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Determination of azelnidipine by LC–ESI-MS and its application to a pharmacokinetic study in healthy Chinese volunteers

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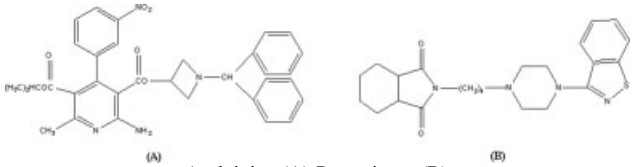
A simple, rapid and sensitive high performance liquid chromatography–electrospray ionization-mass spectrometry (HPLC–ESI-MS) assay for determination of azelnidipine in human plasma using perospirone as the internal standard (IS) was established. After adjustment to a basic pH with sodium hydroxide solution, plasma samples were extracted with diethyl ether and separated on a C_{18} column with a mobile phase of methanol–5 mM ammonium acetate solution (90 : 10, v/v). The lower limit of quantification (LLOQ) was 0.20 ng/ml. After administration of a single dose of azelnidipine 8 mg and 16 mg, respectively; the area under the plasma concentration versus time curve from time 0 h to 96 h (AUC_{0-96}) were (186 ± 47) ng $\cdot$ ml^{-1} $\cdot$ h, (429 ± 145) ng $\cdot$ ml^{-1} $\cdot$ h, respectively; clearance rate (CL/F) were (45.94 ± 11.61), (42.11 ± 14.23) L/h, respectively; peak plasma concentration C_{max} were (8.66 ± 1.15), (19.17 ± 4.13) ng/ml, respectively; apparent volume of distribution (V_d) were (1749 ± 964), (2480 ± 2212) L, respectively; time to C_{max} (T_{max}) were (2.8 ± 1.2), (3.0 ± 0.9) h, respectively; elimination half-life ($t_{1/2\beta}$) were (22.8 ± 2.4), (23.5 ± 4.2) h, respectively; and MRT were (25.7 ± 1.3), (26.2 ± 2.2) h, respectively; The essential pharmacokinetic parameters after oral multiple doses (8 mg, q.d.) were as follows: (C_{max}) ss, (15.04 ± 2.27) ng/ml; (T_{max}) ss, (2.38 ± 0.92) h; (C_{min}) ss, (3.83 ± 0.94) ng/ml; C_{av}, (7.05 ± 1.54) ng/ml; DF, (1.62 ± 0.26); AUCss, (169.19 ± 36.87) ng $\cdot$ ml^{-1} $\cdot$ h.

1. Introduction

Azelnidipine is a new 1,4-dihydropyridine (DHP) derivative. It is a new calcium channel blocker (CCB) with selectivity for L type calcium channels (Koike et al. 2002). Azelnidipine can be used for the treatment of hypertensive patients with or without potential ischemic heart diseases (Kuramoto et al. 2003).



Azelnipine (A) Perospirone (B)

So far, several reports described the pharmacokinetics of azelnidipine. In 2006, Kiyoshi-Kawabata's group used a liquid chromatography/electrospray ionization-tandem mass spectrometry simultaneous to analyze plasma concentrations of azelnidipine and its two metabolites. They also have developed a LC–MS/MS method for enantioselective determination of azelnidipine in human plasma. In the same year, the estimation of azelnidipine in healthy chinese volunteers was first carried out by a Chinese scientist using LC–ESI-MS (Li Ding et al. 2006). In our study, we describe a simple, economic and sensitive LC–ESI-MS method that can determine azelnidipine concentration in human plasma using liquid–liquid extraction and also can be used to evaluate the pharmacokinetics of azelnidipine in humans. Besides, the steady state pharmacokinetics of azelnidipine is first reported.

2. Investigations and results

Under the conditions described in the experimental part, the assay was highly specific, and no endogenous plasma materials interfered with the peak of azelnidipine or perospirone (Fig. 1). Azelnidipine and perospirone were eluted with retention times of 3.7 min and 2.9 min, respectively. The calibration cure was linear from 0.20–30.39 ng/ml ($f = 0.196\ 3 \times c + 0.0153\ 4$, $r = 0.9971$). Relative Standard Deviatien (RSD) values of intraday and interday precision experiments are given in Table 1. The recovery of azelnidipine from human plasma was more than 75% (Table 2). The stability of azelnidipine in plasma was fine including the stability for 4 h at ordinary temperature (0.41 ng/ml, 4.02 ng/ml and 20.13 ng/ml, respectively); the stability of 14 days when frozen and following three freeze/thaw cycles.

The developed method was successfully used for a pharmacokinetic study in which plasma concentrations of azelnidipine in 16 healthy Chinese volunteers (Groups A–B) were

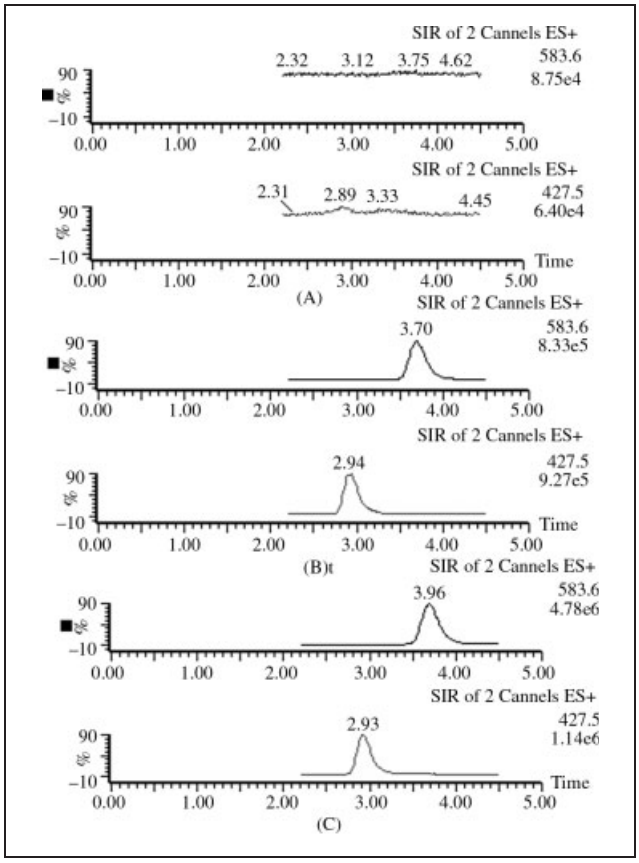


Fig. 1: Chromatograms of azelnidipine using LC-MS; (A) Blank plasma; (B) Blank plasma spiked with azelnidipine and the internal standard; (C) Plasma sample after a single dose oral administration of 8 mg azelnidipine

Table 1: Intraday and interday precision of azelnidipine in plasma

C (ng · ml^{-1})	Mean	RSD (intraday, %)	RSD (interday, %)
0.41	0.48	7.68	10.67
4.02	4.11	6.07	8.16
20.13	20.02	6.47	11.42

RSD: Relative Standard Deviatien

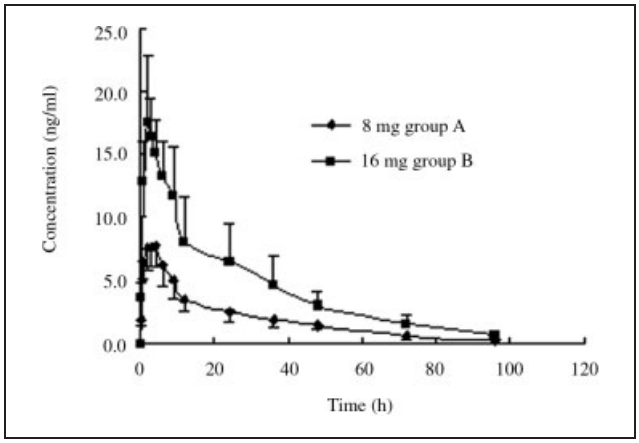


Fig. 2: Plasma concentration-time profile of azelnidipine after an oral administration of 8 mg and 16 mg azelnidipine tablets to 16 healthy volunteers (Groups A–B). Each point represents a mean S.D. (n = 8)

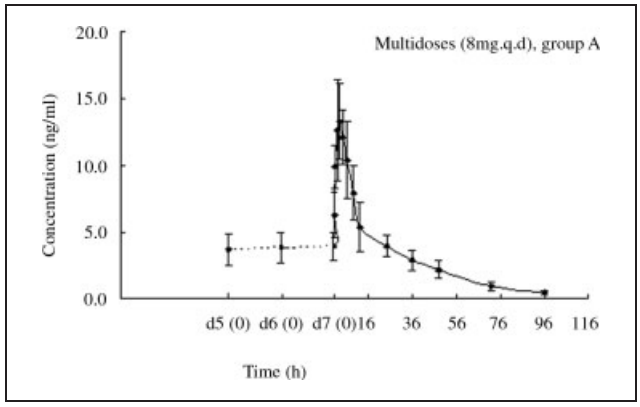


Fig. 3: Mean plasma concentration-time profile of azelnidipine in 8 healthy volunteers (Group A) after oral multiple doses of azelnidipine tablets (8 mg, q.d.). Each point represents a mean S.D. (n = 8)

Table 2: Recovery of azelnidipine in plasma

C (ng · ml^{-1})	Mean (%)	RSD %
0.41	84.23	6.30
4.02	90.31	3.84
20.13	85.64	8.37

Table 3: Pharmacokinetic parameters of azelnidipine in healthy Chinese volunteers (n = 8, mean ± S.D.)

Pharmacokinetic parameter	8 mg	16 mg
AUC_{0-96} (ng · ml^{-1} · h)	186 ± 47	429 ± 145
V_d/L	1749 ± 964	2480 ± 2212
CL/F (L/h)	45.94 ± 11.61	42.11 ± 14.23
C_{max} (ng/ml)	8.66 ± 1.15	19.17 ± 4.13
T_{max} (h)	2.8 ± 1.2	3.0 ± 0.9
$MRT_{(0-96)}$ (h)	25.7 ± 1.3	26.2 ± 2.2
$t_{1/2\beta}$ (h)	22.8 ± 2.4	23.5 ± 4.2

determined up to 96 h after the oral administration of 8 mg and 16 mg azelnidipine tablets. The mean plasma concentration-time curves of single doses are shown in Fig. 2. The mean plasma concentration-time curve of multiple doses was shown in Fig. 3. By using Drug and Statistics software (Version 2.0, Chinese) analysis, the pharmacokinetic parameters are listed in Table 3. The main pharmacokinetic parameters of multiple doses were as follows: (C_{max}) ss, (15.04 ± 2.27) ng/ml; (T_{max}) ss, (2.38 ± 0.92) h; (C_{min}) ss, (3.83 ± 0.94) ng/ml; C_{av}, (7.05 ± 1.54) ng/ml; DF, (1.62 ± 0.26); AUCss, (169.19 ± 36.87) ng · ml^{-1} · h.

3. Discussion

There were no adverse events during the conduct of the study. A lower limit of quantification of 0.20 ng/ml was achieved with this method, which is sensitive enough for the determination of azelnidipine concentration in human plasma. Moreover, the sample extraction procedure is quite simple. The developed method adopts a simple preparation, offers sufficient sensitivity, satisfactory selectivity and good reproducibility. So, it can be successfully applied to pharmacokinetic studies. Sixteen healthy Chinese volunteers received a single dose of azelnidipine 8 mg and 16 mg, respectively, the calculated pharmacokinetic parameter values showed that the C_{max} of azelnidipine in healthy

chinese volunteers increased dose-dependently (C_{max}) and agreed well with previously reported values (Koike et al. 2002). Ding Li et al. (2007) first described the pharmacokinetic profiles of azelnidipine in healthy chinese volunteers after a single oral (containing 8 mg azelnidipine) administration under fasting conditions. Their results regarding C_{max}, T_{max} and $t_{1/2\beta}$ of azelnidipine agree with the present report. But the difference in AUC_{0-96} is significant. These difference may due to using different sample preparation. In this paper, after oral multiple doses, the steady state pharmacokinetics of azelnidipine was first reported. The pharmacokinetic data of Vd, CL/F and $t_{1/2\beta}$ illustrates that azelnidipine is widely distributed and slowly eliminated in the healthy Chinese body.

4. Experimental

4.1. Materials

Azelnidipine standard reference (purity: 99.5%, Batch NO.: 20050915) and azelnidipine tablets (containing 8 mg azelnidipine, Batch NO.: 20050711) were kindly provided by Jian Hua Pharmaceutical Limited (Nanjing, China); The internal standard (perospirone, IS) was purchased from Shang Hai Pu Dong Pharmaceutical Limited (purity: 99.2%, Batch NO.: 20041123, Shanghai, China). Reagent: methanol for HPLC. Tedia Company. Ammonium acetate and diethyl ether for AR. Nanjing Chemical Reagent Co., Ltd. Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA) and was used to prepare all aqueous solutions. Instrument: Masslynx Waters 2695-ZQ 2000 LC-MS system. Drug-free and drug-containing plasma were taken from the volunteers. Plasma was stored at −70 °C until assayed.

4.2. Chromatographic conditions

HPLC Columns: Hanbon C_{18} (dp 5 μm, ID 4.6 × 150 mm). Column temperature: 25 °C. Mobile Phase: methanol-ammonium acetate (5 mM) (90 : 10, v/v). Flow rate: 1.0 ml/min.

4.3. MS spectrometry detection

Electrospray ionization mass spectroscopy (ESI-MS) was carried out on a Masslynx LC-MS series system. The ESI ion source was set in positive ion polarity mode for acquiring all mass spectrometry data. The selective ion monitoring (SIM) was set at m/z 583.6 for azelnidipine and m/z 427.5 for perospirone, respectively. The cone voltage, drying gas flow, drying gas temperature and capillary voltage were set to 40 V, 500 $L \cdot h^{-1}$, 380 °C and 3500 V, respectively.

4.4. Sample preparation for HPLC injection

A 1 ml aliquot plasma sample was added with 30 μl IS (0.1122 μg/ml) solution and 0.1 ml of 0.1 M sodium hydroxide solution. After a thorough vortex mixing for 30 s, mixtures were extracted with 5 ml of diethyl ether, vortex-mixed for 3 min, and centrifuged at 4000 r for 10 min. The organic layer (4 ml) was removed and evaporated under a stream of nitrogen gas in the thermostatically controlled water-bath maintained at 50 °C until completely dry. The dried residue obtained was dissolved in 150 μl of mobile phase, and 20 μl of the supernatant liquid was then injected into the LC–ESI-MS system for analysis. All the process was protected from light.

4.5. Drug administration and sample collection

The pharmacokinetic study protocol used was approved by the State Food and Drug Administration (SFDA, China). Sixteen healthy volunteers including 8 males and 8 females were enrolled in the study. They were randomly divided into two groups, such as Groups A–B. Each group was made up of four males and four females. The age was in a range of 23–28 years and the weight was in a range of 55–65 kg. They were selected after passing a clinical screening procedure including a physical examination and laboratory tests, which included hematology, blood biochemistry, and urine analysis. No volunteers had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or any acute or chronic disease, or an allergy to any drugs. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications. All volunteers avoided using other drugs for at least 2 weeks prior to the study and until after its completion. This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of good clinical practice (GCP). The protocol of this study was approved by the ethics committee of the Nanjing First Hospital Affiliate To Nanjing Medical University (Nanjing, China). All participants signed a written informed consent after they had been informed of the nature and details of the study. Volunteers were hospitalized at 9:00 p.m. 1 day before this study and fasted 10 h before each drug administration. At 8:00 a.m., Groups A–B were administered a single dose of azelnidipine 8 mg and 16 mg with 250 ml water, respectively. A standard lunch was served after 4 h, and an evening meal was provided 12 h after administration. During the 24 h period after drug administration, no strenuous physical or mental activity was permitted. No other food was permitted during the 'in-house' period but liquid consumption was allowed *ad libitum* after lunch (with the exception of alcohol, soda, and coffee drinks, as well as juices). Heparinized blood samples (5 ml) were collected from a suitable forearm vein using an indwelling catheter into heparin containing tubes before (0 h) and 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 36, 48, 72, 96 h after dosing. The blood samples were centrifuged at 4000 r for 10 min, and plasma samples were separated and stored at −70 °C until required for analysis. In the design of multiple doses, Group A received azelnidipine 8 mg with 250 ml water at 8:00 a.m. every day for 7 consecutive oral doses. In days 5, 6 and 7, 5 ml of venous blood before every dosing at 8:00 a.m. was drawn to observe minimum value of steady plasma-drug concentration. In day 7, the procedure was as that of single dose mentioned above.

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