Short Communication

High-performance liquid chromatographic determination of the calcium channel blocker nimodipine in monkey plasma

Mingxin Qian and James M. Gallo

Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA 30602 (USA)

ABSTRACT

A new high-performance liquid chromatographic (HPLC) assay was developed for the determination of nimodipine in monkey plasma. An ethyl acetate extraction procedure was employed with a reversed-phase HPLC separation for the analysis. Absolute recovery of nimodipine from plasma was over 95% with a lower limit of quantitation of 10 ng/ml. This method was applied to a preliminary pharmacokinetic study in which 0.25 mg/kg nimodipine was administered intravenously to three monkeys. Protein binding and stability of nimodipine in monkey plasma were also examined. The pharmacokinetic parameters of nimodipine in monkeys were similar to those obtained in humans and indicate that monkeys are an appropriate animal model for further pharmacokinetic investigations.

INTRODUCTION

Nimodipine is a dihydropyridine calcium channel blocker which has been shown to selectively dilate cerebral arteries and increase cerebral blood flow in animals and humans [1,2]. Its major therapeutic indication is for the prevention and treatment of delayed ischaemic neurological disorders that often occur in patients with subarachnoid hemorrhages. A recent *in vitro* study demonstrated that nimodipine can prevent neu-

ronal toxicity caused by the human immunodeficiency virus (HIV) envelope protein gp 120 [3].

Chromatographic assays for the determination of nimodipine in plasma have been limited [4–6]. These methods utilized gas chromatography (GC) with electron-capture [4,5] and nitrogen [6] detection, and were complicated by uncontrolled oxidation of nimodipine at high temperatures. A highly sensitive and selective high-performance liquid chromatographic (HPLC) method for the determination of nimodipine and its major metabolites was described [4], however, it requires a large amount of plasma and an elaborate and lengthy sample preparation method.

In the present study, a simple and rapid HPLC assay for the determination of nimodipine in

---

Correspondence to: Dr. J. M. Gallo, Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA 30602, USA.

plasma was developed. It offers an alternative to the GC and HPLC procedures available, especially when measurement of nimodipine metabolites is not of interest and plasma volume is limited. In anticipation that nimodipine may be used in combination with anti-HIV drugs, its pharmacokinetics was examined in the monkey, an animal shown to be an appropriate model for the pharmacokinetics of a number of anti-HIV nucleosides [7,8].

EXPERIMENTAL

Chemicals

Nimodipine and the internal standard, nitrendipine, were kindly provided by Miles (West Haven, CT, USA). Stock solutions of both drugs were prepared in methanol (1 mg/ml), protected from light and stored at –20°C. HPLC-grade methanol and ethyl acetate were purchased from J. T. Baker (Phillipsburg, NJ, USA).

Chromatography

The HPLC system consisted of a Waters liquid chromatograph (Waters Chromatography Division, Milford, MA, USA) including a Model 510 pump, a Model 712 WISP autoinjector, a Model 484 absorbance detector and a Model 746 data module. An analytical column (Hypersil ODS, 5 μm, 150 mm × 4.6 mm I.D., Alltech Assoc., Deerfield, IL, USA) preceded by a guard column (2 cm × 2 mm I.D.) filled with 30 ~ 40 μm pellicular RP-18 Perisorb material (Upchurch Scientific, Oak Harbor, WA, USA) was used for all analyses. The mobile phase consisted of methanol–water (65:35, v/v) and contained no salts. The chromatographic analyses were performed at ambient temperature at a flow-rate of 1 ml/min and with the detector wavelength set at 238 nm. Standard curves for plasma were prepared by adding known amounts of nimodipine and nitrendipine to pooled blank plasma. Peak-height ratios of nimodipine to nitrendipine in unknown plasma samples were used to calculate nimodipine plasma concentration from linear regression equations obtained from standards.

Sample preparation

To 100 μl of plasma sample, 10 μl of internal standard (nitrendipine, 10 μg/ml) solution were added into an amber microcentrifuge tube. Ethyl acetate (1 ml) was added to each tube which was then shaken on a horizontal shaker for 3 min, followed by centrifugation at 10 000 g for 10 min. The organic layer was transferred to a clean amber microcentrifuge tube, and evaporated to dryness under nitrogen gas in a 40–50°C water bath. The resultant residue was reconstituted with 200 μl of mobile phase and a 100-μl aliquot was injected onto the HPLC system.

Stability of nimodipine

Photostability of nimodipine in aqueous solutions at concentrations of 0.05, 0.25 and 1 μg/ml was determined after exposure to fluorescence laboratory light and in the dark for 0, 4, 8 and 24 h. Stability studies in monkey plasma stored at – 20°C were performed at nimodipine concentrations of 0.05, 0.02, 1 and 5 μg/ml over a two-month period.

Protein binding of nimodipine in plasma

Binding of nimodipine to monkey plasma proteins was determined by filtration of plasma through a Centrifee (Amicon, Danvers, MA, USA) micropartition system using a YMT membrane with a molecular weight cut-off of 30 000. Prior to filtration, nimodipine was added to heparin-treated monkey plasma at initial concentrations of 0.5, 2 and 10 μg/ml, and then incubated at 37°C for 30 min. The plasma samples were then centrifuged at 1164 g for 25 min, and 100 μl of ultrafiltrate were injected onto the HPLC system. Solutions containing the same nimodipine concentration in phosphate-buffered saline, pH 7.4, were treated in a similar fashion to determine solute retention by the Centrifree system. Quadruplicate samples were run for each concentration.

Pharmacokinetics of nimodipine in monkeys

Three adult male monkeys (Macaca fascicularis), weighing from 4.55 to 4.89 kg, were each administered an intravenous bolus of 0.25 mg/kg

nimodipine, prepared in 0.9% NaCl containing 2% (v/v) Emulphor EL-620. Blood samples were collected by venipuncture at 1, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min after nimodipine administration. Plasma was obtained by centrifugation of the blood samples and kept frozen at −20°C until analyzed. Pharmacokinetic parameters were calculated by non-compartmental methods [9].

RESULTS AND DISCUSSION

HPLC assay

Typical chromatograms of blank plasma and an actual sample obtained from the pharmacokinetic study are shown in Fig. 1. Retention times of nimodipine and nitrendipine were approximately 9 and 7.5 min, respectively. The total run time for each sample injection was about 12 min. There were no interfering peaks in the blank plasma samples.

Standard curves prepared for nimodipine in monkey plasma were linear over a concentration range from 0.01 to 2 μg/ml. The mean (n = 6) calibration curve for nimodipine was y = 0.0052

+ 0.420x, r = 0.999, with a relative standard deviation of the slope equal to 6.8%, where y = peak-height ratio and x = nimodipine sample concentration (μg/ml). Nimodipine plasma concentrations as low as 10 ng/ml could be quantitated with a 100-μl plasma sample. The sensitivity of the assay is sufficient for the determination of steady-state nimodipine concentrations between 15 and 58 ng/ml in patients with subarachnoid hemorrhages [10] and concentrations of nimodipine preventing neurotoxicity of HIV-gp 120 [3].

A simple liquid–liquid extraction procedure with ethyl acetate was developed for nimodipine and nitrendipine based on their lipophilicity. Compared with other extraction procedures [4–6], ethyl acetate has the advantages of excellent extraction recovery and yielding chromatograms without endogenous interferences. The extraction recovery of nimodipine, determined at plasma concentrations of 0.05, 0.2 and 1 μg/ml, was 106, 95 and 96%, respectively. The intra- and inter-day coefficients of variation (C.V.) of the assay are summarized in Table I. The largest C.V. and bias were equal to 7.7% and 11%, respectively.
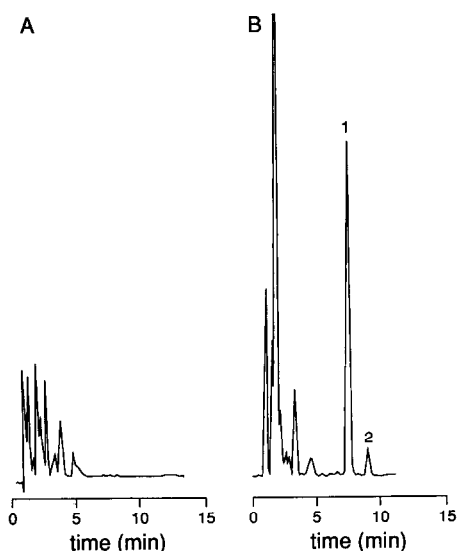

Fig. 1. Chromatograms of nimodipine in monkey plasma. (A) Blank; (B) sample obtained 2 min after intravenous administration of 0.25 mg/kg nimodipine in monkey D1. Peaks: 1 = nitrendipine; 2 = nimodipine. See text for assay conditions.

TABLE I

INTRA- AND INTER-DAY VARIATION OF NIMODIPINE ASSAY IN MONKEY PLASMA

Prepared concentration[a] (μg/ml)	Measured concentration (μg/ml)	C.V. (%)	Bias[b] (%)
Intra-day			
0.050	0.053	3.4	6.0
0.20	0.190	2.2	−5.0
1.0	0.956	4.5	−4.4
Inter-day			
0.050	0.056	3.5	11
0.20	0.20	7.4	0.2
1.0	0.918	7.7	−8.2

[a] n = 5 at all concentrations.

[b] $\text{Bias} = \left[\dfrac{\text{measured concentration} - \text{prepared concentration}}{\text{prepared concentration}} \right] \times 100$.

Stability and protein binding of nimodipine

Unlike nitrendipine and other dihydropyridine analogues reported to be highly light-sensitive [11], there was no measurable photodegradation of nimodipine in aqueous solutions over 24 h, as shown in Fig. 2. Nonetheless, considering the fact that nimodipine degradation rates depend on the light conditions [5], all samples were prepared under minimal light exposure to reduce potential photodecomposition of nimodipine.

In the storage stability study, nimodipine plasma concentrations, over a range of 0.05–5 μg/ml, decreased by 7.75 ± 3.3% after twenty days and 13.7 ± 9.2% after sixty days, indicating that plasma samples should be processed soon after collection and storage at −20°C.

The percentage of nimodipine bound to monkey plasma was 90.3 ± 4.4% at a nimodipine plasma concentration of 0.5 μg/ml, 90.8 ± 0.54% at 2 μg/ml and 90.4 ± 0.67% at 10 μg/ml. The average percentage of nimodipine bound to monkey plasma was 90.5 ± 2.03% (C.V. = 2.2%, n = 11) and was independent of nimodipine plasma concentrations. These results are similar to those reported in other animals and humans [5,12].

Pharmacokinetics of nimodipine in monkeys

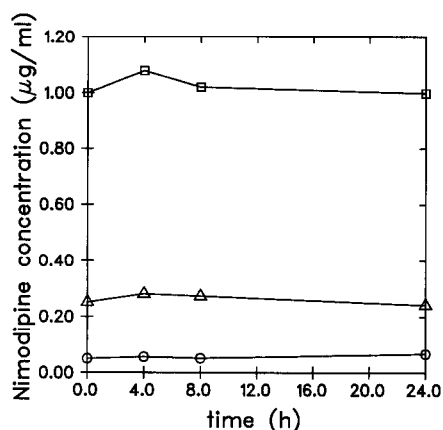The HPLC method was applied to the mea-

Fig. 2. Nimodipine concentrations from photodegradation study in which nimodipine was exposed to laboratory fluorescence light in aqueous solution at concentrations of 0.05 (○), 0.25 (△) and 1 (□) μg/ml.
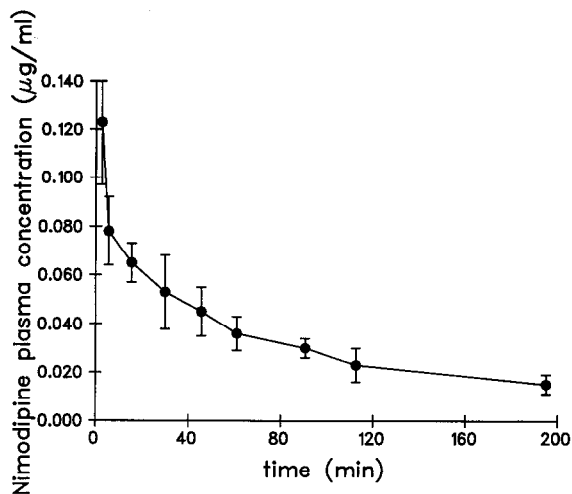
Fig. 3. Mean nimodipine plasma concentrations–time profile in monkeys following intravenous administration of 0.25 mg/kg nimodipine. Each point represents the mean ± S.D. for three monkeys.

surement of nimodipine in plasma samples obtained from three monkeys receiving 0.25 mg/kg nimodipine intravenously. Fig. 3 shows the mean nimodipine plasma concentration–time profile in monkeys. Pharmacokinetic parameters are presented in Table II. The total clearance of nimodipine was 1.98 l/h/kg. The volume distribution at steady-state of 3.6 l/kg indicates that nimodipine distributes into tissues to a moderate extent owing to its highly lipophilic nature. These results are in close agreement with those in humans [13].

CONCLUSION

A simple, rapid and selective HPLC method for measurement of nimodipine plasma concentrations was developed. It offers a suitable alternative to existing GC and HPLC techniques and can be used to quantitate nimodipine for pharmacokinetic investigations.

TABLE II

PHARMACOKINETIC PARAMETERS OF NIMODIPINE FOLLOWING INTRAVENOUS BOLUS ADMINISTRATION OF 0.25 mg/kg NIMODIPINE IN THREE MONKEYS

AUC = area under the concentration–time curve; $t_{1/2}$ = half-life; Cl_T = total clearance; V_{ss} = volume of distribution at steady state.

Animal	AUC (mg h/l)	$t_{1/2}$ (h)	Cl_T (l/h/kg)	V_{ss} (l/kg)
D1	0.117	1.09	2.1	3.18
D2	0.156	1.58	1.62	3.60
R1	0.111	1.36	2.22	4.09
Mean ± S.D.	0.128 ± 0.024	1.34 ± 0.24	1.98 ± 0.32	3.62 ± 0.46

REFERENCES

1 S. Kazda, B. Garthoff, H. P. Krause and K. Schlodmann, Arzneim.-Forsch., 32 (1982) 331.
2 G. S. Allen, H. S. Ahn, T. J. Preziosi and R. Batty, N. Engl. J. Med., 308 (1983) 619.
3 E. G. Dreyer, P. K. Kaiser, J. T. Offerman and S. A. Lipton, Science, 248 (1990) 364.
4 G. J. Krol, A. J. Noe, S. C. Yeh and K. D. Raemsch, J. Chromatogr., 305 (1984) 105.
5 P. Jakobsen, E. O. Mikkelsen, J. Laursen and F. Jensen, J. Chromatogr., 374 (1986) 383.
6 M. T. Rosseel, M. G. Boguert and L. Hugghens, J. Chromatogr., 533 (1990) 224.

7 M. Qian, T. S. Finco, M. Mehta, C. T. Vishwanathan and J. M. Gallo, J. Pharm. Sci., 80 (1991) 1007.
8 M. Qian, T. S. Finco, A. R. Swagler and J. M. Gallo, Antimicrob. Agents Chemother., 35 (1991) 1247.
8 M. Rocci and W. I. Jusko, Comp. Prog. Biomed., 16 (1983) 203.
10 E. Vinge, K. E. Anderson, J. Brandt, B. Ljunggren, L. G. Nilsson and S. Rosendal-Helgesen, Eur. J. Clin. Pharmacol., 30 (1986) 421.
11 F. A. Tucker, P. S. B. Mintz and G. A. Macgregor, J. Chromatogr., 342 (1985) 193.
12 D. Maruhn, H. M. Siefert, H. Weber, K. Ramsch and D. Suwelack, Arzeim.-Forsch., 35 (1985) 1781.
13 M. S. Langley and E. M. Sorkin, Drugs, 37 (1989) 669.