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Development and validation of a highly sensitive LC–MS/MS method for quantification of IC87114 in mice plasma, bronchoalveolar lavage and lung samples: Application to pharmacokinetic study

Anu Marahatta[a], Bidur Bhandary[a], Yong-Chul Lee[b], So-Ri Kim[b], Han-Jung Chae[a,*]

[a] Department of Pharmacology, School of Medicine, Chonbuk National University, Jeonju 560-182, Republic of Korea
[b] Department of Internal Medicine, School of Medicine, Chonbuk National University, Jeonju 560-182, Republic of Korea

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ABSTRACT

IC87114 is a selective PI3Kδ inhibitor. A simple, sensitive and reliable LC–MS/MS method with rapid sample preparation was developed and validated for the determination of IC87114 in mouse plasma, bronchoalveolar lavage, and lung. Chromatographic separation was achieved using an Agilent Zorbax Eclipse XDB-C_{18} column (150 mm × 2.1 mm internal diameter, 3.5 μm particle size). Mass spectrometric detection was conducted by electrospray ionization in positive ion multiple reaction monitoring modes. The calibration curve was linear over a concentration range of 0.01–1000 ng/mL for plasma/BAL and 0.1–250 ng/mL for lung tissue. Recoveries were as high as 97.29%, 102.81% and 89.70% for plasma, BAL fluid and lung sample, respectively. The lower limit of quantification was 0.01 ng/mL. Intra-day and inter-day accuracy and precision were within the acceptable limits of ±15% at all concentrations. Finally, the method was successfully used in a pharmacokinetic study that measured IC87114 in mouse plasma, BAL fluid and lung tissue after administration of a single 1 mg/kg intratracheal dose of IC87114. The percentage change for incurred sample reanalysis (ISR) was within ±15.0% and met the acceptance criteria for ISR.

1. Introduction

Phosphatidylinositide 3-kinases (PI3K) family proteins catalyze the phosphorylation of phosphoinositides at the 3-hydroxyl position and generate lipids that control a wide variety of intracellular signaling pathways. PI3Ks can be classified into class I, class II, and class III subfamilies according to their structure and substrate specificity [1,2]. Class I PI3Ks are responsible for the production of phosphatidylinositol 3-phosphate (PI(3)P), phosphatidylinositol (3,4) bisphosphate (PI(3,4)P2), and phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3). PI3Ks are involved in different cellular functions such as cell growth, proliferation, motility, differentiation, survival and intracellular trafficking [3]. PI3Ks affect key signaling pathways via several downstream molecules such as protein kinase B (PKB/AKT), 3′-phosphoinositide dependent kinase 1 (PDK1), mammalian target of rapamycin (mTOR), and glycogen synthase kinase 3 (GSK3), thus modulating different biological functions such as cell proliferation, cell survival, cell differentiation, migration, metabolism and chemotaxis [4]. There are four isoforms of PI3K class I: α, β, γ, and δ. Among these, the α, β and δ subunits are grouped together as class IA due to their association with the adapter subunit, p85. PI3Kδ isoforms are predominantly expressed in hematopoietic cells [5]. PI3Kδ inhibitors are also novel therapeutic targets to treat rheumatoid arthritis and other inflammatory diseases. Use of PI3Kδ inhibitor has even been shown to enhance the sensitivity of beta(2)-agonist formoterol against oxidative stress-induced corticosteroid resistant chronic obstructive pulmonary disease (COPD) [6].

Asthma is one of the most common chronic diseases in the world and is characterized by reversible airway obstruction and airway hyperresponsiveness [7]. Corticosteroids are the most potent of the anti-inflammatory drugs used to treat asthma. Some reports have linked poor response to corticosteroids in patients with COPD or asthma to oxidative stress. Corticosteroids are also well known to have other side-effects. Hence, there the need exists to develop new drugs to treat pulmonary diseases such as asthma and COPD. Recently a selective inhibitor of PI3Kδ, IC87114 (Fig. 1), has been widely studied for the treatment of pulmonary diseases including asthma and COPD [6,8,9]. This inhibitor was found to reduce the polarized morphology of neutrophils, formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated PIP3 production and chemotaxis. It has also been used to explore the importance of PI3Kδ in neutrophil migration [10].

The objective of this study was to develop and validate a highly sensitive LC–MS/MS method for the quantitative analysis of IC87114 in mouse plasma, BAL fluid and lung samples.

* Corresponding author. Tel.: +82 63 270 3092; fax: +82 63 275 2855.
E-mail address: hjchae@chonbuk.ac.kr (H.-J. Chae).

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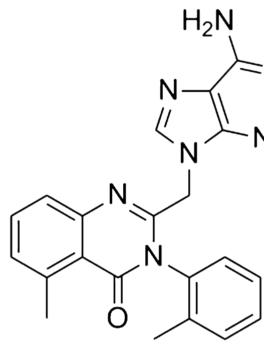

Fig. 1. Chemical structure of IC87114.

2. Experimental

2.1. Chemicals and reagents

IC87114 was purchased from Calbiochem (San Diego, CA). Acetaminophen was purchased from Sigma (St. Louis, MO, USA). Water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). LC–MS grade acetonitrile, methanol and formic acid were from Fischer Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of calibration standards and quality control (QC)

Stock solutions of IC87114 were prepared by dissolving 5 mg of the drug in 1 mL of dimethyl sulfoxide (DMSO) and were stored at −20 °C. Internal standard (IS; acetaminophen) was dissolved in methanol. Calibration standards were prepared by spiking working standards and IS into 50 μL of blank plasma, BAL fluid or lung tissue homogenates from an untreated mouse. The final concentrations of the standard curve samples were 0.01–1000 ng/mL for plasma and BAL, and 0.1–250 ng/mL for lung tissue. The IS concentration was 1 ng/mL in each sample. QC samples were prepared at six concentrations (1000, 500, 100, 10, 0.1, 0.01 ng/mL for plasma/BAL fluid, or 250, 100, 50, 10, 0.1 ng/g for lung tissue). The calibration standard samples and QC samples were stored at −20 °C until analysis.

2.3. Animals

The animal ethics committee at Chonbuk National University of Korea approved all experiments. C57B/L6 mice were obtained from Damul Science (Deajeon, South Korea). Eight-week-old male mice were kept in a fully acclimatized room at constant temperature and humidity on a 24-h light/dark cycle. The animals had free access to food and water.

2.4. Nonsurgical intratracheal administration method

Mice were given a single dose of IC87114 (1 mg/kg) intratracheally (IT) by a nonsurgical method [11]. We choose a dose of 1 mg/kg because of its proven efficacy for treatment of pulmonary disease [9,12,13]. The tongue was pulled out with forceps and held to the side. Using the other hand, the bent gavage needle of a 1 mL syringe containing the drug was inserted into the trachea and the plunger was pushed down to deliver the drug. Blood samples were collected at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 18, 24, 48 and 72 h. Six animals were scarified at each time point.

2.5. Bronchoalveolar lavage

Mice were anesthetized using intraperitoneal pentobarbital sodium. Blood was collected from the eyes of euthanized mice using a capillary. The chest cavity was exposed to allow expansion, after which the trachea was carefully intubated and the catheter secured with ligatures. The lungs were lavaged with 1 mL of 0.9% NaCl. The bronchoalveolar lavage (BAL) fluid was centrifuged at 400 × g for 10 min at 4 °C to pellet the cell fraction, and the supernatant was stored at −80 °C until the measurements were carried out.

Table 1
Multiple reaction monitoring (MRM) transition and condition for IC87114 and IS.

Compound	Precursor ion	Product ion	Frag (V)	CE (V)	Polarity
IC87114	398	146	130	35	Positive
Acetaminophen (IS)	151.900	110	130	20	Positive

2.6. Chromatography

HPLC separation was performed on an Agilent 1100 system (Agilent, Palo Alto, CA, USA). Chromatographic separation was achieved using a Zorbax Eclipse XDB-C18 column (150 mm × 2.1 mm internal diameter, 3.5 μm particle size). Separation of IC87114 and internal standard was carried out by gradient elution. The mobile phase was composed of (A) 10 mM ammonium formate, and (B) acetonitrile. The gradient run started from 10% solvent B for 1 min, increasing up to 100% over 6 min and remaining there for another 1 min, followed by a return to 10% for 15 min. Chromatography was performed at 30 °C with a flow rate of 0.3 mL/min, and the run time was 15 min. The injection volume was 2 μL of each sample.

2.7. Mass spectrometry

An Agilent Technologies 6410 triple quadruple mass spectrometer equipped with electrospray ionization (ESI) in the positive ionization mode was used. The following conditions were found to be optimal for analysis: capillary voltage 4 kV, gas temperature 300 °C, dwell time 200 ms and gas flow 10 L/min. Samples were analyzed by multiple reactions monitoring (MRM). Precursor ions, product ions, and MS/MS parameters are reported in Table 1. Mass hunter software was used to control the LC–MS/MS system and for data analysis.

2.8. Sample preparation procedure

2.8.1. Plasma and BAL fluid

Proteins were precipitated by the addition of 5 μL of IS (100 ng/mL) and 445 μL of methanol to 50 μL plasma/BAL. The resulting solution was vortexed for 30 s and centrifuged at 13,000 × g for 20 min. The supernatant was transferred to a HPLC vial and injected.

2.8.2. Lung

Lung tissues were cut into small pieces with scissors and were homogenized in a phosphate buffer saline solution (PBS) using a Polytron PT 1200C homogenizer (Kinematica INC., Switzerland). Proteins were precipitated by the addition of 5 μL of IS (100 ng/mL) and 445 μL of methanol to 50 μL lung solution. The resulting solution was vortexed for 30 s, centrifuged at 13,000 × g for 20 min and an aliquot of the supernatant was used for analysis.

2.9. Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analytes in presence of other components in the sample. The specificity of the method was evaluated by screening six different lots of blank plasma, BAL fluid and lung. Selectivity was evaluated by analyzing different blank plasma, BAL fluid and