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Development of Jaspine B analysis using LC-MS/MS and its application: Dose-independent pharmacokinetics of Jaspine B in rats

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Abstract: A rapid and simple LC-MS/MS analytical method in determining Jaspine B has been developed and validated in rat plasma. The standard curve value was 25 – 5000 ng/mL and the linearity, inter-day and intra-day accuracy and precision were within 15.0 % of relative standard deviation (RSD). The mean recoveries of Jaspine B ranged from 87.5 % to 91.2 % with less than 3.70 % RSD and the matrix effects ranged from 91.1 % to 108.2 % with less than 2.6 % RSD. The validated LC-MS/MS analytical method of Jaspine B was successfully applied to investigate the dose-escalated pharmacokinetic study of Jaspine B in rats following an intravenous injection of Jaspine B at a dose range of 1 – 10 mg/kg. The initial plasma concentrations and area under plasma concentration curves showed a good correlation with intravenous Jaspine B dose, indicating the dose independent pharmacokinetics of Jaspine B in rats. In conclusion, this analytical method for Jaspine B can be easily applied in the bioanalysis and pharmacokinetic studies of Jaspine B, including its administration at multiple therapeutic doses, or for making pharmacokinetic comparisons for the oral formulations of Jaspine B in small experimental animals as well as in vivo pharmacokinetic–pharmacodynamic correlation studies.

Key words: jaspine B, liquid chromatography-tandem mass spectrometry, pharmacokinetics, rat, dose escalation

1. Introduction

Sphingosine-1-phoshate (S1P), a signaling sphingolipid metabolite and a potent lipid mediator, regulates the proliferation or apoptosis signal in tumor cells[1] and is generated from sphingosine by sphingosine kinase 1 (SphK1). The generation of S1P precursors triggers either a cell's proliferation or death.[1]

Among many pathways in cancer progression, sphingosine metabolites, such as ceramide, sphingosine, and S1P, have been reported to play important roles in tumor proliferation.[2]

Its balance is regulated through the production of sphingosine and then S1P from ceramide via

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ceramidase and SphKs and the increased ratio of S1P/ceramide promotes cancer cell proliferation.[3] Many human cancers, for example, melanoma, have shown high SphK1 activity and S1P level.[4] SphK1 mRNA is overexpressed in various solid tumors, such as breast, brain, lung, stomach, colon, kidney, and ovary cancers, and in patients with acute leukemia.[5-7] The SphK1 downregulation decreased the epidermal growth factor and reduced the metastatic signal in breast cancer.[3]

Thus, SphK1 has been validated as the oncogenic enzymes by interfering ceramide metabolism and inducing S1P level and the necessity of finding a SphK1 inhibitor has been on the rise as the development of novel cancer therapeutics.[2] By searching the SphK1 antagonist, many studies have taken about marine natural products because of their abundance and high potency due to survivor in dilution of seawater.[8] Jaspine B (pachastrissamine) is an anhydrophytosphingosine, extracted from the marine sponge, Pachastrissa spp.[9] Natural Jaspine B and its stereoisomers showed meaningful inhibition activity against SphK1 and SphK2.[10,11] Jaspine B increases the ceramide level in a tumor cell by several mechanisms, leading to the onset of apoptosis and caspases activation[10] and inhibiting melanoma cell growth by impeding the phosphorylation of the Forkhead box O3 (FOXO3) and the production of cyclin-dependent kinase 2[12] and by inducing apoptosis.[13] A novel Jaspine B-ceramide hybrid modulates sphingolipid metabolism by retaining the ceramide concentration and decreasing the sphingosine level in the cells, leading to the cytotoxicity of Jaspine B-ceramide hybrid molecules.[14] Jaspine B also inhibited ceramide synthase and induced cell death in gastric cancer cells.[15]

Based on the mechanism of action on the anticancer effect, Jaspine B has been screened for various tumor types of different tissue origins and has shown differential efficacy in various cancer cells, with its cellular accumulation at a steady-state, correlating with its cytotoxic efficacy.[16] Moreover, the intravenous injection of Jaspine B (200 mg/mouse, equivalent to 6.7 mg/kg) on the fourth, eighth, twelfth day dramatically decreased the metastatic melanoma cell growth in the lungs of the Jaspine B-injected mice.[12] Despite of these in vivo and in vitro anti-proliferative effects of Jaspine B, the pharmacokinetics features and its optimal dose finding was not investigated intensively. As the first step in the dose-escalating study, this study aimed to develop and validate a simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for measuring Jaspine B in biological samples and to investigate the pharmacokinetic features of Jaspine B in relation to the dosing regimen.

2. Experimental

2.1. Chemicals and reagents

Jaspine B was synthesized according to the method of Kwon et al. with a purity of over 99 %, as confirmed by nuclear magnetic resonance and mass spectroscopy results.[16,17] Berberine chloride (internal standard (IS)) and formic acid were purchased from Sigma (St. Louis, MO, USA). Acetonitrile, water, and methanol of high-performance liquid chromatography (HPLC) grade were purchased from Tedia (Fairfield, OH, USA).

2.2. Preparation of the stock and working solutions

A stock solution of Jaspine B was prepared in acetonitrile with a 1 mg/mL concentration. The working solutions of Jaspine B for calibration standard and quality control (QC) samples were prepared by serial dilution of the stock solution with acetonitrile at concentrations of 0.25, 0.5, 0.75, 2, 5, 7.5, 10, 25, 30, and 50 μg/mL. A stock solution of berberine was prepared in acetonitrile as 1 mg/mL and diluted with acetonitrile at a concentration of 5 ng/mL. All stock and working solutions were stored at -20 °C during the study.

2.3. Preparation of calibration standards and quality control samples

Calibration standards were prepared by spiking blank rat plasma with working solutions of Jaspine B to obtain the final concentrations of 25, 50, 200, 750, 1000, 2500, and 5000 ng/mL. The QC samples were

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prepared at a concentration of 75 (low QC), 500 (middle QC), and 3000 (high QC) ng/mL in the same manner as that of the preparation of the calibration standards. All calibration standards and QC samples were stored at -20 °C during the study.

2.4. Sample preparations

A 50 μL aliquot of rat plasma samples was mixed with a 250 μL IS solution (50 ng/mL berberine in acetonitrile) in a tube and vortexed for 10 min. The mixtures were centrifuged at 16,000 × g for 10 min at 4 °C. A 150 μL aliquot of supernatant was transferred to an autosampler vial.

2.5. LC-MS/MS conditions

The Jaspine B concentrations in the rat plasma samples were analyzed using an Agilent 6430 Triple Quad LC-MS/MS system (Agilent, Wilmington, DE, USA) coupled with an Agilent Infinity 1260 HPLC system. The separation was performed on a Synergi Polar-RP column (2.0 mm × 150 mm, 4 μm pore size; Phenomenex, Torrance, CA, USA) using a mobile phase consisting of water and acetonitrile (15:85, v/v) with 0.1 % formic acid at a 0.2 mL/min flow rate. The column temperature was maintained at 30 °C, and the injection volume was 2 μL. Mass spectra were recorded by electrospray ionization in the positive mode. Quantification was performed using multiple reaction monitoring (MRM) at m/z 300.1→270.2 for Jaspine B and m/z 336.2→320.1 for berberine (IS).

2.6. Method validations

2.6.1. Selectivity

The selectivity was determined by the comparison of chromatogram between the blank rat plasma from six different rats and corresponding rat blank plasma containing 25 ng/mL of Jaspine B.

2.6.2. Precision and accuracy

Interday precision and accuracy were determined using the analysis of the two sets of QC samples (low, middle, and high QC) on five independent days. Intraday precision and accuracy were determined by the analysis of the six sets of QC samples (low, middle, and high QC) on the same days. Precision was expressed as the relative standard deviation (RSD, %), whereas accuracy was expressed as the percentage of the measured QC concentration to the neat solution.

2.6.3. Matrix effect and recovery

The matrix effect of Jaspine B was determined by the comparison of the peak area obtained from the postextraction blank plasma spiked with three QC concentrations of Jaspine B with peak area from the corresponding concentration of the neat solution. The matrix effect of IS was determined in the same manner as the procedure of Jaspine B using the concentration of 5 ng/mL of IS. The recovery of Jaspine B was determined by the comparison of the peak area obtained from the preextraction samples spiked with three QC concentrations of Jaspine B with that from the postextraction blank plasma spiked with corresponding QC concentrations of Jaspine B. The recovery of IS was determined in the same manner using the procedure of Jaspine B using the concentration of 5 ng/mL of IS.

2.6.4. Stability

The stability of Jaspine B in the rat plasma was determined using the three sets of QC samples (low, middle, and high QC) under various conditions. The short-term stability was determined by placing the QC samples at 25 °C for 6 h, whereas the post-treatment stability was evaluated by placing the extracted QC samples in the autosampler at 10 °C for 8 h. For the freeze–thaw cycle stability, QC samples were analyzed after three cycles of freezing and thawing the samples. One cycle of the freeze–thaw step means storing the QC samples at -80 °C for over 12 h and thawing the QC samples at 25 °C for 6 h. After undergoing three cycles of the freeze-thaw step, the concentrations of the QC samples were determined using the freshly prepared calibration standards.

2.7. Pharmacokinetic study

The experimental protocols involving animals were

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approved by the Animal Care and Use Committee of the Kyungpook National University (No. 2020-0093) and conducted in accordance with the National Institutes of Health guidance for the care and use of laboratory animals.

Male Sprague–Dawley rats (weighing 220 – 250 g, 7 weeks) were purchased from Samtako (Osan, Kyunggi-do, Korea), provided with cereal-based maintenance pellet chow (Altromin, Lage, Germany) and water ad libitum and maintained in a light-controlled room (light: 07:00 – 19:00, dark: 19:00 – 07:00), kept at a 22 ± 2 °C temperature with a 55 ± 5 % relative humidity in Animal Center for Pharmaceutical Research, College of Pharmacy, Kyungpook National University.

For the pharmacokinetics study, Jaspine B was dissolved in the mixture of 20 % DMSO and 80 % distilled water and injected intravenously via the rat tail vein at 1, 2, 5, and 10 mg/kg doses (four rats per group). Blood samples (approximately 100 μL) were collected using sparse sampling method at 5, 15, 30 min, 1, 2, 4, 6, 12, 24 h via the retro-orbital vein after the intravenous injection of Jaspine B and centrifuged at 16,000 × g for 1 min to separate the plasma samples. The aliquots of the plasma samples

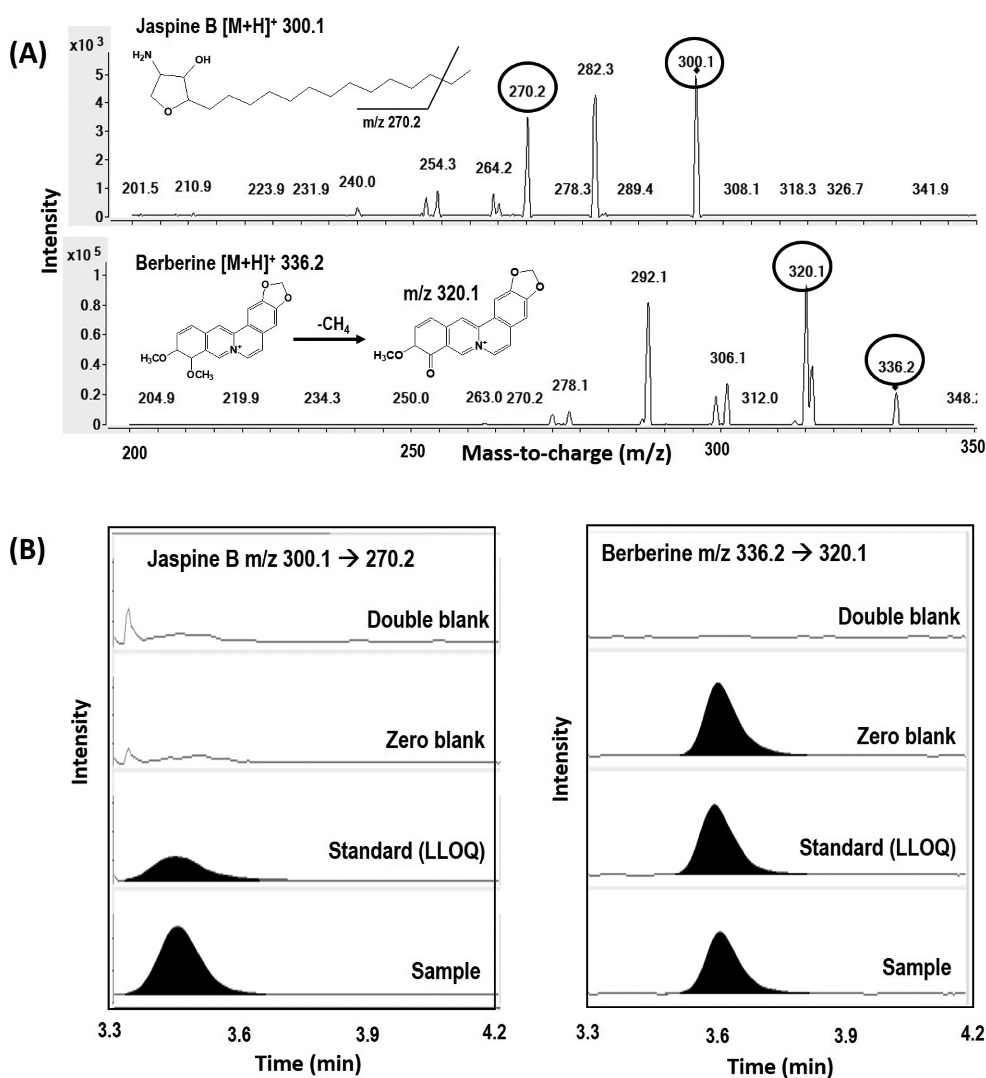

Fig. 1. (A) Product ion scan and (B) multiple reaction monitoring (MRM) chromatogram of Jaspine B and berberine (IS).

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(50 μL) were stored at -80 °C until the analysis.

2.8. Data analysis and statistics

Non-compartmental pharmacokinetic analysis was performed using the WinNonlin software (version 5.1; Pharsight Co., Mountain View, CA, USA), having the value of $p < 0.05$ deemed statistically significant using one-way ANOVA test among the pharmacokinetic parameters from four different doses. All data are expressed as the mean ± standard deviation (SD).

3. Results

3.1. LC-MS/MS analysis of Jaspine B

The mass spectrometer was optimized in a positive ionization mode and the precursor and product ion was selected based on the mass scan mode following the direct injection of Jaspine B working solution (*Fig.* 1(A)). In case of berberine, the precursor and product ion was consistent with the previously published papers.[18,19]

3.2. Selectivity

Jaspine B selectivity was confirmed in six different rat blank plasma and rat plasma samples obtained after Jaspine B administration. The representative MRM chromatograms of Jaspine B and IS (*Fig.* 1(B)) showed that both Jaspine B and IS peaks were well-separated, with no interfering peaks at the respective retention times. The retention times of Jaspine B and IS were 3.46 and 3.64 min, respectively, with a total run time of 4.2 min.

3.3. Precision and accuracy

The QC samples were analyzed using the calibration curve prepared in the same run. The precision and accuracy of intraday and interday assays are shown in *Table* 1. For the interday experiments, the precision and accuracy ranged from 3.36 % to 10.3 % and 102.6 % to 109.0 %, respectively. Further, for the intraday experiments, the precision and accuracy ranged from 1.67 % to 4.31 % and 104.5 % to 112.4 %, respectively (*Table* 1).

Table 1. Intra- and inter-day precision and accuracy of Jaspine B in rat plasma

Inter-day (n = 5)			
QC (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (RSD, %)
75	76.95 ± 7.91	102.6	10.3
500	519.3 ± 35.3	103.9	6.80
3000	3269 ± 109.9	109.0	3.36
Intra-day (n = 6)			
QC (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (RSD, %)
75	83.21 ± 0.10	110.9	1.67
500	522.5 ± 22.5	104.5	4.31
3000	3372 ± 85.4	112.4	2.53

Data are expressed as the mean ± SD.

Table 2. Matrix effect and recovery of Jaspine B in rat plasma

QC (ng/mL)	Recovery (%)		Matrix effect (%)	
	Measured concentration	RSD	Measured concentration	RSD
75	98.1 ± 3.1	3.1	81.0 ± 2.9	3.6
500	92.2 ± 4.6	5.0	109.0 ± 10.0	3.1
3000	88.2 ± 5.3	6.1	97.1 ± 13.8	14.2
5 (IS)	106.2 ± 4.1	3.8	89.7 ± 2.0	2.2

Data are expressed as the mean ± SD (n = 5).

3.4. Matrix effect and recovery

The mean recoveries of Jaspine B in the low, medium, and high QC samples ranged from 88.2 % to 98.1 % with less than 6.1 % RSD. The matrix effects ranged from 81.0 % to 109.0 % with less than 14.2 % RSD. These results indicate no significant interference occurrence during the ionization and sample precipitation processes. The matrix effect and recovery of the IS at 5 ng/mL was 106.2 % and 89.7 %, respectively (*Table* 2).

3.5. Stability

Jaspine B in rat plasma samples was stable for up to 6 h at 25 °C with 5.0 – 11.1 % RSD and also stable after being subjected to three cycles of freeze-thaw step with 1.3 – 4.3 % RSD. The Jaspine B samples were also stable after being stored for 8 h in the autosampler after sample preparation with 0.4 – 4.0 % RSD (*Table* 3). The results suggested that Jaspine B

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Table 3. Stability of Jaspine B in rat plasma

Stability	QC (ng/mL)	Measured concentration (ng/mL)	RSD (%)
Short term stability (6 h, 25 ºC)	75	70.7 ± 7.8	11.1
	500	494.7 ± 24.9	5.0
	3000	3000 ± 162	5.4
Post-treatment stability (8h, 10 ºC)	75	67.6 ± 2.3	3.4
	500	430.9 ± 17.3	4.0
	3000	2773 ± 10.5	0.4
Free-thaw cycle stability (-80 ºC/25 ºC, 3 Cycles)	75	62.9 ± 2.7	4.3
	500	469.0 ± 16.5	3.5
	3000	2832 ± 37.0	1.3

All data are expressed as the mean ± SD (n = 5).

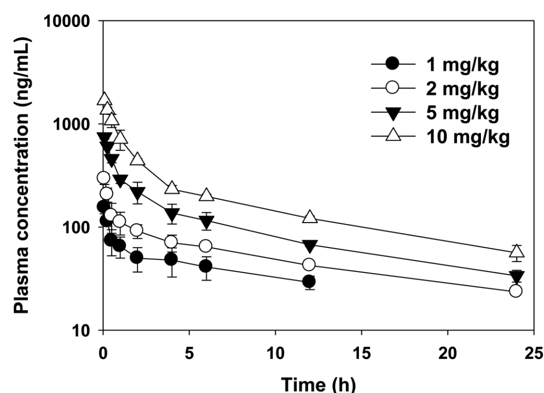

Fig. 2. Plasma concentration vs. time profile of Jaspine B in rats following the intravenous injection of Jaspine B at a single dose of 1 mg/kg (●), 2 mg/kg (○), 5 mg/kg (▼), and 10 mg/kg (△), respectively. Data are expressed as mean ± SD from four rats per group

has no stability issues during the analysis.

3.6. Pharmacokinetic

The mean plasma concentration-time profiles of Jaspine B following the escalated intravenous dose from 1 to 10 mg/kg were shown in *Fig.* 2, while their related pharmacokinetic parameters were presented in *Table* 4. In all the administered doses of Jaspine B, the plasma concentration declined very fast at the distribution phase and declined slowly at the elimination phase, consistent with the large volume of distribution (Vd) of this compound. The Vd and clearance (CL) values were not significantly altered by the escalated dose of Jaspine B, suggesting the compound's linear elimination kinetics (*Table* 4). Similarly, the dose-

Table 4. Pharmacokinetic parameters of Jaspine B in rats

Dose (mg/kg)	1	2	5	10	P value
C_0 (ng/mL)	182.6 ± 38.5	356.0 ± 84.6	882.1 ± 68.7	1873 ± 54.8	-
C_0/D (ng/mL/mg/kg)	182.6 ± 38.5	178.0 ± 42.3	176.4 ± 13.7	187.3 ± 5.5	0.972
AUC (ng·h/mL)	548.2 ± 106	1282 ± 41.4	2772 ± 356	5557 ± 219	-
AUC/D (ng·h/mL/mg/kg)	548.2 ± 106	641.3 ± 20.7	554.4 ± 71.2	555.7 ± 21.9	0.241
$T_{1/2}$ (h)	13.5 ± 6.6	12.7 ± 1.3	13.8 ± 4.4	13.9 ± 2.1	-
MRT (h)	7.2 ± 0.3	8.2 ± 0.3	7.4 ± 0.5	7.0 ± 0.5	-
CL (L/h/kg)	1.9 ± 0.4	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	0.304
Vd (L/kg)	13.5 ± 2.5	12.7 ± 0.8	13.4 ± 1.6	12.6 ± 1.1	0.857

Data were expressed as mean ± SD from four rats per group.
P value indicates static comparison among four groups using one-way ANOVA test.
C_0: initial plasma concentration; C_0/D: dose normalized C_0
AUC: Area under plasma concentration-time curve; AUC/D: dose normalized AUC
MRT: mean residence time; CL: clearance (Dose/AUC); Vd: volume of distribution (MRT?CL)

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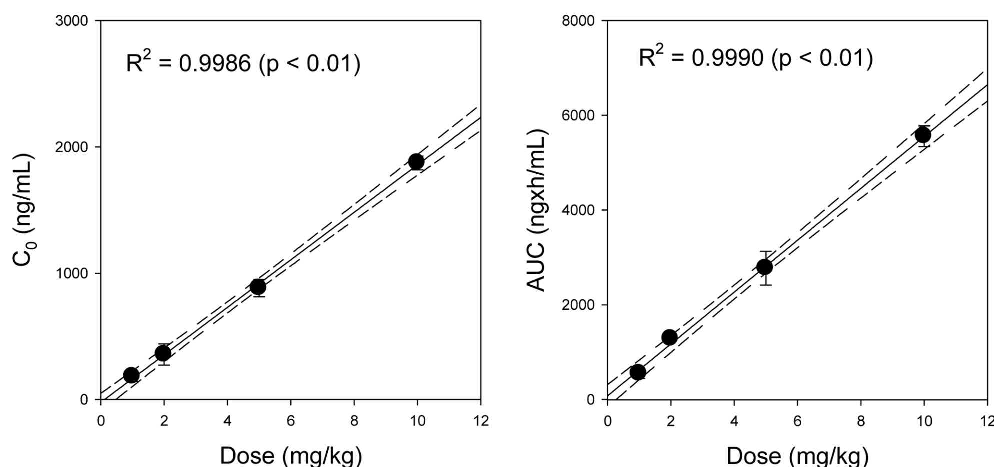

Fig. 3. Correlations between C_0 and AUC values for Jaspine B and intravenous dose of Jaspine B. Lines were generated from a linear regression analysis. Dotted lines represent 90 % confidence intervals around the geometric mean value. R^2 represents the correlation coefficient of linear regression analysis.

normalized plasma concentrations (C_0/D) and the dose-normalized area under the plasma concentration (AUC/D) were not significantly different by the escalated dose from 1 to 10 mg/kg of Jaspine B, showing the dose-independent pharmacokinetics of Jaspine B following the compound's intravenous injection (*Table* 4).

The correlation analysis on the C_0 and AUC of Jaspine B in relation to the Jaspine B dose revealed a good correlation between the C_0 and AUC of Jaspine B and the Jaspine B dose. The results suggested the dose-independent pharmacokinetics of Jaspine B following the compound's intravenous injection (*Fig.* 3).

4. Discussion

With the accumulated evidence of the antitumor activity of Jaspine B in various cancer cells and its relation to sphingosine metabolism[10,11,15] and apoptotic signal induction,[12] one animal study was performed using metastatic melanoma tumor-bearing mice. The intravenous injection of Jaspine B (200 μg/mouse, equivalent to 6.7 mg/kg) dramatically decreased the metastatic melanoma cell growth in the lungs of the Jaspine B-injected mice.[12]

However, the Jaspine B dose could not be optimized based on its plasma and tumor tissue concentration. Previously, we reported that Jaspine B showed varying degrees of cytotoxicity (IC_{50} from 2.60 μM to over 100 μM) in different cancer cell types, such as breast cancer cells, renal and ovarian carcinoma, melanoma, and hepatoma cells, in which its differential steady-state cellular concentration was involved.[16]

The results suggested the importance of Jaspine B concentration in tumor cells and the in vivo pharmacokinetics for the evaluation of its therapeutic effect. By pursuing the pharmacokinetic-efficacy relationship of Jaspine B, its dose and dosing regimen could be optimized for better efficacy with least toxicity. As a first step for the pharmacokinetic study for the dose-escalation study, we firstly developed analytical methods for Jaspine B and applied this validated analytical method to the in vivo animal study following the intravenous injection at the doses from 1 to 10 mg/kg.

The analytical method for Jaspine B using an LC-MS/MS system has been developed using a small volume (50 μL) of the plasma samples and the lower limit of the quantitation of 25 ng/mL with simple sample preparation method. We used a protein-precipitation method involving acetonitrile containing an IS and then directly injected an aliquot of the supernatant after the centrifugation of protein-precipitated plasma samples. Finally, we developed and fully validated a rapid and simple analytical

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method for Jaspine B in the biological samples with a total run time of 4.2 min, which can be easily applied in the bioanalysis and pharmacokinetic study of Jaspine B in small experimental animals as well as pharmacokinetics-efficacy optimization.

Moreover, as shown in *Fig.* 3 and *Table* 4, the pharmacokinetics of Jaspine B showed dose-independent linear pharmacokinetics, suggesting a possible linear relationship between the dose and plasma exposure (C_0 and AUC). Therefore, this study could be the first step toward the pharmacokinetics and the further evaluation of pharmacokinetic-efficacy relation studies using the inhibitory coefficient in cancer cells and the target plasma and tumor tissue concentration.

5. Conclusions

We developed and validated the analytical method for Jaspine B using LC-MS/MS system through the inter- and intra-day precision and accuracy, matrix effect, extraction recovery, and the stability test. We also applied this validated analytical method to the pharmacokinetic study in rats following the intravenous injection at the doses of 1, 2, 5, and 10 mg/kg. The pharmacokinetics of Jaspine B showed linear relationship between the intravenous dose of Jaspine B and the plasma exposure.

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