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Enantioselective high-performance liquid chromatographic determination of nicardipine in human plasma

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Abstract

A sensitive method for the enantioselective high-performance liquid chromatography (HPLC) determination of nicardipine in human plasma is described. (+)-Nicardipine, (−)-nicardipine and (+)-barnidipine as an internal standard are detected by an ultraviolet detector at 254 nm. Racemic nicardipine in human plasma was extracted by a rapid and simple procedure based on C_{18} bonded-phase extraction. The extraction samples were purified and concentrated on a pre-column using a C_1 stationary phase and the enantiomers of nicardipine are quantitatively separated by HPLC on a Sumichiral OA-4500 column, containing a chemically modified Pirkle-type stationary phase. Determination of (+)− and (−)-nicardipine was possible in a concentration range of 5–100 ng ml^{-1} and the limit of detection in plasma was 2.5 ng ml^{-1}. The recoveries of (+)- and (−)-nicardipine added to plasma were 91.4–98.4% and 93.3–96.7%, respectively, with coefficients of variation of less than 9.0 and 9.4% respectively. The method was applied to low level monitoring of (+)- and (−)-nicardipine in plasma from healthy volunteers. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Nicardipine

1. Introduction

Nicardipine hydrochloride, 2-(*N*-benzyl-*N*-methylamino) ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedecarboxylate monohydrochloride (I) (Fig. 1), is one of the most used calcium entry-blocking agents with potent oral vasodilating activity [1]. It is used for the treatment of hypertension [2] and cerebrovascular disease [3]. The pharmacological potency of the (+)-enantiomer of nicardipine has been found to be three times higher than that of the (−)-enantiomer [4], which

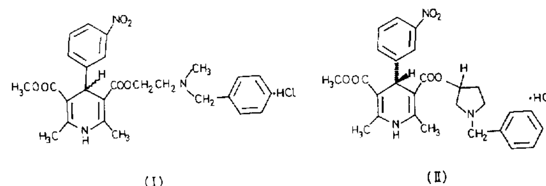

Fig. 1. Chemical structure of racemic nicardipine (I) and (+)-barnidipine (II).

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implies a different pharmacokinetic behaviour of both enantiomers.

Several methods have been reported for the determination of nicardipine in plasma [5–7]. Recently, enantioselective chromatographic determination of dihydropyridine calcium antagonists in plasma has been reported using gas chromatography [8,9], gas chromatography–mass spectrometory (GC–MS) [10,11] and HPLC [12,13]. Optical resolution of dihydropyridine enantiomers, containing nicardipine, has been reported by Ohkubo et al. [14] and other papers [15] using a (+)-poly(triphenylmethyl methacrylate) and xylan- or cellulose (phenylcarbamate)-type column. However, we could not obtain highly sensitive enantiospecific determination of dihydropyridines with the above columns because of peak broadening on the chromatogram. Recently, enantioselective determination of nicardipine has been reported by Iwaoka et al. [16]. However, in this study, serum (+)- and (−)-nicardipine were separated by HPLC [15] and detected by GC–MS after isolation of (+)- and (−)-nicardipine. This method is very tedious and time-consuming.

Therefore, we established a chromatographic separation of high sensitivity with sharp peaks of dihydropyridine enantiomers in our previous study using a Pirkle-type column [17]. A liquid–liquid extraction method was used in the previous papers [8–13], however, such a method involves tedious procedures. Recently, we described an extraction method using a solid bonded phase for the efficient recovery of several drugs from plasma or serum samples [18–20].

This method is satisfactory in terms of simplicity and rapidity. In the present paper, we describe a solid-phase extraction method for nicardipine in plasma and an enantioselective HPLC method. This method was applied to an enantioselective pharmacokinetic study in healthy volunteers.

2. Experimental

2.1. Reagents and materials

Nicardipine was obtained from Sigma (St. Louis, Mo, USA) and (+)-barnidipine (II) (Fig. 1) was kindly donated by Yamanouchi (Tokyo, Japan). The Sep-Pak C_{18} cartridge was purchased from Waters (Milford, MA, USA). All solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals of analytical grade were purchased from Wako Pure Industries or Nakarai Tesque (Kyoto, Japan).

2.2. Apparatus

The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) equipped with a Jasco UV-880 ultraviolet detector (Jasco). The wavelength of the detector was set at 254 nm. Test samples were introduced using a Rheodyne Model 7120 injector (Rheodyne, Cotati, CA, USA) with an effective volume of 100 μl. Another Rheodyne Model 7120 injector was used as the switching valve. The HPLC precolumn (10×4.6 mm I.D.) contained a trimethylsilirated silica stationary phase (5 μm) which was prepared in our laboratory. The analytical column (250×4.6 mm I.D.) contained a Sumichiral OA-4500 stationary phase (5 μm) (Sumika Chemical Analysis Service, Osaka, Japan). The mobile phase consisted of *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (250:40:10:1, v/v), and the flow-rate was 1 ml min^{-1} at ambient temperature.

2.3. Preparation of sample

(+)-Barnidipine (100 ng) in methanol (10 μl) was added to the plasma sample (1 ml) as an internal standard, the plasma sample was then diluted with 5 ml of a 1 *M* NaCl solution and the solution was briefly mixed. The mixture was applied to a Sep-Pak C_{18} cartridge that had previously been activated with 5 ml of methanol and water. The cartridge was then washed with 10 ml of water and 10 ml of 40% methanol, and then the desired fraction was eluted with 5 ml of 80% methanol. The eluate was dried by evaporation in a vacuum at 60°C. The residue was dissolved in 0.2 ml of chloroform and 50-μl extracts were loaded onto the precolumn for the elimination of interfering substances from the plasma sample. After washing for 0.5 min, (+)-, (−)-nicardipine and (+)-barnidipine were eluted from the precolumn and then led to the analytical column by a column-

switching technique using the previously described mobile phase.

2.4. Calibration graphs

Known amounts of racemic nicardipine in the range of 10–200 ng ml^{-1} [5–100 ng ml^{-1} of (+)- and 5–100 ng ml^{-1} of (−)-nicardipine] were added to blank plasma samples. These plasma samples were treated according to the extraction procedure described above. The peak-height ratios of (+)-nicardipine and (−)-nicardipine to (+)-barnidipine were measured and plotted against the respective concentration of analyte.

2.5. Preparation of quality control and calibration samples

Duplicate samples were prepared in 1.0 ml of plasma, by adding aliquots of the stock solution of racemic nicardipine to drug-free plasma at four different concentrations – 10, 50, 100 and 200 ng ml^{-1} of racemic nicardipine, equal amounts of (+)- and (−)-nicardipine – to determine the accuracy and precision of the method. These samples are designated quality control samples. The quality control samples were stored at −40°C. Calibration samples containing 10, 20, 50, 100 and 200 ng ml^{-1} of racemic nicardipine were prepared. The calibration samples were treated in the same manner as the quality control samples. For each validation run, a quality control sample was thawed and extracted.

2.6. Recovery experiments

Plasma was spiked by adding a known amount of racemic nicardipine to drug-free plasma to obtain a total volume of 1 ml. These samples were extracted by the above described method. Control samples were prepared by adding a known amount of racemic nicardipine to 1 ml of methanol. These control samples were not extracted, but directly dried by evaporation at 60°C, and the residues reconstituted in 0.2 ml of chloroform. An external standard instead of the internal standard was added to all of the samples before being dried by evaporation. Recoveries were determined by comparison between solid-phase extraction and no extraction as control.

2.7. Method validation

The accuracy of the method was determined by injection of the racemic nicardipine calibration samples and the four different quality control samples after extraction on five separate days. Calibration curves of (+)- and (−)-nicardipine were required to have a correlation value of at least 0.997. The accuracy was calculated as a percentage of the nominal concentration. Accuracy=(conc. obs./conc. nominal)·100%. The same data used in the accuracy determinations was used for the calculation of the between-run percentage relative standard deviation [% R.S.D., R.S.D.=(S.D./mean)·100%]. The within-run % R.S.D. resulted from analysis of five quality control samples at each concentration with injection on the same day. The detection limit of the HPLC assay after extraction was estimated as the drug quantity in plasma giving a signal five times the baseline noise. The lower limit of quantitation was defined as the quantity of the sample after preparation and extraction, which could be quantified with deviation and precision of less than 20%.

2.8. Drug administration and sampling

Racemic nicardipine tablet (40 mg) (20 mg of Perdipine brand of nicardipine, Yamanouchi Pharmaceutical, Tokyo, Japan) was orally administered to seven healthy male volunteers. Blood samples (5 ml) were collected by venipuncture at 0.5, 1, 2, 3, 4 and 6 h after administration. Plasma samples were separated by centrifugation at 1900 *g* for 15 min and stored at −40°C until analysis.

3. Results

We established that racemic nicardipine was clearly resolved into its enantiomers on a Pirkle-type chiral column (Sumichiral OA-4500) with a nonaqueous mobile phase (*n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid, 250:140:10:1, v/v). The resolution of (+)- and (−)-nicardipine in human plasma was 1.2 and the time of analysis was less than 38 min. A typical chromatogram of a standard mixture of racemic nicardipine and (+)-barnidipine in plasma is shown in Fig. 2. Low

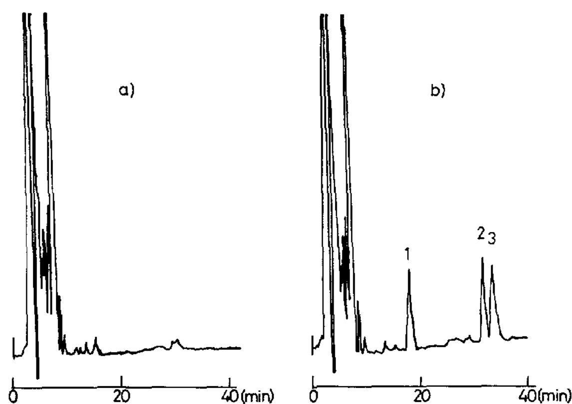

Fig. 2. Typical chromatogram of (a) plasma blank, (b) added standards in plasma. Peaks: 1=(+)-barnidipine (I.S.), 2=(+)-nicardipine, 3=(−)-nicardipine.

interference from endogenous components of plasma was obtained by the present extraction method employing a column-switching technique using a C_1 short column.

Calibration graphs for (+)- and (−)-nicardipine in human plasma were linear in the range 5–100 ng ml^{-1} with a correlation coefficient of at least r=0.997. The limits of detection for (+)- and (−)-nicardipine were each 2.5 ng ml^{-1} (signal-to-noise ratio=5). The results of recovery studies are shown in Table 1. The recovery of (+)- and (−)-nicardipine was determined by adding the four known levels of 5, 25, 50 and 100 ng ml^{-1} to blank plasma. The recovery values were 91.4–98.4% for (+)-nicardipine and 93.3–96.7% for (−)-nicardipine in plasma. Coefficients of variation were less than 9.0% for (+)-nicardipine and less than 9.4% for (−)-nicardipine.

Representive plasma concentration–time profiles for (+)- and (−)-nicardipine after administration of 40 mg of racemic nicardipine are shown in Fig. 3. (+)-Nicardipine concentration is higher than the (−)-nicardipine concentration at each sampling time. The mean pharmacokinetic parameters for the enantiomers were calculated for seven healthy volunteers and are shown in Table 2. The maximum concentration and the $AUC_{0\rightarrow6}$ were about 2.3-fold higher for (+)-nicardipine than for (−)-nicardipine. There was no difference between (+)- and (−)-nicardipine in the other parameters.

4. Discussion

Initially, our efforts were directed towards developing an efficient enantiomer separation – i.e. a sharp peak on a HPLC chromatogram – since no sufficient sensitivity in biological samples could be obtained by the previous chiral column HPLC detection system, because of peak broadening of both peaks on the chromatogram. Therefore, these methods needed a more sensitive GC–MS detection system; however, off-line HPLC–GC–MS detection is very tedious and GC–MS is very expensive. In a previous paper [17] we established enantiomer separation of 1,4-dihydropyridines using Pirkle-type columns (Sumichiral OA series), giving a satisfactory chromatogram with sharp peaks suitable for on-line ultraviolet detection. Our present enantio-separation HPLC system gave a higher detection limit (2.5 ng ml^{-1} in plasma) than previous papers. Under our chromatographic conditions, (+)-nicardipine eluted at 33.7 min and (−)-nicardipine at 35.8 min. The

Table 1
Accuracy and precision of nicardipine enantiomers in human plasma

Added (ng ml^{-1})	(+)-Nicardipine				(−)-Nicardipine			
	Found (mean±S.D.) (ng ml^{-1})	Accuracy (%)	Between-run precision (%)	Within-run precision (%)	Found (mean±S.D.) (ng ml^{-1})	Accuracy (%)	Between-run precision (%)	Within-run precision (%)
5	4.9±0.79	98.4	7.9	6.8	4.8±0.44	94.8	4.7	6.7
25	24.5±4.41	98.0	9.0	6.6	23.9±4.54	95.6	9.4	8.3
50	45.7±2.63	91.4	2.9	5.6	48.4±6.91	96.7	7.1	8.3
100	92.8±5.35	92.8	2.9	4.7	93.3±6.49	93.3	5.5	3.6

n=5.

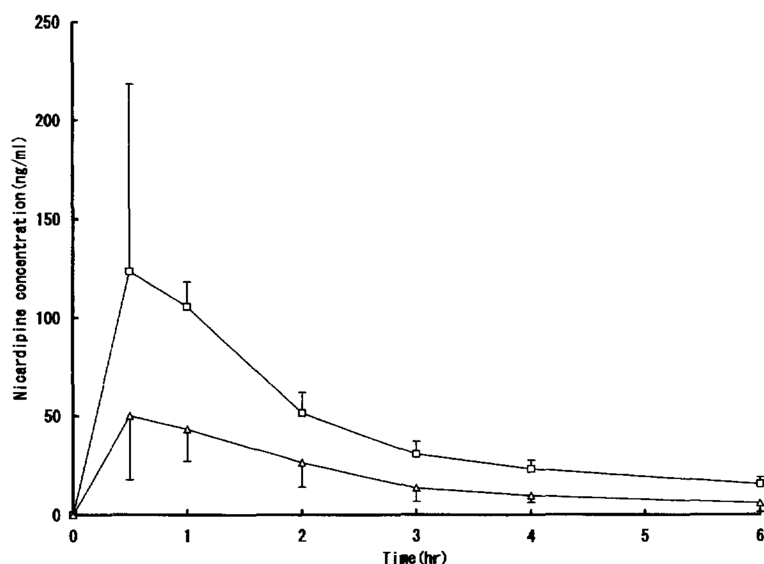

Fig. 3. Mean plasma concentration–time profile of (+)-nicardipine and (−)-nicardipine after a single 40-mg oral dose of racemic nicardipine to seven healthy volunteers. □: (+)-nicardipine, △: (−)-nicardipine.

observed stereochemical selectivity (α) was 1.05 and the stereochemical resolution factor (*R*) was 1.2. Therefore, this HPLC system could be applicable to the measurement of (+)- and (−)-nicardipine in human plasma using an ultraviolet detection system.

Subsequently, we directed our project towards establishing an extraction method for racemic nicardipine and (+)-barnidipine, and elimination of endogenous substances from plasma. Bocker et al. [21] reported a simple extraction method for nitrendipine and its metabolites in an incubation mixture of liver microsome using a Bond Elut C_{18} extraction column. However, in our present study, the high interference peak of endogenous components in plasma was not removed with Sep-Pak C_{18} extraction. In our previous paper, we described a serum sample direct injection HPLC system coupled with a column-switching method for analysis of several drugs [22–24], and a solid-phase extraction method of manidipine coupled with a column-switching technique [25]. No interference of the chromatogram was obtained by this method. Therefore, we used a column-switching technique after extraction in the present study and no interference of the chromatograms was observed at the retention time of the (+)-, (−)-nicardipine and (+)-barnidipine (Fig. 2). The plasma concentration of nicardipine was reported by Higuchi et al. [26].

The lowest plasma concentration of the nicardipine enantiomers for therapeutic drug monitoring may be at 2.5 ng ml^{-1} for each enantiomer in the dose range of 10–40 mg. The sensitivity and calibration range of the present method are appropriate for therapeutic drug monitoring of nicardipine enantiomers in patients. The results of recovery studies (Table 1) show that the proposed method is satisfactory with respect to accuracy and precision.

The method described was used to study the

Table 2
Pharmacokinetic parameters of nicardipine enantiomers after a oral racemic 40-mg dose to seven healthy volunteers

	C_{max} (ng ml^{-1})	T_{max} (h)	*Ke* (h^{-1})	$T_{1/2}$ (h)	$AUC_{(0\rightarrow\infty)}$ (ng·h ml^{-1})
(+)-Nicardipine	152.4±68.0	0.8±0.3	0.41±0.03	1.7±0.1	273.2±18.7
(−)-Nicardipine	65.1±21.3	0.7±0.03	0.46±0.05	1.6±0.2	116.9±10.9

n=7.

pharmacokinetics of (+)- and (−)-nicardipine in an oral dose of 40 mg racemate given to seven healthy volunteers (Fig. 3). The pharmacokinetic parameters obtained in the present study (Table 2) are similar to those of a previous paper [26]. The $AUC_{0\to\infty}$ value of (+)-nicardipine (273.2±18.7 ng·h ml^{-1}) plus (−)-nicardipine (116.9±10.9) is the same value as in the previous paper (403.66 ng·h ml^{-1}) [27]. The values of $T_{1/2}$ and T_{max} are similar to the previously found values. However, the C_{max} and $AUC_{0\to\infty}$ were higher for (+)-nicardipine than for (−)-nicardipine in the present study. The stereoselective activity of 1,4-dihydropyridine isomers has been described in previous papers [27]. The pharmacokinetic analysis of 1,4-dihydropyridine enantiomers is useful for understanding the stereoselective contribution of both enantiomers to the pharmacological activity and to understand the different influence each enantiomer has on the pharmacokinetics. From the results, it was shown that the proposed method for the determination of (+)- and (−)-nicardipine could be applied to pharmacokinetic study in patients receiving racemic nicardipine treatment. Further pharmocokinetic studies of drug interaction will be carried out in these laboratories, and the details will be reported elsewhere.

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