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Application of liquid chromatography–tandem mass spectrometry to study the effect of docetaxel on pharmacokinetics and tissue distribution of apatinib in mice

Siqi Feng[1], Jingwei Zhang[1], Ying Wang, Runbin Sun, Dong Feng, Ying Peng, Na Yang, Yue Zhang, Haoxue Gao, Huilin Gu, Guangji Wang, Jiye Aa*, Fang Zhou*

State Key Laboratory of Natural Medicines, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, China

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ABSTRACT

Apatinib, a highly selective small-molecule inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2), has attracted many attentions due to its anticancer activity in various malignancies containing non-small-cell lung cancer (NSCLC). Our previous preclinical study confirmed the enhanced anti-tumor efficacy of combined treatment between apatinib and docetaxel for NSCLC. However, the effects of docetaxel on pharmacokinetics and tissue distribution of apatinib are not clear. In present study, a reliable HPLC-MS/MS method was established for determination of apatinib. This method had a good linearity in the range of 1–5000 ng/mL, and the recovery and matrix effect were 100.1–103.5%, 77.6–83.5%, respectively. Plasma exposure level of apatinib and the values of C_{max}, AUC_{0-12h}, $T_{1/2}$, and MRT were not affected by multi-dose of docetaxel. The tissue distributions (kidney, heart, lung, spleen) of apatinib in combined treatment group were lower at 0.25 h but higher at 2 h, and that in intestine and liver were not significantly changed compared with control group. However, pretreatment with docetaxel had no significant effect on AUC_{0-4h} of apatinib in tissues in mice. In conclusion, plasma and tissues exposure levels of apatinib were not affected by long-termed treatment with docetaxel, indicating that docetaxel is less likely to increase the side effect of apatinib such as hypertension, hand-foot syndrome and so on.

1. Introduction

Apatinib is a small molecule tyrosine kinase inhibitor (TKI) which selectively inhibits VEGFR-2 [1]. It has been approved as a third or fourth line medicine and used in the therapy for advanced gastric cancer. In current, more and more clinical research demonstrated the efficacy of apatinib for the treatment of cancers, such as non-small cell lung cancer (NSCLC), advanced hepatocellular carcinoma and breast cancer [2–4]. Apatinib has attracted many attentions due to its encouraging anticancer activity in clinic [5,6].

In recent years, some anti-angiogenesis drugs were reported to have a relapse or metastases after discontinuation such as sunitinib, sorafenib [7–9]. In addition, no prolonged overall survival was described in some patients because of acquired resistance. Therefore, combination of two or more anti-tumor agents is a more effective strategy to overcome acquired resistance and the limited efficacy of mono anti-angiogenic therapy [10,11]. Today, many studies focus on the possibility of combined treatment of apatinib with other chemotherapeutic drugs in second line or first line for cancer patients [12–14].

Our previous study demonstrated the anti-tumor effect of apatinib was enhanced when combined with docetaxel in A549 xenograft mice [15]. However, docetaxel and apatinib are both the substrate of CYP3A4 [16,17], which has potential for occurring drug–drug interaction. Therefore, whether the pharmacokinetic process of apatinib is affected by combined treatment with docetaxel needs further investigation.

In present study, a HPLC-MS/MS method was established and validated for determination of apatinib. This method was then applied to investigate the pharmacokinetic behavior of apatinib with or without long-term administration of docetaxel in mice and to evaluate the effect of docetaxel on tissue distribution of apatinib. This study can provide more evidence for estimating pharmacokinetic characteristics and guidance for the clinical combined treatment of apatinib and docetaxel.

* Corresponding authors at: State Key Laboratory of Natural Medicines, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing, Jiangsu, China.
E-mail addresses: jiyea@cpu.edu.cn (J. Aa), zf1113@163.com (F. Zhou).
[1] Siqi Feng and Jingwei Zhang contributed equally to this work.

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2. Materials and methods

2.1. Chemicals and reagents

Apatinib (purity > 98%) was obtained from Jiangsu Hengrui Medicine Co. (Jiangsu, China). Ponatinib (purity > 98%) as internal standard (IS) was obtained from Nanjing Sanhome Pharmaceutical Co. Ltd. (Jiangshu, China). The methanol was HPLC grade and purchased from Merck Company (Darmstadt, Germany). Ultra-pure water was produced by a Milli-Q system of Millipore (Bedford, USA). Other reagents and solvents were analytical grade.

2.2. Instrumentation and conditions

The HPLC system was comprised of a online degasser, two LC-20AD XR pumps, a SIL-20AC XR autosampler and a CTO-20A column oven (Shimadzu, Kyoto, Japan). Apatinib and ponatinib (IS) were separated by an Agilent ZORBAX SB-C18 column (2.1 × 150 mm, 3.5 μm, Agilent Technologies, USA). The temperature of column oven was maintained at 40 °C during the separation. The mobile phase was as follow: (A) aqueous phase: 0.1‰ formic acid in water; (B) organic phase: methanol. The gradient elution was at a flow rate of 0.25 mL/min and 5 μL injection volume. Compounds were eluted in the following gradient conditions: the initial proportion was 45% B and hold for 0.5 min, and increasing to 85% B in one minute and maintained for 1.5 min, followed by decreasing to 45% B within 0.5 min, and the whole gradient time was 7.5 min.

The mass spectrometric detection was performed on an Sciex API 4000 triple-quadrupole mass spectrometer (CA, USA) with an electrospray ionization (ESI) source in positive mode. Quantification was acquired by multiple reaction monitoring (MRM) mode of *m/z* 398.30 → 184.30 at 3.98 min for apatinib and m/z 533.40 → 433.00 at 4.00 min for IS. After optimization, the detective conditions of mass spectrometer were as follows: internal source voltage 3000 V, spray temperature 550 °C, ion Source Gas 1 50 Arb, Ion Source Gas 2 55 Arb, Curtain Gas 26 Arb, and Collision Gas 10 Pa. The collision energy for analyte and IS was 50 eV and 35 eV, respectively.

2.3. Animal

Male Balb/c mice of 4–6 weeks and 18–22 g were purchased from Shanghai Slike Experimental Animals Co. (Shanghai, China). All mice had free access to food and water and were cared under standard conditions (12/12 h light/dark cycle, humidity of 60% ± 5%, 22–25 °C) according to Animal Facility Guidelines of the China Pharmaceutical University.

2.4. Standard and sample preparation

2.4.1. Stock solutions, calibration standards and quality control (QC) samples preparation

The standard stock solutions of apatinib and IS were both dissolved in methanol at the concentration of 1 mg/mL. Subsequently, the working standard solution of apatinib was diluted the concentrations to 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10,000, 20,000, 50,000 ng/mL with methanol. These working solutions were stored at 4 °C until analysis. 5 μL of each standard solution was added to 45 μL of blank plasma or tissue homogenates (liver, heart, kidney, spleen, lung and intestine) biological matrix to obtain final concentrations of 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 ng/mL. Quality control (QC) samples (20, 5000 and 40,000 ng/mL) were obtained by the same way as the calibration standards.

2.4.2. Sample preparation

An aliquot of 50 μL sample was added to a new eppendorf tube, and vortexed with 150 μL methanol containing IS for 3 min to precipitate protein. After centrifugation at 18,000 rpm for 10 min, 5 μL of supernatant was injected to the HPLC–MS/MS system for separation and MS detection. The plasma samples with concentrations above the upper linear range were diluted 1:10 (v/v) by blank mice plasma prior to analysis to be within the validated range. Around 100 mg of tissue were accurately weighed to obtain various tissue homogenate (2 mL/0.2 g tissue). 50 μL of tissue homogenate was operated as the plasma.

2.5. Method validation

The specificity of the proposed method was evaluated by five lots of blank mouse plasma sample added with apatinib (500 ng/mL), blank biological matrices with apatinib and the IS to exclude potential endogenous interference.

Extraction recovery and matrix effect were determined at three concentrations of apatinib. The extraction recovery was calculated by peak area ratios of extracted samples to those of post-extracted samples. Matrix effect is calculated by the peak area ratios of post-spiked samples with those of standard solutions (at equivalent concentrations). The matrix effect was considered as regular if the ratio was in the range of 85–115%.

Calibration curves were prepared with blank mouse plasma at eight concentrations in the range of 1–5000 ng/mL of apatinib. The standard curves of apatinib were constructed by plotting the peak area ratios of apatinib to IS vs. the concentrations of apatinib with the weighted least square method (w = 1/x) based on analysis software. The lower limit of quantification (LLOQ) was determined with a precision lower than 15% and accuracy of 85–115%.

Precision and accuracy were assessed by analyzing five replicates at four different concentrations (2, 50, 500, 5000 ng/mL) on the same day and on three consecutive days. Accuracy is calculated against the calibration curve. The precision was assessed as intra- and inter-day relative standard deviation (RSD) not exceeding 15%. The accuracy was calculated as the relative error (RE) between the mean measured concentration and the nominal concentration and the RE value should be within 85–115%.

The stability of analyte in biological matrix was accessed by determining five replicates under various storage conditions: three freeze–thaw cycles, 12 h at room temperature, long-term storage stabilities (−70 °C for 30 days) and post-preparative stability in the autosampler for 24 h. The analyte was considered stable when calculated bias was within 85–115%.

2.6. Pharmacokinetics and tissue distribution study

Male mice were randomized into two groups (24 mice per group): (a) Control (saline, every four days for three times, i.p.); (b) Docetaxel (8 mg/kg, every four days for three times, i.p.). Mice of the two groups were all treated with apatinib (150 mg/kg) at the third day after two days of the last time treatment of saline or docetaxel. All animals were fasted overnight prior to the experiment. The dosing solutions of apatinib for intragastric administration were prepared by suspending in 0.5% carboxymethyl cellulose sodium salt (CMC-Na). The 24 mice were randomly divided into 4 sub-groups (n = 6). Blood samples were collected from the mice of each sub-group at the time points of 0 and 6 h, 1 and 12 h, 0.25 and 2 h, 4 and 10 h after oral administration of apatinib. In addition, liver, heart, kidney, spleen, lung and intestine of mice were collected after 0.25, 1, and 4 h treatment of apatinib (n = 6). Whole blood was centrifuged at 8000 rpm for 5 min immediately and plasma was collected and stored at −70 °C until analysis.

2.7. Statistical analysis

Statistical analysis was performed by GraphPad Prism 6.0 (GraphPad software, Inc., San Diego, CA). Experimental values are expressed as mean ± SD. Statistical analysis was conducted using a