HPLC Method for the Determination of Nicorandil in Human Plasma

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Abstract − The present study is to determine of sensitive nicorandil analysis method using HPLC and measure the pharmacokinetics parameters (bioavailability, C_{max}, T_{max}, Ke, $T_{1/2}$) of nicorandil (5 mg, Tab; Choongwae Pharma Corporation). Plasma (500 ul) was mixed with furosemide (internal standard, 500 ug/ml). Detection wavelength was 256 nm. The mixture of 0.01 M ammonium acetate and acetonitrile 80:20 (v/v) was used mobile phase. The HPLC separation was accomplished on ODC reverse HPLC column. The nicorandil was analyzed by a HPLC system, which consists of CAPCELL PAK C18 column (5 μm, 4.6×150 mm) and a chromatography data analysis S/W, using a isocratic mobile phase (mixture of 0.01 M ammonium acetate and acetonitrile 80:20 <v/v>) at 1.0 ml/min. Its sensitivity, selectivity, accuracy and precision must be adequate for the bioavailabilty study of nicorandil, and the linearity ($r^2 \geq 0.9994$) of nicorandil was also proved in the range of 0.05 ug/ml − 3 ug/ml. The pharmacokinetic parameters of nicorandil (5 mg) tablets were measured as the follow. AUC: 0.19 ug/ml·hr, C_{max}: 0.14 ug/ml, t_{max}: 0.58 hr, Ke: 0.11 hr$^-$, $t_{1/2\beta}$: 6.76 hrs. This method is simple and sensitive HPLC method using UV detector for determination of nicorandil in human plasma.

Keywords: Nicorandil, Pharmacokinetic parameters, HPLC

INTRODUCTION

Nicorandil has been proposed for use as direct coronary vasodilators in the treatment of both vasospastic and chronic stable angina (Hamilton and Weston, 1989; Lablanche *et al.*, 1993; Why and Richardson, 1993). Studies of nicorandil (Bachert and Fung, 1993; Berdeaux *et al.*, 1992; Frukawa *et al.*, 1981; Holzmann, 1983; Kukovetz *et al.*, 1992) demonstrate that its relaxant effect on coronary arterioles is inhibited by the K^+ channel blocker, glyburide, and thus is likely due to K^+ channel activation, with attendant cellular hyperpolarization of vascular smooth muscle. However, this agent also exerts a nitrate-like effect, stimulating guanylyl cyclase to increase cyclic GMP, primarily in epicardial coronary arteries, including stenotic segments (Meisheri, 1991). Drugs that have their primary site of vasodilation at the arteriolar level generally are not of benefit in the treatment of angina (Anonymous, 1992). The efficacy of nicorandil may be primarily due to its nitrate-like effect.

Various kind of methods for determining nicorandil in human plasma have been reported (Andrensek *et al.*, 1999; Bachert and Fung, 1993; Mawatari *et al.*, 1996; Ojha and Pargal, 1999). These methods are HPLC method using fluorescence detector (Mawatari *et al.*, 1996) and liquid chromatographic method (Ojha and Pargal, 2003).

In the study a sensitive and simple HPLC method using UV detector was performed for the quantitation of nicorandil in human plasma and the bioavailability study was performed by using the HPLC in healthy human volunteers after oral administration of nicorandil.

MATERIALS AND METHODS

Nicorandil tablets as the test product (Sigmat^{TM} tablet : nicorandil, a 5 mg tablet) was obtained from Choongwae Pharma Corporation (Seoul, Korea). Furosemide used as the internal standard, ammonium acetate, dichloromethane, sodium bicarbonate and ethyl acetate were purchased from Sigma (St. Louis, MO, USA). Acetonitrile was purchased from Merck. All agents were of analytical grade.

After approval of pre-planed proposal by Korea Food

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and Drug Administration (KFDA), male volunteers who submitted the agreement to attend to this project were medically examined and 9 volunteers were selected by a medical doctor in Hanyang Medical Center (Seoul, Korea), based on clinical examination including seropathological (hemoglobin, hematocrit, WBC, platelet), serochemical (blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, akaline phosphatase) and urological (specific gravity, color, pH, sugar, alumin, bilirubin, RBC, WBC) data. An exclusion criterion for selecting volunteers includes taking frequently any medicine such as hypertensive agent and vitamins or nutrient aids. They were accommodated at the clinical pharmacokinetic room at the Hanyang Medical Center one day before blood collection. They were fasted for at least 12 hr before administration of tablets. Lunch and dinner were allowed, respectively, 4 and 12 hr after drug intake. Physical and biological examinations were carried out before and after completion of the study.

A 22-gauge i.v. catheter on arm vein was established on the arm vein of each volunteer and 7 ml of the blood were collected for blank. According to the prescription directed by a doctor, two tablets (a 5 mg tablet nicorandil) were orally taken by each volunteer of the designated group with 240 ml of water. Blood was collected into EDTA-treated tubes (Vacutainer) at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hr after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood collection time. Blood was centrifuged to obtain plasma. The plasma was stored at -70°C until analyzed.

Conditions of analysis

Nicorandil analysis was performed by HPLC with UV detector (Gilson 151). Gilson 234 autosampler was used and Gilson Unipoint 3.0 data module was supported by computer. The detector was set at 256 nm. Symmetry C_{18} (4.6 × 150 mm, 5 um, Shiseido, Japan) column was used. The mobile phase for nicorandil analysis consists of acetonitrile and ammonium acetate (0.01 M) (2 : 8, v/v). The flow rate of the mobile phase was set to 1.0 ml/min.

Ploting of calibration curve

The stock solution of nicorandil was prepared by dissolving it in acetonitrile as 20 ug/ml. Plasma sample were made. The concentrations of nicorandil of plasma sample were 0.05, 0.1, 0.5, 1, 2 and 3 ug/ml. Furosemide, internal standard, was dissolved in acetonitrile as 500 ug/ml. In 10 ml centrifuge tube 500 ul of the 0.05, 0.1, 0.5, 1, 2 and 3 ug/ml of nicorandil solution was added, respec-

tively. 50 ul of furosemide (500 ug/ml), 20 ul of 60% $HClO_4$ and 0.5 ml of dichloromethane were added. The tube was centrifused at 13000 rpm and 4°C for 10 min. To 400 ul of supernatant, 50 ul of 1 M Na_2CO_3 and 1 ml of ethyl acetate were added. After shaking 900 ul of supernatant was evaporated in -60°C nitrogen. The evaporated supernatant dissolved in 0.5 ml of mobile phase was filtered. 20 ul of filtered solution was applied to the instrument. Calibration curves were made by plotting the concentrations of nicorandil added at x-axis and peak area ratios of the nicorandil to internal standard at y-axis. Intra-a day (within-a day) and inter-days (between-days) precisions and accuracies were obtained from the five repeated experiment, respectively.

Processing of plasma sample

The frozen plasma samples were thawed at room temperature, vortex-mixed, and 500 ul of the sample was added to the eppendorf tubes. 50 ul of furosemide (500 ug/ml in acetonitrile) was added to human plasma sample. The rest of the clean-up procedure was the same as described above. The nicorandil plasma concentrations in human volunteers were determined based on the calibration curves from peak area ratios of nicorandil to the internal standard.

Calculation of pharmacokinetic parameters

Pharmacokinetic parameters, AUC, C_{max}, T_{max}, Ke, $t_{1/2}$, were determined from the time-plasma concentrations of nicorandil. The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from time-plasma concentration curves of nicorandil. The area under the curve of time-plasma concentrations of nicorandil until the last sampling time ($AUC_{0\ to\ last}$) was determined by the equation of $AUC_{0\ to\ inf} = AUC_{0\ to\ last} + C_{last}/\beta$, where β, is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time (Shargel and Yu, 1993).

Data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Nicorandil is a nicotinamide ester that has vasodilating properties in normal coronary arteries but complex effects in patients with angina. Clinical studies suggest that both preload and afterload. It also provides some myocardial protection via preconditioning by activation of cardiac K_{ATP} channels. One large trial showed a significant reduction in relative risk of fatal and nonfatal coronary events in patients receiving the drug (Anonymous, 1992).

The plasma concentrations of nicorandil in healthy volunteers were determined and validated by HPLC with a UV detector. The HPLC chromatograms obtained from either its internal standard and nicorandil were showed in Fig. 1. Retention times of internal standard and nicorandil were about 9.3 and 5.4 min, respectively and no interfering peaks were observed at these times, showing good separation between peaks. Total run time for determing one sample was within 15 min. Precision and accuracy were presented in Table I. The lower limit of quantitation for nicorandil in human plasma was decided to be 0.05 ug/ml, at which the within-a day and between-days precision were less than 20%, and 0.3 ug/ml, at which the accuracy were less than 20%. The signal to noise ratios for nicorandil peaks were larger than 5. The linearity of nicorandil calibration curve was good (r^2=0.9994) within the equation of y (ratio of peak area

Table I. Nominal list and personal items of volunteers

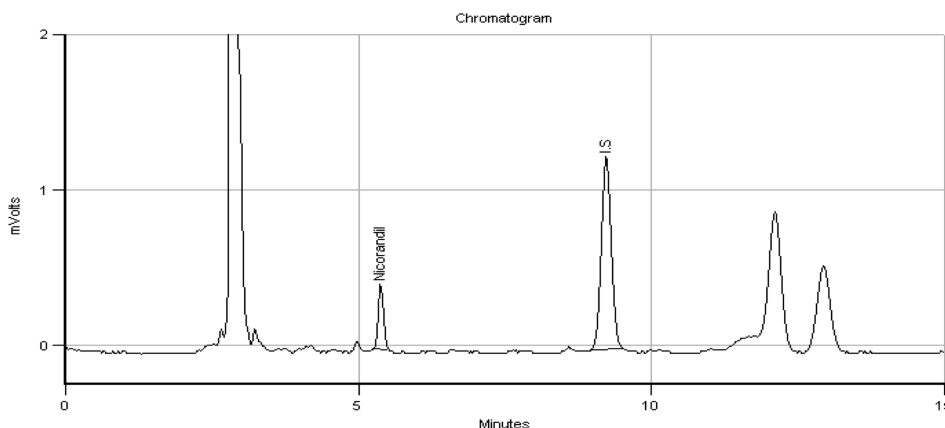| No.	Age(year)	Body Weight(kg)	Height(cm)
A1	30	63.1	171.6
A2	30	60.7	172.4
A3	26	77.4	177.4
A4	25	74	175
A5	26	67.6	166.2
A6	25	66.6	178.7
A7	24	88	172.9
A8	25	73.5	172.8
A9	24	73.1	175.8
MEAN	26.1	71.6	173.6
SD	2.3	8.3	3.7

Fig. 1. Chromatograms obtained by HPLC with a UV detector from human plasma blank with the internal standard (I.S.) and nicorandil. Furosemide was used as the internal standard. Nicorandil was eluted at 5.4 min and furosemide at 9.3 min.
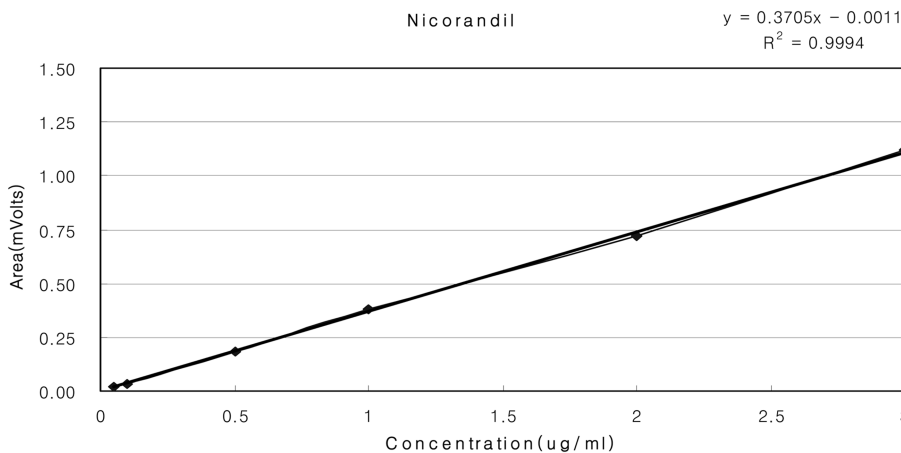

$$y = 0.3705x - 0.0011$$
$$R^2 = 0.9994$$

Fig. 2. The linearity of nicorandil calibration curve : y(ratio of peak area nicorandil to internal standard)=0.3705 × concentration of nicorandil - 0.0011 (r^2=0.9994).

Table II. Precision and accuracy for the determination of nicorandil in the human plasma

Concentrations (ug/ml) of nicorandil	Precision(CV%)		Accuracy (%)
	Within-a day (n=5)	Between-days (n=5)	
0.05 ug/ml (lower limit of quantitation for nicorandil)	14.13	13.82	111.15
0.1 ug/ml	5.93	4.78	95.91
0.5 ug/ml	3.91	4.26	100.22
1.0 ug/ml	2.46	4.81	102.23
2.0 ug/ml	2.21	4.12	97.37
3.0 ug/ml	1.59	4.02	100.92
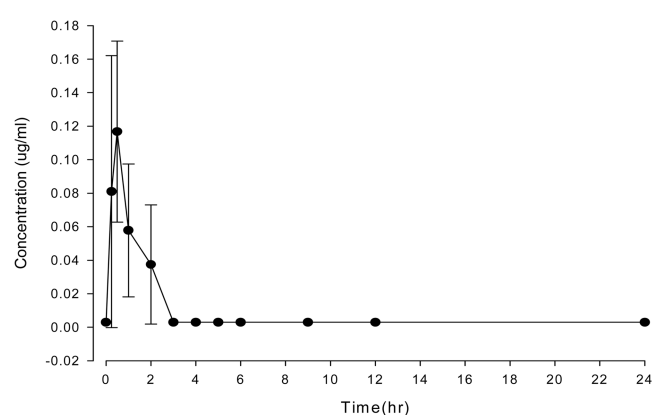
Fig. 3. The time-plasma concentration curves of nicorandil in human volunteers after oral administration of nicorandil to healthy volunteers. Mean (±SD) values of plasma nicorandil concentrations of 9 volunteers were represented.

Table III. Pharmacokinetic parameters obtained after oral administration of nicorandil (as total 5 mg) to human healthy volunteers

Subject	Parameter				
	AUC (ug·hr/ml)	C_{max} (ug/ml)	T_{max} (hr)	Ke	$t_{1/2}$ (hr)
A1	0.16	0.08	2.0	0.07	10.56
A2	0.12	0.10	0.5	0.08	8.43
A3	0.14	0.13	0.25	0.11	6.49
A4	0.24	0.16	0.5	0.13	5.48
A5	0.20	0.17	0.5	0.12	5.87
A6	0.16	0.10	0.5	0.10	6.73
A7	0.14	0.18	0.25	0.09	7.68
A8	0.32	0.22	0.25	0.16	4.27
A9	0.26	0.16	0.5	0.13	5.31
Mean	0.19	0.14	0.58	0.11	6.76
STD	0.07	0.05	0.54	0.03	1.90

nicorandil to internal standard)=0.3705 × (concentration of nicorandil) − 0.0011 at concentrations ranging from 0.05 to 3 ug/ml (Fig. 2). This data suggest that the method was suitable to determine the plasma concentrations of nicorandil and applicable to the pharmacokinetic and bio-availability studies.

From the time-plasma concentrations of nicorandil in healthy human after oral administration of nicorandil (Fig. 3), principal pharmacokinetic parameters were determined. The parameters for individual subjects are seen in Table II. The value of AUC_t was 0.19 ± 0.07 ug·hr/ml. The value of C_{max} was 0.14 ± 0.05 ug/ml. The value of T_{max} was 0.58 ± 0.54 hr.

Various kind of methods for determining nicorandil in human plasma have been reported (Andrensek *et al.*, 1999; Bachert and Fung, 1993; Mawatari *et al.*, 1996; Ojha and Pargal, 2003). These methods are HPLC methods using fluorescence detector (Mawatari *et al.*, 1996) and liquid chromatographic method (Ojha and Pargal, 2003).

This method is more simple and sensitive HPLC method using UV detector than other methods such as HPLC methods using fluorescence detector and liquid chromatographic method for determination of nicorandil in human plasma.

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