Acta Pharmaceutica Sinica B 2013;3(3):180–184

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

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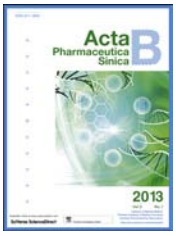

ORIGINAL ARTICLE

Determination of ifenprodil by LC–MS/MS and its application to a pharmacokinetic study in healthy Chinese volunteers

Jing Yang, Chengtao Lu, Wei Song, Jiankang Li, Yi Ding, Yanrong Zhu, Jinyi Cao, Likun Ding, Yanyan Jia, Aidong Wen*

Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Shanxi 710032, China

Received 22 November 2012; revised 27 December 2012; accepted 28 March 2013

KEY WORDS

Ifenprodil;
Liquid/liquid extraction;
LC–MS/MS;
Pharmacokinetics;
Intravenous infusion;
Human;
Plasma

Abstract This paper reports the development and validation of an assay for ifenprodil based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) and its application to a pharmacokinetic study involving single and multiple intravenous infusions to healthy Chinese volunteers. After sample preparation of plasma by liquid–liquid extraction with ethyl acetate, the analyte and internal standard, urapidil, were separated by reversed phase chromatography in a run time of 4 min and detected by positive ion electrospray ionization followed by multiple reaction monitoring of the precursor-to-product ion transitions at *m/z* 326.2→308.1 for ifenprodil and *m/z* 388.4→205.3 for IS. The assay was linear in the concentration range 0.2–50.0 ng/mL with recovery >76.4%. In the pharmacokinetic study of single intravenous infusions of 5, 10 and 15 mg ifenprodil, peak plasma concentrations and areas under the plasma concentration–time curve were both linearly related to dose. In the pharmacokinetic study of multiple once daily intravenous infusions of 10 mg ifenprodil for 7 days, pharmacokinetic parameters were similar to those after the single dose showing that ifenprodil does not accumulate on repeated administration.

*Corresponding author. Tel./fax: +86 29 84773636.
E-mail address: adwen_2004@hotmail.com (Aidong Wen).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

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http://dx.doi.org/10.1016/j.apsb.2013.04.001

1. Introduction

Ifenprodil [(1*RS*, 2*SR*)-4-[2-(4-benzylpiperidin-1-yl)-1-hydroxypropyl]phenol hemi-(2*R*,3*R*)-tartrate] (Fig. 1A) is a new class of *N*-methyl-D-aspartate (NMDA) receptor antagonist that selectively inhibits NMDA receptors containing the NR2B subunit[1,2]. The drug has been widely used in the treatment of cerebrovascular diseases and peripheral arterial obliterative disease[3–5]. However, pharmacokinetic studies of ifenprodil are limited to one involving a single dose administration[6] and there is no literature reporting the pharmacokinetics of ifenprodil after multiple doses. The aim of the present study was to develop and validate an assay for ifenprodil based on liquid chromatography–tandem mass spectrometry (LC–MS/MS)[7] and apply it to a pharmacokinetic study of ifenprodil involving single and multiple intravenous infusions to healthy Chinese volunteers.

2. Materials and methods

2.1. Reagents and materials

Ifenprodil tartrate for injection and ifenprodil reference standard (purity 98.7%) were obtained from Sihuan Kebao Pharmaceutical Co., Ltd. (Bejing, China). Urapidil for use as internal standard (IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was deionized and purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade and used as received. Drug-free human plasma from healthy volunteers was kindly provided by the Blood Center of Xijing Hospital (Shanxi, China) and was stored at −20 °C until use.

2.2. Instrumentation

LC–MS/MS was performed using an Agilent 1200 series HPLC equipped with a Spursil C18-EP column (150 mm × 2.1 mm i.d., 3 μm) maintained at 35 °C and an Agilent 6410 Triple Quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent Technologies, USA) operating in the positive ionization mode. All data were acquired and processed using Agilent 6410 Quantitative Analysis processing software.

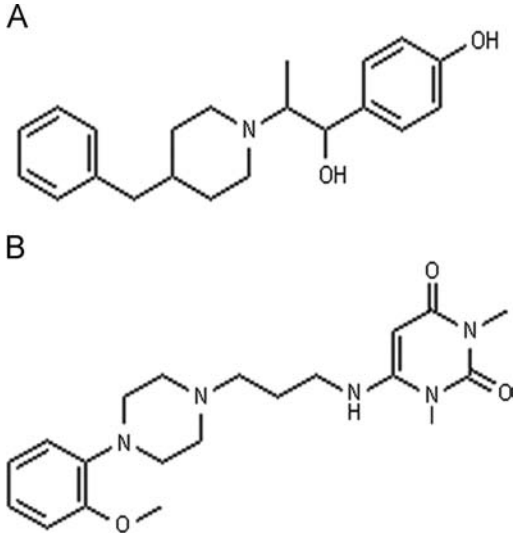

Figure 1 Chemical structures of (A) ifenprodil and (B) urapidil.

2.3. Preparation of the calibration standards and quality control (QC) samples

A stock solution of ifenprodil was prepared in methanol and serially diluted with methanol to give standard solutions with concentrations of 0.01, 0.1, 1.0, 10.0 and 100.0 μg/mL. In a similar manner, an IS working solution (203.2 ng/mL) was prepared by diluting a stock solution of urapidil (101.6 μg/mL). All solutions were kept at −20 °C until use. Calibration standards (0.20, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 ng/mL) and QC samples (0.5, 4 and 40 ng/mL) were prepared by spiking 0.5 mL blank plasma samples with different volumes of working solutions.

2.4. Sample preparation

Frozen human plasma samples were thawed at ambient temperature and 0.5 mL aliquots mixed with 50 μL IS working solution. The mixture was then subjected to liquid–liquid extraction (LLE) by vortex-mixing for 3 min with 4 mL ethyl acetate and centrifuging at 4000 rpm for 10 min. The organic layer was removed and evaporated under a stream of nitrogen gas at 40 °C. The residue was reconstituted in 120 μL mobile phase, vortex-mixed for 1 min, centrifuged at 16,000 rpm for 3 min and 5 μL of supernatant injected into the LC–MS/MS system.

2.5. LC–MS/MS conditions

The mobile phase was methanol–10 mM ammonium acetate solution (90:10, *v/v*) delivered at a flow rate of 0.2 mL/min. Detection was by multiple reaction monitoring (MRM) of the precursor-to-product ion transitions at *m/z* 326.2 → 308.1 for ifenprodil and *m/z* 388.4 → 205.3 for IS. Optimized MS parameters were as follows: nebulizer pressure 40 psi; drying gas temperature 350 °C; dry gas flow rate 10 L/min; dwell time per transition 200 ms; EMV 200 V; fragmentor voltages 140 V (ifenprodil) and 150 V (IS); collision energies 19 eV (ifenprodil) and 45 eV (IS).

2.6. Assay validation

Assay validation was carried out according to the USFDA guidelines[8]. Specificity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Linearity was determined by weighted ($1/C^2$) least-squares linear regression of calibration curves based on eight calibration standards prepared from three batches of plasma. The acceptance criteria for the calibration curve were a correlation coefficient (r) ≥ 0.99 and that each back-calculated concentration was within 15% of the nominal value. The lower limit of quantitation (LLOQ) was defined as the lowest concentration on the calibration curve at which the precision (as relative standard deviation, RSD) was 20% and accuracy (as relative error, RE) was ±20%. Precision and accuracy of the method were determined by assay of 5 replicates of QC samples on three validation days. Intra- and inter-batch precisions (as RSD calculated by one-way analysis of variance) were required to be <15% with accuracy (as RE) of ±15%. Recovery of ifenprodil was determined by comparing peak areas for ifenprodil in 5 replicates of QC samples with those of blank plasma extracts to which ifenprodil was added after extraction at corresponding