to pharmacokinetic studies for using K tablets efficiently and safely in man.

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