Journal of Chromatography, 617 (1993) 59–63
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6896

Determination of benidipine hydrochloride in human plasma by capillary column gas chromatography–negative ion chemical ionization mass spectrometry

Hiroshi Magara*, Hiroyuki Kobayashi and Satoshi Kobayashi

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411 (Japan)

(First received February 8th, 1993; revised manuscript received April 22nd, 1993)

ABSTRACT

A highly sensitive and specific determination method of a new calcium antagonist, benidipine hydrochloride, in human plasma has been developed using capillary column gas chromatography with negative ion chemical ionization mass spectrometry. A deuterated analogue of benidipine hydrochloride was used as an internal standard. Benidipine hydrochloride was extracted from plasma with diethyl ether under basic conditions, back-extracted with 0.5 *M* sulphuric acid, and reextracted with diethyl ether under basic conditions. For gas chromatography, a solventless injection port was used, and the whole extract was injected. Fragment ions at *m/z* 488 (benidipine) and *m/z* 493 (internal standard) were used as monitoring ions for selected-ion monitoring. The detection limit was 0.02 ng/ml with 0.5 ml of plasma. The coefficient of variation of intra-day assay was 17.4% and 12.1% at the 0.02 and 0.1 ng/ml levels, respectively. This method has made it possible to detect the plasma level of unchanged drug up to 12 h after an 8-mg oral dose of benidipine hydrochloride to humans.

INTRODUCTION

Benidipine hydrochloride, (±)-(*R**)-3-[(*R**)-1-benzyl-3-piperidyl]methyl-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride (Fig. 1A), is a newly developed potent dihydropyridine calcium antagonist, and exhibits slow onset and long-lasting antihypertensive and antianginal activity in experimental animals [1–3] and patients [4,5].

Because benidipine hydrochloride has a group with high electron affinity, the unchanged drug in biological fluids has been determined by gas chromatography with electron-capture detection (GC–ECD) [6]. However, human plasma levels of the unchanged drug cannot be monitored accurately after oral administration of benidipine hydrochloride at clinical doses (2–12 mg) because of its detection limits (0.2 ng/ml) [6]. Because the GC–ECD method involves oxidation of benidipine hydrochloride to the more stable pyridine analogue prior to analysis, the pyridine analogue, which is also present in plasma as a metabolite, is also determined as unchanged drug [7].

Recently, negative ion chemical ionization mass spectrometry (NICIMS) has been applied to the highly sensitive determination of compounds containing a group with high electron affinity. Dihydropyridine calcium antagonists can be determined sensitively by capillary column GC–NICIMS [8–11].

This paper describes a highly sensitive method for the determination of benidipine hydrochlo-

* Corresponding author.

0378-4347/93/$06.00

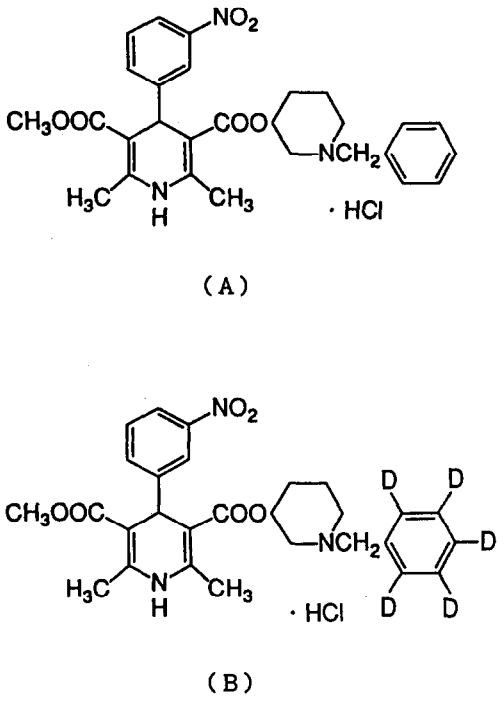

Fig. 1. Molecular structures of (A) benidipine hydrochloride and (B) benidipine-d_5 hydrochloride.

ride in human plasma by capillary column GC–NICIMS without oxidation to the pyridine analogue.

EXPERIMENTAL

Materials

Benidipine hydrochloride and benidipine-d_5 hydrochloride (Fig.1B) were synthesized and supplied by the laboratory of medicinal chemistry in our laboratories. Diethyl ether, 0.5 *M* sulphuric acid, sodium hydroxide and sodium hydrogencarbonate were of analytical grade and purchased from Kanto Chemicals (Tokyo, Japan).

Standard solutions

Standard solutions of benidipine hydrochloride were prepared by dissolving it in methanol and diluting to appropriate concentrations. Benidipine-d_5 hydrochloride was used as the internal standard for the assay. It was dissolved in methanol and diluted to 100 ng/ml with methanol. Storage of these solutions at 4°C did not result in any detectable decomposition for 2 months.

Gas chromatography–mass spectrometry

The GC–MS system consisted of a JMS SX-102 (JEOL, Tokyo, Japan) coupled to an HP-5890A (Hewlett Packard, Palo Alto, CA, USA) with a solventless injector (JEOL). The column was a methylsilicon-coated fused-silica capillary, ULTRA 1 (4 m × 0.2 mm I.D., film thickness 0.11 μm, Hewlett Packard). The column was directly connected to the ion source of the mass spectrometer so that the end of the column was within 1.0 cm of the ion source centre. The column oven temperature was maintained at 220°C for 1 min, then increased at 40°C/min to 300°C. The injector was operated at 270°C, and the transfer line at 300°C. Helium was used as the carrier gas at 25 kPa of inlet pressure. The mass spectrometer, equipped with a pulsed NICI accessory, was operated with using isobutane as a reagent gas. The ionization current, ion source temperature, accelerating voltage and ionization energy were 0.3 mA, 300°C, −10 kV, and 200 eV, respectively. The mass spectrometer was set to monitor *m/z* 488 ($[M - 16]^-$, benidipine) and *m/z* 493 ($[M - 16]^-$, benidipine-d_5).

Subjects

Five male Japanese volunteers, aged 22.0 ± 1.6 (mean ± S.D.) years and weight 66.4 ± 9.4 kg, were recruited for the study. Subjects were judged to be healthy on the basis of medical histories, physical examinations, 12-lead electrocardiograms, urinalysis, and routine laboratory tests of serum biochemistry and haematology. All subjects gave written informed consent, and the study protocol was approved by the ethics committee of Tokyo Musashino Hospital (Tokyo, Japan).

The subjects were given an oral dose of benidipine hydrochloride in the form of an 8-mg tablet with 150 ml of water after overnight fasting. Blood samples of 10 ml were collected into heparinized tubes before administration and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after administration.

The plasma was separated immediately and stored at −20°C until analysis.

Sample preparation

A 10-μl volume of internal standard solution (benidipine-d_5 hydrochloride, 1 ng per 10 μl in methanol), 0.5 ml of plasma sample and 0.5 ml of saturated sodium hydrogencarbonate aqueous solution were added to a 10-ml silanized centrifuge tube, and mixed. Benidipine and benidipine-d_5 were extracted three times with 2 ml of diethyl ether by 1 min agitation, and back-extracted with 1.5 ml of 0.5 *M* sulphuric acid. The organic layer was pippetted out. The aqueous layer was added to 360 μl of 5 *M* sodium hydroxide, and extracted with 5 ml of diethyl ether. The organic layer was transferred to a 10-ml silanized glass centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 20 μl of ethyl acetate, and the total solution was applied to the solventless injection port and injected into the GC–MS system.

Pharmacokinetic analysis

The plasma level–time data were used to determine the maximum plasma level (C_{max}), and the time to reach the maximum level (T_{max}). The estimation of the elimination rate constant (k) was obtained by the linear least-squares regression method, using the terminal portion of the plasma level–time curve; the half-life ($T_{1/2}$) was calculated as $0.693/k$. The area under the plasma level–time curve (AUC) was calculated by the trapezoidal rule and was extraporated to infinity ($AUC_{0-\infty}$) using the last detectable plasma level and the elimination rate constant. The apparent total clearance (Cl/F, F = absorbed fraction) was calculated by dose/AUC.

RESULTS AND DISCUSSION

Fig. 2 shows the NICI mass spectra of benidipine hydrochloride and benidipine-d_5 hydrochloride. In the spectrum of benidipine hydrochloride, the fragment ion m/z 488 ($[M - OH]^-$) was the base peak, and the relative abundance of the molecular ion m/z 505 (M^-) was lower than that of m/z 488. In the spectrum of benidipine-d_5 hydrochloride, the fragment ion m/z 493 ($[M - OH]^-$) was the base peak, and the relative abundance of the molecular ion m/z 510 (M^-) was lower than that of m/z 493. Therefore, the fragment ions m/z 488 (benidipine) and m/z 493 (benidipine-d_5) were used for selected-ion monitoring (SIM). It was considered that there was no mutual contribution, because the m/z 493 peak was not found at the retention time of benidipine-d_5 hydrochloride after benidipine hydrochloride was injected, and no m/z 488 peak appeared at the retention time of benidipine hydrochloride after benidipine-d_5 hydrochloride was injected.

Fig. 3 shows the SIM chromatograms obtained from human plasma samples. When benidipine-d_5 hydrochloride was added to blank plasma, the chromatogram of m/z 488 showed no peak that would obstruct the determination of benidipine hydrochloride. The calibration curve of benidipine hydrochloride in plasma was non-linear around 0.5 ng/ml in the range 0.02–5 ng/ml, so the calibration curve was constructed in two parts, 0.02–0.5 ng/ml and 0.5–5 ng/ml; each curve showed good linearity (r = 0.999, r = 0.999). The limit of detection, 0.02 ng/ml at a signal-to-noise ratio of 3, was slightly higher than that of nilvadipine (0.01 ng/ml) [8], another calcium antagonist of the dihydropyridine analogue type. Table I shows the intra-day assay variation. The coefficient of variation (C.V.) was 5.5% at 1 ng/ml, 12.1% at 0.1 ng/ml and 17.4% at the limit of detection (0.02 ng/ml). The relative error was 5.4% at 1 ng/ml, 7.0% at 0.1 ng/ml and 15.0% at 0.02 ng/ml. Consequently, this method was considered to be fully applicable for pharmacokinetic studies in humans.

Plasma levels of the unchanged drug after an 8-mg oral dose of benidipine hydrochloride to five healthy volunteers are shown in Fig. 4. Pharmacokinetic parameters are shown in Table II. Benidipine hydrochloride was rapidly absorbed, with a T_{max} of 0.60 ± 0.22 (mean ± S.D.) h and C_{max} were 2.282 ± 0.336 ng/ml. After T_{max}, the drug disappeared biphasically. The $T_{1/2}$ was 4.51 ± 2.29 h, and $AUC_{0-\infty}$ was 4.09 ± 0.84 ng · h/ml.

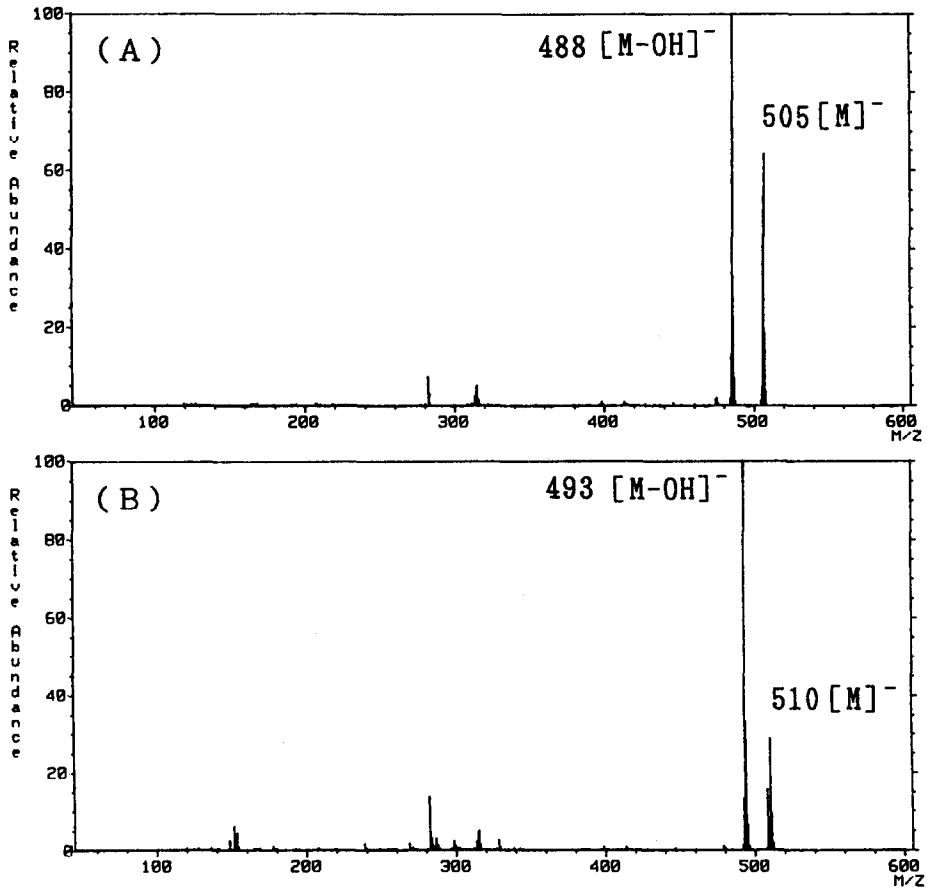

Fig. 2. NICI mass spectra of (A) benidipine hydrochloride and (B) benidipine-d_5 hydrochloride.

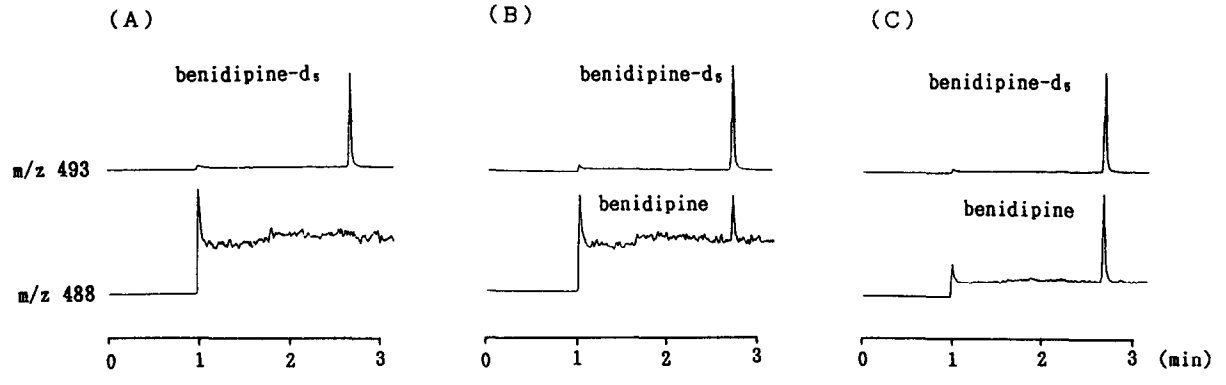

Fig. 3. Typical SIM chromatograms of (A) human blank plasma, (B) plasma spiked with benidipine hydrochloride (0.05 ng/ml), and (C) plasma collected from a healthy volunteer 4 h after an 8-mg oral dosing of benidipine hydrochloride. (The calculated level of benidipine hydrochloride was 0.253 ng/ml.)

TABLE I

PRECISION AND ACCURACY OF INTRA-DAY ASSAY

Added (ng/ml)	Found (mean ± S.D., n = 4) (ng/ml)	C.V. (%)	Relative error (%)
0.020	0.023 ± 0.004	17.4	15.0
0.050	0.060 ± 0.006	10.0	20.0
0.100	0.107 ± 0.013	12.1	7.0
1.000	1.054 ± 0.058	5.5	5.4

TABLE II

PHARMACOKINETIC PARAMETERS OF BENIDIPINE HYDROCHLORIDE

	T_{max} (h)	C_{max} (ng/ml)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/ml)	Cl/F (l/h)
Mean	0.60	2.282	4.51	4.09	2020
S.D. (n = 5)	0.22	0.336	2.29	0.84	370

The GC–ECD method that has been used for the determination of benidipine hydrochloride in human plasma cannot accurately determine the level of unchanged drug after oral administration to humans of benidipine hydrochloride at clinical doses (2–12 mg) because of its detection limit (0.2 ng/ml). However, this method has made it possible to detect benidipine hydrochloride upon 12 h after an 8-mg oral dose.

CONCLUSION

We have developed a highly sensitive and selective method for determination of benidipine hydrochloride in human plasma using GC–NICIMS. The method has made it possible to investigate precisely the pharmacokinetics of benidipine hydrochloride in humans at clinical doses (2–12 mg).

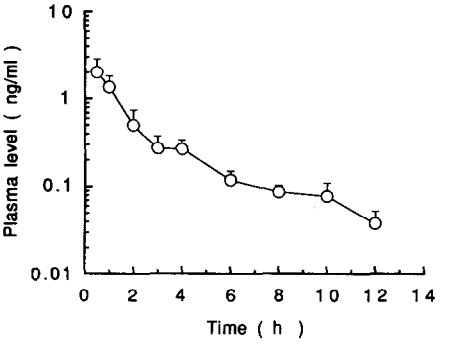

Fig. 4. Plasma levels of the unchanged drug after oral administration of benidipine hydrochloride (8 mg) to healthy volunteers. Each point with a bar represents the mean ± S.D. of five volunteers.

ACKNOWLEDGEMENT

We thank Miss M. Suzuki for her technical assistance.

REFERENCES

1 A. Karasawa, K. Kubo, T. Oka and N. Nakamizo, *Arzneim.-Forsch.*, 38 (1988) 1684.
2 A. Karasawa, J. Ikeda, K. Yamada, K. Kubo, T. Oka and N. Nakamizo, *Arzneim.- Forsch.*, 38 (1988) 1695.
3 A. Karasawa, K. Kubo, T. Oka and N. Nakamizo, *Arzneim.-Forsch.*, 38 (1988) 1702.
4 H. Noda, Y. Itou and T. Fujita, *Jpn. Pharmacol. Ther.*, 18 (1990) 21.
5 H. Tsukiyama and K. Otsuka, *Jpn. Pharmacol. Ther.*, 18 (1990) 31.
6 Y. Uji, T. Sugimoto, H. Kobayashi and S. Kobayashi, *Jpn. Pharmacol. Ther.*, 18 (1990) 7.
7 H. Kobayashi, S. Kobayashi, A. Inoue, T. Oka and N. Nakamizo, *Arzneim.-Forsch.*, 38 (1988) 1730.
8 Y. Tokuma, T. Fujiwara and H. Noguchi, *J. Chromatogr.*, 345 (1985) 51.
9 M. Ahnoff, M. Ervik and L. Johansson, *J. Chromatogr.*, 394 (1987) 419.
10 C. Jean and R. Laplanche, *J. Chromatogr.*, 428 (1988) 61.
11 Y. Ueno, E. Matsushima, M. Maniwa and T. Marunaka, *J. Chromatogr.*, 434 (1988) 123.