

Pharmacokinetics and Bronchopulmonary Disposition of PI3K δ Inhibitor IC87114 after Intratracheal Administration in a Severe Asthma Model

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Marahatta, *et al.*: Pharmacokinetics of PI3K Inhibitor in Asthma

Type 2 helper T cell-predominant asthma is a refractory type of asthma that has motivated biomedical industries to develop novel drugs. The phosphoinositide 3-kinase pathway has been implicated in asthma and chronic obstructive pulmonary disease. IC87114 (2-[(6-aminopurin-9-yl)methyl]-5-methyl-3-[2-methylphenyl]quinazolin-4-one) was recently developed as a PI3K δ inhibitor. In a neutrophil type 2 helper T cell dominant asthma model, pharmacokinetic analysis of IC87114 was performed. Following intratracheal administration of 1 mg/kg IC87114 to ovalbumin/lipopolysaccharide-sensitized/challenged mice, airway hyper-responsiveness and structural analysis was performed and the plasma concentration time course and bronchoalveolar lavage fluid concentration of IC87114 were described using a compartment model with a first-order elimination rate constant. Plasma had a three-fold higher C_{max} compared to bronchoalveolar lavage fluid. The half-life of IC87114 in plasma and bronchoalveolar lavage fluid was 2.37 and 10.25 h, respectively. Enhanced airway hyper-responsiveness and increased peribroncholar and perivascular inflammatory cell infiltrates in the lungs were significantly reduced in the IC87114-administered group compared with the ovalbumin/lipopolysaccharide-exposed group. Modelling approaches sufficiently explained the relationship between systemic circulation and bronchoalveolar lavage fluid concentration of IC87114 with $K_{bronchoalveolar\ lavage/plasma}$ (approximately 0.38 h⁻¹) in ovalbumin/lipopolysaccharide-sensitized/challenged mice. Our results will be useful for understanding the relationship between the pharmacokinetics and pharmacodynamics of IC87114.

Key words: IC87114; pharmacokinetic; bronchoalveolar lavage fluid; compartmental

Asthma is a chronic airway disease that originates in childhood and is related to allergies, dysregulated type 2 helper T cells (TH2) inflammatory response, and increased eosinophils. Classically, the best treatment for asthma is to target inflammation. However, there is accumulating evidence regarding the complexity of asthmatic populations with steroid-resistant asthma and difficult or refractory asthma that do not exhibit typical asthmatic features^[1,2]. In fact, severe asthma might be mediated by neutrophils^[3,4]. Thus, unmet medical needs related to refractory asthma have motivated biomedical industries to develop novel drugs.

The phosphoinositide 3-kinase (PI3K) pathway has been implicated in many diseases including cancer, rheumatoid arthritis, asthma, and chronic obstructive pulmonary disease. PI3Ks phosphorylate inositol lipids within cell membranes as an early step in the signalling cascades initiated by numerous ligand-receptor

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November–December 2017

Indian Journal of Pharmaceutical Sciences

Accepted 15 October 2017

Revised 04 April 2017

Received 16 June 2016

Indian J Pharm Sci 2017;79(6):994-1000

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interactions^[5]. Thus, PI3Ks have been the focus of intensive drug discovery and development efforts. The discovery of the quinazolinone purine series, which is exemplified by ICOS Corporation (Bothell, WA) compound IC87114 (2-[(6-aminopurin-9-yl)methyl]-5-methyl-3-[2-methylphenyl]quinazolin-4-one)^[6], indicates the feasibility of targeting PI3Ks. In an ovalbumin (OVA) model of asthma, treatment with PI3K δ inhibitor IC87114 reduces eosinophil recruitment^[7,8]. More recently, it has become apparent that pharmacological inhibition of PI3K δ with IC87114 also reduces IL-17 levels in an asthma mouse model^[9]. In addition, IC87114 blocks TNF α -stimulated elastase exocytosis from neutrophils^[10].

Pharmacokinetic studies are required to develop practical guidelines for newly developed agents. Although pharmacokinetic studies of IC87114 have been performed^[11-13], they focused on sensitive liquid chromatography with tandem mass spectrometry (LC-MS/MS) method development, and thus, in this study, pharmacokinetic analysis was performed with normal rats to obtain analytical information.

Intratracheal drug delivery is preferred for asthma patients, although systemic distribution is observed under organ-specific delivery conditions. In this study, we performed a pharmacokinetic study of IC87114 following intratracheal administration of 1 mg/kg in a neutrophil-dominant TH2-type asthma model. In addition, we developed a pharmacokinetic model to explain the relationship between plasma concentration and bronchoalveolar lavage (BAL) fluid concentration.

MATERIALS AND METHODS

IC87114 was purchased from Calbiochem (San Diego, CA, USA). Acetaminophen, phosphate-buffered saline (PBS), OVA, lipopolysaccharides (LPS), and methacholine (MeCh) were purchased from Sigma (St. Louis, MO, USA). Isoflurane was purchased from Hana Pharm. Co. Ltd (Seoul, South Korea). Similarly, ketamine was purchased from Yuhan Pharmaceuticals (Seoul, South Korea) and xylazine was purchased from Bayer Korea (Seoul, South Korea) respectively. Water was purified using the Milli-Q system from Millipore (Bedford, MA, USA). LC-MS-grade acetonitrile, methanol, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Experimental animals:

All experiments were conducted using adult (8-10 w old, 20-23 g weight) female C57B/L6 mice purchased

from Damul Sciences (Dajeon, Korea). The mice were placed in cages (5 mice per cage) and were kept in a fully acclimatized room at constant temperature and humidity on a 12:12-h light/dark cycle, with *ad libitum* feeding. The animal ethics committee at Chonbuk National University of Korea approved all experiments (CBU 2013-0025) on the basis of the 3 R's (replacement, refinement and reduction). Animals were deeply anesthetized with isoflurane before being killed by cervical dislocation. Efforts were made to minimize the number of animals used and their suffering.

Mouse asthma model protocol:

All mice were sensitized intranasally with 75 μ g OVA plus 10 μ g LPS on days 0, 1, 2, 3 and 7 and challenged with OVA alone on days 14, 15, 21 and 22. On day 22 (3 h after the last airway challenge with OVA), 1 mg/kg IC87114 or vehicle (0.05 % dimethyl sulfoxide, DMSO) diluted with 0.9 % NaCl, was administered in a volume of 30 μ l by a nonsurgical intratracheal method^[14].

Nonsurgical intratracheal method:

Mice were given a single intratracheal dose of IC87114 (1 mg/kg). We selected this dose based on previous reports confirming its efficacy in asthma models^[8,10]. Mice were lightly anesthetized with gaseous isoflurane (Matrix, Orchard Park, NY) and placed in a supine position on an angled board with their necks extended for intratracheal administration. The tongue of the mice was gently pulled outwards using forceps. With the other hand, a 1-ml syringe containing the drug, fitted with a bent gavage needle, was inserted into the trachea and the plunger was pushed to deliver the drug. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally before collecting blood. Blood samples were collected retroorbitally using a capillary tube 0, 0.083, 1, 2, 4, 8, 12, 18, 24, 48 and 72 h after dosing and mice were euthanized using cervical dislocation. The chest cavity was exposed to allow expansion, after which the trachea was carefully incubated and catheter secured with ligatures. One millilitre of 0.9 % NaCl was slowly infused into the lungs and withdrawn. Six animals were sacrificed at each time point.

Measurement of airway hyper reactivity (AHR):

AHR was assessed by whole-body plethysmography (OCP 3000, Allmedicus, Seoul, South Korea), and airflow obstruction was induced with a MeCh aerosol spray. Each group of mice, DMSO or OVA/LPS

with or without IC87114 was exposed for 3 min to aerosolized saline followed by exposure to increasing concentrations of aerosolized MeCh (0, 6.25, 12, 25 and 50 mg/ml) dissolved in isotonic saline. Following each nebulization, the enhanced pause (PE) was recorded for 3 min. PE values measured during each 3-min sequence were averaged and evaluated for each dose of MeCh. PE data were plotted as the change from baseline per dose of MeCh in PBS (0, 6.25, 12, 25, and 50 mg/ml) nebulized through the inlet of the main chamber. Data were recorded using FinePoint software version v2.4.6 (Buxco® Research Systems). Each dose was nebulized for 2 min and the ensuing airway response was recorded for 5 min. The percent increase in PE over baseline PE was calculated for each concentration of MeCh, and the values were used to compare results among experimental groups.

Analysis of BAL samples:

Mice were euthanized via cervical dislocation. The abdominal cavity was opened and the chest cavity was dissected to expose the lung and trachea for expansion. The trachea was exposed by a 1 cm incision on the ventral neck skin for insertion of the cannula. A 1-ml volume of 0.9 % NaCl was slowly infused into the lungs and withdrawn. Six animals were sacrificed at each time point. BAL samples (1 ml) were centrifuged (600× g, 3 min) and the supernatants were stored at -20° for cytokine analysis. The cell pellets from both samples were pooled for total counts (model Z1, Beckman-Coulter, Miami, FL, USA) after lysis of erythrocytes (Zap-Oglobin II, Beckman-Coulter, Fullerton, CA, USA). Slides were loaded with 1×10⁵ cells, centrifuged (Eppendorf, 5415R; 700× g, 3 min), and stained with Diff-Quick (Baxter, Detroit, MI, USA). Differentials (300 cells) were counted under light microscopy (Jena, Germany). The concentration of IC87114 in plasma and BAL fluid was measured using a previously reported LC-MS/MS method^[12].

Non-compartmental pharmacokinetic analysis:

The validated method was applied to a pharmacokinetic study in mice. Blood and BAL fluid samples were collected at 0, 0.083, 1, 2, 4, 8, 12, 18, 24, 48 and 72 h. Non-compartmental pharmacokinetic analyses were performed with WinNonlin Standard Edition software, version 5.3 (Pharsight Corp., Mountain View, CA, USA). The area under the plasma and BAL fluid concentration versus time curves from time zero to the last measurable concentration (AUC_t) was calculated using the linear trapezoidal rule and extrapolated

to infinity (AUC_∞). The peak plasma and BAL fluid concentration (C_{max}) and the time to reach C_{max} (T_{max}) of IC87114 were taken directly from the data. A terminal elimination rate constant (λ_z) was calculated using log-linear regression of the terminal phase, and the elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. Apparent total body clearance (Cl/F) and volume of distribution (V_z/F) were calculated as $dose/AUC_\infty$ and $dose/(K_{el} \times AUC_\infty)$, respectively. All results are presented as the mean±standard deviation (SD).

Compartmental pharmacokinetic analysis:

The relationship between plasma concentration and BAL fluid concentration of IC87114 after intratracheal administration in OVA/LPS-sensitized/challenged mice was explained using a BAL fluid-plasma compartment model with first-order rate elimination kinetics. The differential equations used to describe the changes in the amounts of IC87114 in the different compartments following intratracheal administration are given by Eqns. 1, 2, and 3: $dA_1/dt = K_{21} \times A_2 - (K_{12} + K_{13}) \times A_1$, $dA_2/dt = K_{12} \times A_1 - K_{21} \times A_2$, $dA_3/dt = K_{13} \times A_1 - K_{el} \times A_3$, where A_1 and A_2 represent the amount of IC87114 in the central and peripheral BAL fluid compartments; A_3 represents the amount of IC87114 in the central plasma compartment; K_{el} is the first-order elimination rate constant of IC87114 from the plasma compartment; K_{12} and kcp are the rate constant of transport of IC87114 from the central to the peripheral BAL fluid compartment and from the peripheral to the central BAL fluid compartment, respectively; and K_{13} is the rate constant dictating the transport of the drug from the central BAL fluid compartment to the plasma compartment.

Equations were solved numerically and fitted to the data using ADAPT 5 (Biomedical Simulation Resource, Los Angeles, CA, USA) with maximum likelihood estimation under the assumption that the SD of the measurement error was a linear function of the measured quantity. The BAL fluid concentration (C_b) and plasma concentration (C_p) of IC87114 are as Eqns. 4 and 5: $C_b = A_1/V_1$; $C_p = A_3/V_3$, where V_1 and V_3 are the volume of distribution of IC87114 for BAL fluid and plasma, respectively. The final model accommodated the relationship between BAL fluid and plasma concentration of IC87114 in the asthma model.

The following information, obtained using ADAPT 5, was used to evaluate the goodness of fit and the quality of the parameter estimates: visual examination of the distribution of residuals, Akaike's

information criterion, coefficient of variation of parameter estimates, parameter correlation matrices, and sums of squares of residuals. We used a coefficient of variation of <0.5 and a correlation coefficient threshold of 0.9 as criteria for evaluating the numerical identifiability of parameter estimates^[15].

RESULTS AND DISCUSSION

The calibration curves were linear over the following concentration ranges: 0.01-1000 ng/ml for plasma/BAL fluid. The calibration curves of IC87114 were satisfactory, with correlation coefficients of 0.9993 and 0.9998 for plasma and BAL fluid, respectively.

Intra- and inter-day precision and accuracy results are presented in Table 1. Precision is presented in terms of the percent relative SD and the accuracy as percent bias. The intra-day precision for the lowest concentration of IC87114 (1 ng/ml) was 9.75 % for plasma and 6.58 % for BAL fluid. Likewise, the inter-day precision was 13.44 % for plasma and 9.79 % for BAL fluid. The intra-day and inter-day variation and accuracy for various concentrations of IC87114 were all within acceptable range. The average extraction recoveries of IC87114 were 93.9-99.9 % for plasma and 93.9-102 % for BAL fluid (Table 2). The matrix effects of IC87114 were 96.4-98.8 % and 97.9-101.0 % for plasma and BAL fluid, respectively.

In an initial study of airway hyper-responsiveness, mice underwent the allergy protocol (n=6 per group) followed by aspiration after the last allergen challenge (fig. 1A). In order to determine the effects of IC87114 (a PI3K δ inhibitor), mice were sampled at 0, 5 min, 1, 4, 8, 12, 18, 24, 48, and 72 h after IC87114 administration.

Airway responses to MeCh (12.5 mg/ml) were measured. One hour before exposure to MeCh, the OVA/LPS and OVA/LPS with IC87114 groups showed asthma-like symptoms (data not shown). Expectedly, PE values were significantly increased in response to MeCh in OVA/LPS-sensitized/challenged mice. In contrast, IC87114 exposure significantly reduced PE values in OVA/LPS-exposed mice.

The reduction in airway hyper-responsiveness described above correlated with histological changes in the lung parenchyma. Lungs from OVA/LPS-sensitized/challenged mice exhibited widespread peribronchiolar and perivascular inflammatory cell infiltrates compared with controls (fig. 2). However, the administration of IC87114 resulted in a significant reduction in inflammatory cell infiltration and inhibition of morphological alterations. Together, these results indicate that treatment with IC87114 efficiently inhibited infiltration of inflammatory cells and attenuated allergic airway inflammation.

C_{max} values were 816.89 \pm 331.96 and 340.24 \pm 204.21 μ g/ml for plasma and BAL fluid. Plasma pharmacokinetic data were slightly higher in the present study compared to data observed in a previous study by Marahatta *et al.*^[12]. The plasma sample had a 2.4-fold higher drug concentration compared to BAL fluid. T_{max} was delayed in BAL fluid by 0.15 h compared to plasma samples. Plasma IC87114 reached C_{max} at 0.57 h post dose and then was eliminated with a terminal half-life of 2.37 h. Subsequently, the ∞ BAL fluid concentration of IC87114 gradually decreased with a $t_{1/2}$ of 10.25 h. AUC_{72h} was 5-fold lower in BAL fluid. Clearance and volume of distribution were

TABLE 1: INTRA AND INTER-DAY PRECISION AND ACCURACY OF IC87114 QUANTITATION

Sample	Concentration (ng/ml)	Intra-assay		Inter-assay	
		Precision (% RSD)	Accuracy (% bias)	Precision (% RSD)	Accuracy (% bias)
Plasma	0.1	9.75	5.01	13.44	11.01
	10	1.26	3.32	6.73	6.78
	1000	1.11	2.13	3.92	3.74
BAL fluid	0.1	6.58	8.60	9.79	9.12
	10	2.12	5.67	3.18	6.30
	1000	1.18	3.10	2.75	5.45

BAL is bronchoalveolar lavage, RSD is relative standard deviation for n=5 observations

TABLE 2: RECOVERY AND MATRIX EFFECT OF IC87114 IN PLASMA AND BAL FLUIDS SAMPLES

Concentration (ng/ml)	Recovery (%)		Matrix effect (%)	
	Plasma	BAL fluid	Plasma	BAL fluid
0.05	97.8 \pm 5.6	100.1 \pm 5.1	96.4 \pm 3.2	98.2 \pm 7.6
0.5	99.9 \pm 6.4	102.0 \pm 3.1	98.8 \pm 2.5	97.9 \pm 3.7
2	93.9 \pm 2.2	93.9 \pm 2.6	98.1 \pm 4.3	101.0 \pm 7.2

Data are presented as mean \pm standard deviation (SD) for n=5 observations

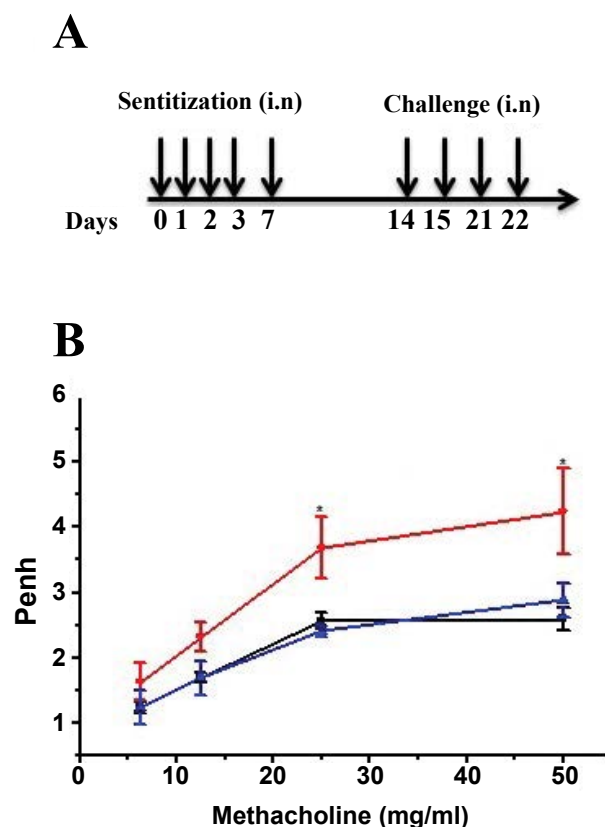


Fig. 1: Experimental design and time course of airway reactivity against MeCh

A) Mice were sensitized intranasally with OVA (75 μ g) and LPS (10 μ g) on days 0, 1, 2, 3 and 7 and challenged intranasally with OVA (50 μ g) alone on days 14, 15, 21 and 22. On day 22 (3 h after the last airway challenge with OVA), 1 mg/kg IC87114 or vehicle (0.05 % DMSO) diluted with 0.9 % NaCl was administered intranasally in the OVA/LPS-sensitized condition in a volume of 30 μ l. B) Airway reactivity in response to increasing doses of nebulized MeCh was assessed by whole-body plethysmography. —■— control; —●— OVA/LPS; —▲— IC87114. Each point represents the mean \pm SEM of six experiments. * p < 0.05. OVA sensitized/challenged mice

489.25 \pm 188.77 ml/h/kg and 1628.58 \pm 586.41 ml/kg. Thappali *et al.*^[13] reported a C_{max} of 1374 ng/ml at a dose 5 mg/kg. In this study, $AUC_{0-\infty}$ was 20143 h \times ng/ml, similar to a recent study in a healthy model^[12]. The non-compartmental pharmacokinetic parameters of IC87114 are listed in Table 3. Similarly, final estimates of the compartmental parameters are listed in Table 4.

In order to analyse the pharmacokinetics and bronchopulmonary disposition of IC87114, we established a BAL fluid-plasma compartment model using first-order kinetics (fig. 3). The plasma and BAL fluid concentration-time profiles of IC87114 after a single intratracheal dose of 1 mg/kg in OVA/LPS-sensitized/challenged mice are shown in fig. 4. The solid lines represent the resulting fit of the average plasma and BAL fluid concentration-time profiles for IC87114, demonstrating that the measured data were consistent with the compartment model. In addition, we found that a BAL fluid-plasma compartment model with a first-order elimination constant best described the time course of drug concentrations in both BAL fluid and plasma.

Compartmental pharmacokinetic parameters were calculated using ADAPT 5 software. The BAL fluid-plasma compartment model provided a good fit for the data. Time versus BAL fluid concentration curves showed a clear distribution in the peripheral compartment. Thus, a high K_{cp}/K_{pc} ratio (estimated to be 5.28) was established to explain rapid distribution into the peripheral compartment^[16]. In addition, the relationship between BAL fluid and plasma concentration of IC87114 was explained using the K_{13}

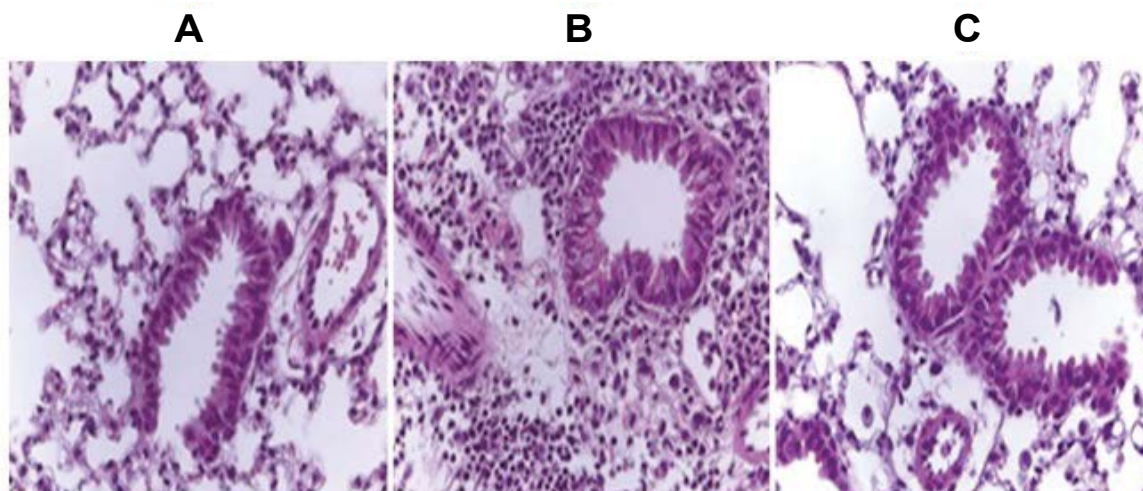


Fig. 2: IC87114 attenuates structural changes induced by OVA/LPS sensitized/challenged mice

A. Control; B. OVA/LPS; C. OVA/LPS/IC87114. Histological examination of lung tissue sections stained with haematoxylin and eosin was performed. Magnification is X200

parameter, which was estimated to be 1.64 (26.43 %).

TABLE 3: NON-COMPARTMENTAL PHARMACO KINETIC PARAMETERS OF IC87114

Parameter (units)	Plasma	BAL fluid
T_{max} (h)	0.57±0.39	0.72±0.99
C_{max} (ng/ml)	816.89±331.96	340.24±204.21
AUC_{72h} (h×ng/ml)	2252.05±699.03	422.08±96.92
AUC_{inf} (h×ng/ml)	2256.76±691.36	473.39±185.74
λ_z (h^{-1})	0.31±0.09	0.09±0.06
$t_{1/2}$ (h)	2.37±0.64	10.25±6.42
Cl_t/F (ml/h/kg)	489.25±188.77	N.A.
V_z/F (ml/kg)	1628.58±586.41	N.A.

Non-compartmental pharmacokinetic parameters following intratracheal administration of 1 mg/kg IC87114 in ova-sensitized/challenged mice. N.A. is not applicable. C_{max} is peak plasma concentration, T_{max} is the time to reach C_{max} , AUC_{72h} is area under the plasma concentration-versus-time curve from time zero to 72 h. AUC_{inf} is AUC extrapolated to infinity, λ_z is terminal elimination rate constant, $t_{1/2}$ is elimination half-life. Cl_t/F is apparent total body clearance and V_z/F is volume of distribution. Data are presented as mean±standard deviation (SD)

TABLE 4: FINAL ESTIMATED PARAMETERS FROM THE BAL FLUID–PLASMA COMPARTMENT MODEL IN OVA-SENSITIZED/CHALLENGED MICE

Parameter (units)	Estimates	%CV
K_{12} (h^{-1})	4.05	23.57
K_{21} (h^{-1})	0.77	29.37
K_{13} (h^{-1})	1.64	26.43
K_{el} (h^{-1})	1.39	1.71
V_1/F (l)	5.61	3.76
V_2/F (l)	0.20	24.82

CV is coefficient of variation

To evaluate the quality of the final model, Monte Carlo simulation with 1000 subjects was performed. The mean pharmacokinetic parameter vector and diagonal elements of the variance components of the analysis were embedded in the PRIOR subroutine of ADAPT 5 software using programs by D'Argenio and

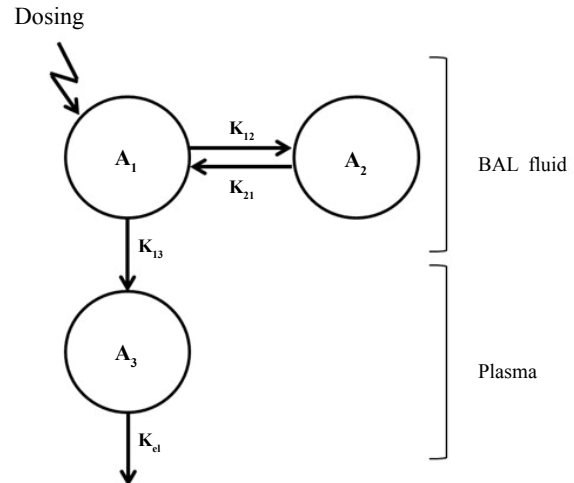


Fig. 3: Compartmental model for BAL fluid and plasma concentrations of IC87114

BAL fluid and plasma concentrations of IC87114 in OVA/LPS-sensitized/challenged mice after intratracheal administration of a single 1 mg/kg dose. A_1 and A_2 represent the amount of IC87114 in the central and peripheral compartments of BAL fluid; A_3 represents the amounts of IC87114 in the central compartment of plasma; K_{el} is the first-order elimination rate constant of IC87114 from the plasma compartment; K_{12} and K_{21} are the rate constants of IC87114 transport from the central to the peripheral compartment of BAL fluid and from the peripheral to the central compartment of BAL fluid, respectively; and K_{13} is the rate constant dictating the transport of IC87114 from the central compartment of BAL fluid to the plasma compartment

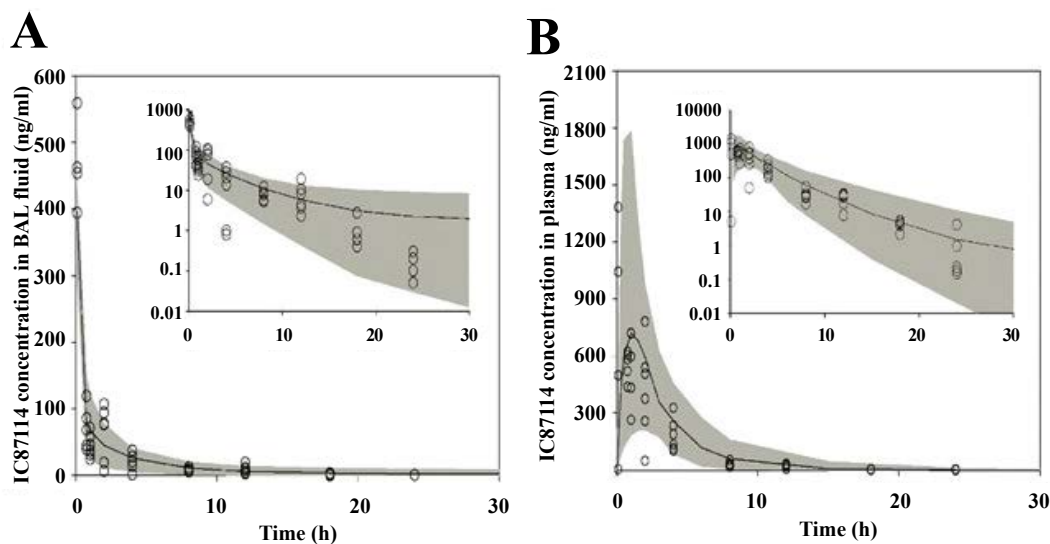


Fig. 4: BAL fluid and plasma concentration vs. time profiles of IC87114

BAL fluid and plasma concentrations of IC87114 following a single intratracheal dose of 1 mg/kg IC87114 to OVA/LPS-sensitized/challenged mice (n=6). Solid lines indicate the final model. Shaded grey represents the 95th and 5th percentiles of the population prediction using Monte Carlo simulation

Schumitzky^[17]. Population simulations were performed using the process noise option. Fig. 4 shows the model-predicted IC87114 concentration averages in BAL fluid and plasma, which described the experimental data well between the 5th and 95th percentiles of the prediction interval.

The pharmacokinetics and bronchopulmonary disposition of IC87114 were investigated using non-compartmental and compartmental approaches in OVA/LPS-sensitized/challenged mice. A BAL fluid-plasma compartment model with first-order elimination kinetics was used to describe the relationship between systemic circulation and bronchopulmonary disposition after intratracheal administration of 1 mg/kg IC87114 in OVA/LPS-sensitized/challenged mice. These findings regarding the pharmacokinetic characteristics of IC87114 in a disease animal model will be useful for investigating the relationship between exposure and response to IC87114.

Acknowledgments:

This work was supported by the Ministry for Health and Welfare, Republic of Korea (Korea Healthcare Technology R&D Project (A121931) and also supported by the National Research Foundation (2017M3A9G7072719).

Conflict of interest:

The authors declare that they have no conflict of interest.

Financial support and sponsorship:

Nil.

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