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An UPLC–MS/MS method for the quantitation of alectinib in rat plasma

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ABSTRACT

Currently, crizotinib is the first generation drug, which has been used in the treatment of ALK-rearranged non-small cell lung cancer (NSCLC). However, more and more patients are found in crizotinib-resistance. In the last year, alectinib has been approved for treatment of patients with crizotinib-resistance.

In this study, we aim to develop and validate a simple, rapid and sensitive tandem mass spectrometry (UHPLC–MS/MS) method for determination of alectinib in rat plasma. Diazepam was chosen as an internal standard (IS). Protein precipitation by acetonitrile was utilized to prepare plasma samples. Chromatographic separation was achieved on a RRHD Eclipse Plus C18 (2.1 × 50 mm, 1.8 μ) column with a gradient mobile phase consisting of acetonitrile and water (containing 0.1% formic acid). The analytes were detected by an electrospray ionization (ESI) source in positive mode. A dynamic multiple reaction monitoring (MRM) method was developed to detect specific precursor and product ions. The target fragment ions were *m/z* 483.2 → 396.1 for alectinib and *m/z* 285.0 → 192.9 for diazepam (IS). Linear calibration plots were achieved in the range of 1–500 ng/ml for alectinib (R^2 = 0.997) in rat plasma. Mean recoveries of alectinib in rat plasma ranged from 84.2% to 92.2%. The intra- and inter-day precision was below 9.3% and accuracy was from −1.4% to 12.1%. No obvious matrix effect was found. This method shows a good performance: accuracy, precision and stability. It has been fully validated and successfully applied to pharmacokinetic study of alectinib.

1. Introduction

Lung cancer is a common cancer disease, resulting in more than one million deaths each year [1]. Most lung cancers (~90%) are non-small cell lung cancers (NSCLC), which comprise a number of subtypes driven by various activated oncogenes [2]. Generally, this disease could not be diagnosed until it is locally advanced or metastatic, resulting in a high mortality rate [3]. Anaplastic lymphoma kinase (ALK) has been identified as a drug target. In the clinical practice, it plays a fundamental role in 3%–7% of NSCLCs [4,5]. ALK, a member of the insulin receptor tyrosine kinase family, is encoded by the ALK gene on chromosome 2p23 [6]. Soda et al. first reported the ALK gene in a resected adenocarcinoma specimen from a 62 years old male smoker and found that a small inversion of ALK gene within chromosome 2p results in the formation of a fusion gene comprising portions of the EML4 gene [7]. Various variants of EML4-ALK have been identified in NSCLC [8,9]. Crizotinib, the first-generation of ALK inhibitor, was used for ALK-positive non-small-cell lung cancer. However, brain metastases are widespread existing at diagnosis of ALK-positive NSCLC. Due to the poor penetration of crizotinib through the blood-brain barrier, it is hard to achieve therapeutic drug concentrations in the central nervous system (CNS) [10–12]. Also, Sasaki and Choi et al. revealed that the EML4-ALK C1156Y, L1196M and F1174L mutations were identified as the cause of crizotinib-resistance [13,14].

To overcome the crizotinib-resistance, the second-generation ALK-inhibitors have enter the clinic. Ceritinib (in the US in April 2014) [15] has shown a good effect in ALK-positive NSCLC treatment in early phase of clinical development and in CNS metastases patients, interestingly, its activity of antitumor was observed both in crizotinib-native patients and crizotinib-refractory patients [16].

Similar to ceritinib, alectinib (Alecensa®; Fig. 1), a novel and highly selective oral ALK inhibitor, also shows antitumor activity in crizotinib-resistance, and it has been approved by FDA in 2015 [17,18], also, it is currently under evaluation by EMA (status June 2016). Alectinib can selectively inhibit the kinase activity of ALK and shows a strong antitumor activity to treat patients with advanced ALK-positive NSCLC [17,19,20]. In addition, it exerts activity against leukocyte tyrosine kinase receptor (LTK) and cyclin-G-associated kinase (GAK) [21]. Compared to the first-generation ALK inhibitor

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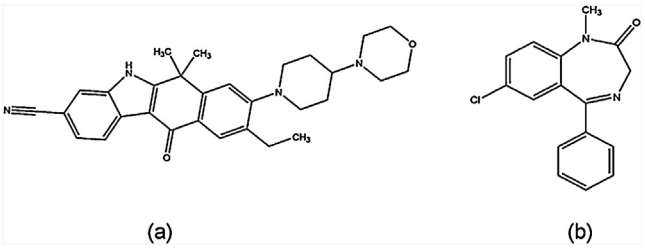

Fig. 1. The chemical structures of (a) alectinib and (b) diazepam (IS).

crizotinib, the alectinib has shown better treatment in crizotinib-resistant patients [22,23]. Kinoshita et al. reported that this effect is due to high antitumor activity both in vitro and vivo against tumor cell lines with some types of ALK gene alteration. Alectinib is also active against the gatekeeper Leu1196Met mutation in EML4-ALK [21], which is playing an important role in emerge of the crizotinib-resistant [13]. Also, alectinib shows a good treatment on crizotinib-induced interstitial lung disease and esophageal ulceration [24,25].

There are many studies about the treatment of alectinib in ALK-inhibitors resistance and crizotinib-induced diseases, but few about determination of the alectinib in plasma. Morcos et al. reported the method of LC–AMS to determine the concentration of [^{14}C]-alectinib in human plasma [26]. To the best of our knowledge, there was no published report about the determination of the concentration of alectinib in rat plasma. We aim to develop a sensitive and validated assay to examine the preclinical plasma pharmacokinetics of alectinib for avoiding drug toxicity and obtaining a better therapeutic efficacy. We also explore the pharmacokinetic properties of alectinib by the accurate, stable and selective method via UHPLC–MS/MS. This method was validated for selectivity, linearity, precision, accuracy, recovery, stability, and was successfully applied to measure concentration of alectinib in rat plasma.

2. Experimental

2.1. Materials

Alectinib and diazepam (internal standard, IS) were obtained from Sigma (St. Louis, MO, USA). LC-grade acetonitrile, methanol and formic acid were purchased from Merck Company (Darmstadt, Germany). Ultrapure water was produced by the Millipore purification system (Bedford, MA, USA).

2.2. Instrumentation and conditions

Chromatographic separation was performed on an Agilent 1290 Infinity Liquid Chromatography (UHPLC) system with a RRHD Eclipse Plus C18 (2.1 × 50 mm, 1.8 μ) column at the flow rate of 0.4 ml/min and a constant temperature of 35 °C to separate compounds. The dwell volume was 0.16 ml. Column void volume was 0.13 ml. Mobile phase consisting of 0.1% formic acid (A) and acetonitrile (B) was applied. The gradient elution was used as follows: 0.0–0.8 min linear increase to 95% B, 0.8–1.8 min maintained at 95% B, and 1.8–2.5 min linear decrease to 25% B. The injection volume was 2 μl.

Detection was performed using the Agilent 6490 Triple Quadruple LC/MS The system was equipped with an electrospray ionization (ESI) source in positive mode. A dynamic multiple reaction monitoring (MRM) method was developed to detect specific precursor and product ions (quantifier and qualifier) of the alectinib and IS inside their retention time window. In addition to a specific MRM transition, the retention time and the ratio of quantifier and qualifier product ions were used to assure the specific detection of analyte. In this study, to determine the analyte accurately and assure the specific detection, the most intense fragment was used as quantifier, and the second-one was used for qualification. MS parameters of alectinib and diazepam are shown in Table 1.

2.3. Standard and sample preparation

The stock solution of alectinib was dissolved in methanol to make the calibration standards. The working solutions of the alectinib were obtained by diluting the corresponding stock solutions to the suitable concentration levels.

The alectinib calibration standards were prepared by appropriate working solutions with blank rat plasma. To each plot of the calibration was added 10 μl of corresponding working solution to 90 μl of the blank rat plasma. The final concentrations of the calibration standards were 1, 5, 10, 20, 50, 100, 200 and 500 ng/ml. The concentrations of QC samples were prepared independently in the same way at three levels (2, 40, 800 ng/ml).

Quality control (QC) samples with concentration of 1.0 mg/ml were prepared by dissolving 10 mg in 10 ml methanol. Stock solution of IS was prepared by dissolving in the methanol to the final concentration of 10 mg/ml. The working solution of IS (100 ng/ml) was made from the stock solution using methanol for dilution. All working solutions, stock solutions, calibration standards and QCs were stored at −20 °C.

2.4. Sample preparation

The frozen plasma samples were placed to room temperature before analysis. After it thaw completely, 100 μl of plasma samples and 200 μl of acetonitrile were added for protein precipitation, then 30 μl of internal standard were pipetted into 1.5 ml polypropylene tubes. The tubes were vortex mixed for 2 min for fully blending. After centrifuging at 12,000 g for 10 min, the supernatant (100 μl) was diluted by 100 μl of ultrapure water and in a new tube. The tube was gently mixed for 30 s to fully blending, then pipetted 50 μl into an UHPLC vial for separating.

2.5. Method validation

Before determining the alectinib in rat plasma, the method was validated for specificity, precision, linearity, accuracy, recovery, matrix effect and stability according the United States Food and Drug Administration bioanalytical method validation guidances [27].

2.5.1. Specificity

The specificity of the method was evaluated by analyzing six different blank plasma samples from six rats, blank plasma-spiked alectinib and IS, as well as the plasma samples obtained after oral administration.

2.5.2. Linearity and lower limit of quantification (LLOQ)

To evaluate the linearity, calibration standards of eight different concentrations of alectinib (1–500 ng/ml) were measured and assayed on three different days. The linearity of alectinib was evaluated by the weighted ($1/x^2$) least-squares linear regression of the peak area ratios against concentrations. The lowest concentration of alectinib on the calibration curves was defined as the LLOQ.

2.5.3. Accuracy and precision

To analyze the accuracy and precision of the method, three different concentrations of the QC samples (2, 40 and 800 ng/ml) in rat plasma were determined in three separate days. RSD (relative standard deviation, %) and RE (the percentage of the measured con-