Development and validation of an analytical method to quantify baphicacanthin A by LC-MS/MS and its application to pharmacokinetic studies in mice

So Yeon Jeon[1,†], San Kim[1,†], Jin-Hyang Park[2], Im-Sook Song[2],
Young Taek Han[1, ★], and Min-Koo Choi[1, ★]

[1]*College of Pharmacy, Dankook University, Cheon-an 31116, Korea*
[2]*BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit,
Vessel-Organ Interaction Research Center (VOICE), College of Pharmacy and Research Institute
of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Korea*

Abstract: In this study, we developed and validated a sensitive analytical method to quantify baphicacanthin A in mouse plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The standard calibration curves for baphicacanthin A ranged from 0.5 to 200 ng/mL and were linear, with an r^2 of 0.985. The inter- and intra-day accuracy and precision and the stability fell within the acceptance criteria. Besides, we investigated the pharmacokinetics of baphicacanthin A following its intravenous (5 mg/kg) and oral administration (30 mg/kg). Intravenously injected baphicacanthin A showed biphasic elimination kinetics with high clearance and volume of distribution values. Furthermore, baphicacanthin A showed a rapid absorption but low aqueous solubility (182.51 ± 0.20 mg/mL), resulting in low plasma concentrations and low oral bioavailability (2.49 %). Thus, we successfully documented the pharmacokinetic properties of baphicacanthin A using this newly developed sensitive LC-MS/MS quantification method, which could be used in future lead optimization and biopharmaceutic studies.

Key words: baphicacanthin A, analytical method validation, LC-MS/MS, bioavailability

1. Introduction

Phenoxazinone alkaloids are tricyclic heterocycle compounds; they are common natural products produced by various organisms such as insects, fungi, and Australian marsupials.[1] Phenoxazinone alkaloids can protect mammalian tissues from oxidative damage[2] and display antitumor activity by intercalating human DNA.[3] For example, actinomycin D, one of the most famous natural phenoxazinone, intercalates guanine-cytosine rich regions of DNA in various tumors.[4] Moreover, phenoxazinone derivatives have also shown antiviral, anti-inflammatory, antimicrobial, and anti-Alzheimer activity,[5] proving the strong potential of phenoxazinones in drug development.

Baphicacanthin A, another phenoxazinone alkaloids,

†These authors contributed equally to this work

★ Corresponding author
Phone : +82-(0)41-550-1438, +82-(0)41-550-1431   Fax : +82-(0)41-559-7899
E-mail : minkoochoi@dankook.ac.kr, hanyt@dankook.ac.kr

was recently isolated from the roots of *Baphicacanthus cusia*.[6,7] The crude extract of *B. cusia* has been used to treat mumps, epidemic cerebrospinal meningitis, and severe acute respiratory syndrome (SARS).[6,8] In addition, aurantiamide acetate, isolated from *B. cusia* roots, exhibited anti-inflammatory and antiviral effects by inhibiting the nuclear factor-κB (NF-κB) signaling pathway in Influenza A virus-infected cells.[9] Because of these potent antiviral activities, a method of isolation and purification of the extract of *B. cusia* was developed and identified 30 alkaloid compounds.[7] Among them, 28 were known compounds such as triterpenoids, lignans, phenylethanoids, and flavonoids and two were new alkaloids, which were named baphicacanthin A and baphicacanthin B.[7] Recently, synthetic methods for these two compounds were reported.[6,10] Considering the efficacy of phenoxazinone derivatives from *B. cusia*, it is necessary to investigate the pharmacological activity and druggability of baphicacanthin compounds. Therefore, this study aimed to develop and validate a sensitive and reproducible liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification method and document the biopharmaceutical, pharmacological, and pharmacokinetic properties of biphicacanthin compounds.

Several analytical methods exist for aminophenoxazinones (baphicacanthin A structural analogs). Researchers extracted phenoxazinone derivatives from plasma, urine, feces, and cancer tissues using solid phase extraction from sample of 0.5 mL. The lower limit of quantification (LLOQ) was 2.0 – 8.0 ng/mL and the matrix effect was 84.8 % – 128 %.[11-13] To develop a sensitive LC-MS/MS analytical method for baphicacanthin A applicable to pharmacokinetic studies in mice, we needed to extract the compound from much smaller samples (30 μL). We thus used liquid-liquid extraction, which has good drug sensitivity and a favorable matrix effect.[14]

2. Experimental

2.1. Chemicals and reagents

We synthesized baphicacanthin A (*Fig.* 1) using the method described by Ahn *et al*.[6] We confirmed the
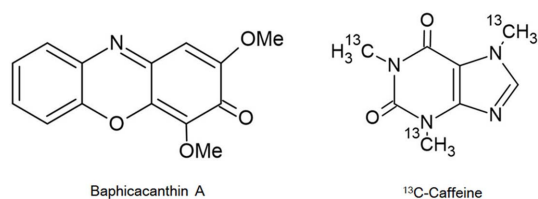

Fig. 1. Chemical structures of baphicacanthin A and ^{13}C-caffeine (internal standard, IS).

purity using nuclear magnetic resonance spectroscopy and mass spectroscopy, as previously described.[6] The internal standard (IS) was ^{13}C-Caffeine, purchased from Sigma-Aldrich (St. Louis, MO, USA). We obtained acetonitrile and water from Tedia (Fairfield, CT, USA) and ethyl acetate from J. T. Baker (Phillipsburg, NJ, USA). All solvents and chemicals were of HPLC and reagent grade.

2.2. Preparation of the stock and working solutions

We prepared 2 mg/mL stock solutions by dissolving baphicacanthin A in acetonitrile. We prepared the baphicacanthin A working solutions by serial dilution of the stock solution with acetonitrile to obtain final concentrations of 0.5, 1, 2, 5, 20, 50, 100, and 200 ng/mL for the calibration standards and 1.5, 30, and 150 ng/mL for the quality control (QC) samples. We dissolved ^{13}C-caffeine in water to obtain a 20 ng/mL solution. The stock and working solutions stored at -20 °C during the analysis.

2.3. Preparation of calibration standards and quality control samples

We evaporated 30 μL aliquots of the working solution for calibration standards and QC samples and reconstituted them in 30 μL of blank mouse plasma. The final concentrations of the calibration standards were 0.5, 1, 2, 5, 20, 50, 100, and 200 ng/mL and the concentrations of the QC samples were 1.5, 30, and 150 ng/mL.

2.4. Sample preparation

We combined the calibration standards or QC samples (30 μL) with 20 μL of the ^{13}C-caffeine solution

(20 ng/mL in water) and 400 μL of ethyl acetate. The mixture was vigorously vortexed for 10 min and then centrifuged at 16,000 × g for 5 min. Next, we transferred the supernatant to a clean tube and evaporated it to dryness using a gentle nitrogen. We then reconstituted the residue in 150 μL of mobile phase, vortexed the mixture for 10 min, and centrifuged it at 16,000 × g for 5 min. Finally, we transferred 120 μL of the supernatant to a vial, and injected a 10 μL of this solution into the LC-MS/MS system.

2.5. Instrument conditions

We analyzed the mouse plasma samples using an Agilent Infinity 1260 Infinite II HPLC system (Agilent Technologies, Wilmington, DE, USA) coupled to an Agilent 6430 triple quadrupole tandem mass spectrometer with an electrospray ionization source. We performed chromatographic separation on a Luna C18 column (150 × 2.0 mm, 5 μm; Phenomenex, Torrance, CA, USA). We eluted the compounds with an isocratic mobile phase consisting of water and acetonitrile (20:80, v/v) containing 0.1 % formic acid at a flow rate of 0.2 mL/min on a column maintained at 30 °C.

The mass spectrometer operated in positive ion mode with multiple reaction monitoring (MRM) transitions at m/z 257.9→169.0 for baphicacanthin A with an optimized fragmentor voltage of 100 V and a collision energy of 35 eV, and at m/z 198.0→139.9 for ^{13}C-caffeine (IS) with an optimized fragmentor voltage of 115 V and a collision energy of 25 eV.

2.6. Method validations

2.6.1. Selectivity and linearity

We assessed selectivity using blank plasma samples from six different mice. We compared their signals with those of the corresponding LLOQ samples and IS solution (20 ng/mL)

We plotted the ratios between the peak areas of baphicacanthin A and that of the IS to the baphicacanthin A concentrations (0.5 − 200 ng/mL) and fitted the standard calibration curves using least square linear regression with a weight of $1/x^2$.

2.6.2. Precision and accuracy

We determined the inter-day precision and accuracy using two sets of QC samples (0.5, 1.5, 30, and 150 ng/mL) on five independent days. We determined intra-day precision and accuracy by analyzing six sets of QC samples (0.5, 1.5, 30, and 150 ng/mL) on the same days. The precision was expressed as the coefficient of variance (CV, %), whereas accuracy was expressed as the percentage of the measured QC concentration to the nominal QC concentration.

2.6.3. Extraction recovery and matrix effect

We determined the extraction recovery and matrix effect using QC samples at three different concentrations (1.5, 30, and 150 ng/mL) and the IS solution (20 ng/mL).

To calculate the extraction recovery, we spiked the pre-extraction samples and the post-extraction blank plasma with the three QC samples and compared their peak areas.

Finally, to determine the matrix effect of baphicacanthin A, we divided the peak areas from the post-extraction blank plasma spiked with QC samples by those from neat solutions of the corresponding concentrations. We determined the extraction recovery and matrix effect of IS using the same procedure, but with the 20 ng/mL solution instead of the baphicacanthin A QC samples.

2.6.4. Stability

We assessed the stability of baphicacanthin A in mouse plasma using three QC samples concentrations (1.5, 30, and 150 ng/mL) under three conditions: For bench-top stability, we placed the QC samples at 25 °C for 6 h. For freeze-thaw stability, we analyzed QC samples that underwent three freeze–thawing cycles. One freeze–thaw cycle consisted in storing the QC samples at -80 °C for over 12 h and then at 25 °C for over 6 h. Finally, for autosampler stability, we placed the extracted QC samples in the autosampler at 6 °C for 24 h.

2.7. Pharmacokinetic experiments

All the experimental procedures involving animals

were approved by the Animal Care and Use Committee of the Kyungpook National University (No. 2019-0126) and were conducted following the National Institutes of Health guidelines for the care and use of laboratory animals.

We purchased male ICR mice (7 – 8 weeks old, 30 – 35 g) from Samtako (Osan, Korea). They had a one-week acclimation period at the animal facility of Kyungpook National University with *ad libitum* access to food and water and fasted for 12 h before the pharmacokinetic experiments. We randomly divided eight mice into two groups. The first group received 5 mg/kg of baphicacanthin A intravenously (5 mg/kg dissolved in a 2 mL mixture of DMSO/saline [20:80, v/v]) *via* the tail vein. The second group received 30 mg/kg of baphicacanthin A orally (30 mg/kg suspended in 5 mL of a 0.5 % carboxymethyl cellulose suspension). Next, we collected blood samples *via* the Retro-Orbital plexus using a heparinized collection tube at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h post-administration using a sparse sampling method. We immediately centrifuged the blood samples at 16,000 × g for 5 min to obtain plasma samples, which we stored at -80 °C until analysis.

Next, we mixed the plasma samples (30 μL) with 20 μL of IS solution and 400 μL of ethyl acetate. We vigorously vortexed the mixture for 10 min and then centrifuged it at 16,000 × g for 5 min. We then transferred the supernatant into a clean tube, evaporated it to dryness using a gentle nitrogen stream, and reconstituted the residue in 150 μL of mobile phase. We vortexed the mixture for 10 min and then centrifuged it at 16,000 × g for 5 min. Finally, we transferred 120 μL of supernatant to a vial and injected 10 μL of the solution into the LC-MS/MS system.

2.8. Solubility

We weighed 5 mg of baphicacanthin A and added 1 mL of water. We vortexed the tube for 24 h on a Multi Reax shaker (Heidolph, Schwabach, Germany) and filtered the solution through a PVDF syringe filter (pore size 0.45 μm, Hyundai Micro, Seoul, Korea). We diluted the filtrate 50 times with acetonitrile, then 20 times with the mobile phase. Next, we added

an equal volume of ^{13}C-caffeine solution (20 ng/mL in water) to the solution. Finally, we vigorously vortexed the mixture was for 5 min and injected 10 μL of the mixture into the LC-MS/MS system.

2.9. Plasma protein binding

We determined the extent of baphicacanthin A (150 ng/mL, high QC) protein binding in mouse plasma using a rapid equilibrium dialysis kit (ThermoFisher Scientific Korea, Seoul, Korea) following the manufacturer's protocol.[15,16] Briefly, we placed 50 μL of mouse plasma containing 150 ng/mL of baphicacanthin A in the inner sample chambers of the kit's inserts and 300 μL of phosphate-buffered saline (PBS) in the outer chambers. A semipermeable membrane with a molecular weight cut-off of 8,000 Da separated the chambers. We then incubated the samples for 4 h at 37 °C on a shaking incubator at 300 rpm and collected 25 μL aliquots from both the sample and buffer chambers. Next, we treated the samples with equal volumes of fresh PBS or blank plasma to match the sample matrices. We then mixed 30 μL of the matrix-matched samples with 20 μL of IS solution (20 ng/mL ^{13}C-caffeine in water) and 400 μL of ethyl acetate. Finally, these mixtures underwent the steps described in section 2.4 (plasma sample preparation).

2.10. Data analysis and statistics

All data are expressed as the mean ± standard deviation (SD). The pharmacokinetic parameters, including the area under the concentration-time curve (AUC), clearance (CL), volume of distribution (V_d), and elimination half-life ($T_{1/2}$), were calculated through the non-compartmental method using WinNonlin 5.1 software (Pharsight Co., Mountain View, CA, USA). The absolute oral bioavailability (BA) was calculated using the equation: BA (%) = (dose normalized AUC_{PO}/dose normalized AUC_{IV}) × 100 %.

3. Results and Discussion

3.1. LC-MS/MS analysis of baphicacanthin A

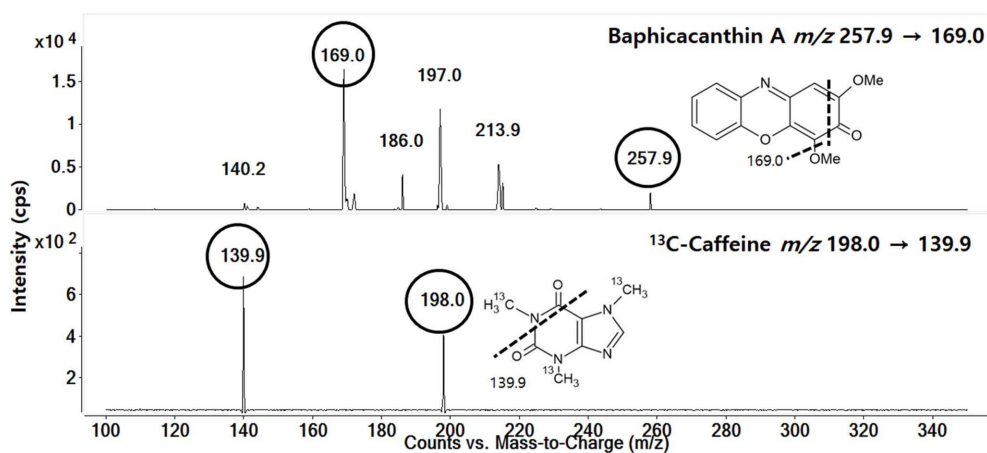To optimize the electrospray ionization conditions of baphicacanthin A and IS, we injected each compound

Fig. 2. Product ion mass spectra of baphicacanthin A and ^{13}C-caffeine (IS).
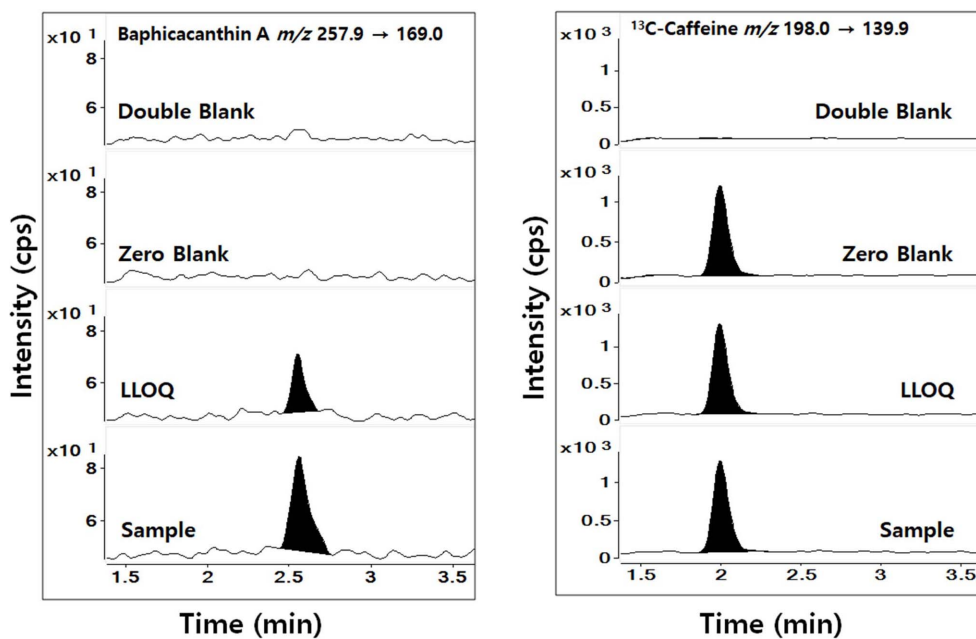

Fig. 3. Representative multiple reaction monitoring (MRM) chromatograms of baphicacanthin A and ^{13}C-caffeine (IS) in double blank, zero blank, LLOQ samples (0.5 ng/mL), and plasma samples 4 h after oral administration of baphicacanthin A.

Table 1. Back-calculated concentrations of baphicacanthin A in calibration standards (n=6)

	Nominal concentrations (ng/mL)								Slope	r²
	0.5	1	2	5	20	50	100	200		
Back-calculated concentration (ng/mL)	0.51	0.96	1.75	4.80	20.80	48.58	112.90	198.23	0.048	0.985
SD	0.044	0.12	0.14	0.34	1.32	3.82	7.28	24.04	0.0065	0.0118
Accuracy (%)	102.40	96.00	87.63	96.03	104.02	97.16	112.90	99.12	-	-
CV (%)	8.56	11.98	7.91	7.07	6.37	7.87	6.45	12.13	13.51	1.20

Table 3. Extraction recoveries and matrix effects of baphicacanthin A and IS

Analyte	Nominal concentration (ng/mL)	Extraction recovery (%)	CV (%)	Matrix effects (%)	CV (%)
Baphicacanthin A	1.5	68.53 ± 5.84	8.53	82.11 ± 7.17	8.74
	30	66.91 ± 7.79	11.65	76.29 ± 10.21	13.38
	150	70.84 ± 8.22	11.61	86.00 ± 10.46	12.16
IS	20	96.77 ± 10.47	10.82	55.54 ± 5.98	10.77

Data represented as mean ± SD (n = 6).

directly into the mass spectrometer ionization source. The ionization of baphicacanthin A and IS were optimal in the positive mode. As shown in *Fig.* 2, the MRM transition of baphicacanthin A was selected from the precursor ion ([M+H]$^+$, *m/z* 257.9) and the most frequent product ion (*m/z* 169.0). The MRM transition of IS was selected from the precursor ion ([M+H]$^+$, *m/z* 198.0) and the most frequent product ion (*m/z* 139.9), which was consistent with the previous reports.[17,18]

3.2. Selectivity and linearity

Fig. 3 shows the typical chromatograms of double blank, zero blank, LLOQ samples (0.5 ng/mL), and plasma samples 4 h after the oral administration of baphicacanthin A. Baphicacanthin A and IS had retention times of 2.6 and 2.0 min, respectively. We observed no significant interfering endogenous peaks or matrix interference around the retention times of baphicacanthin A and IS in LLOQ samples compared with the chromatograms from six different blank plasma. The signal-to-noise ratio of baphicacanthin A was > 5.0 in the LLOQ samples.

Between 0.5 and 200 ng/mL, the calibration standard curve of baphicacanthin A was linear, and the linear regression analysis with a weighting of 1/concentration^2 yielded a coefficient of determination (r^2) of 0.985 (*Table* 1).

3.3. Precision and accuracy

Table 2 summarizes the intra- and inter-day precision and accuracy for baphicacanthin A from four QC samples concentrations. The intra- and inter-day precision and accuracy for baphicacanthin A were 2.57 % – 14.84 % and 85.20 % – 112.42 %, respectively,

which satisfied the acceptance criteria of the FDA guidance.[19]

3.4. Extraction recovery and matrix effect

Table 3 summarizes the extraction recoveries and matrix effects results. Using QC samples at three different baphicacanthin A concentrations, we calculated an extraction recovery of 66.91 % – 70.84 % with a CV of 8.53 % – 11.65 %, suggesting that the extraction procedure was efficient and reproducible.

Using QC samples at three different baphicacanthin A concentrations (1.5, 30, and 150 ng/mL), we found matrix effects ranging from 76.29 % to 86.00 %, with a CV from 8.74 % to 13.38 %, suggesting that the co-eluting substances did not significantly interfere with the ionization of baphicacanthin A. The extraction recovery and matrix effects of IS were also high and reproducible (*Table* 3).

3.5. Stability

Table 4 presents the stability experiments results. The accuracy of baphicacanthin A concentration measurements ranged from 87.17 % to 110.27 %, and the precision ranged from 1.27 % to 12.42 % for the three different stability tests. None of the test conditions significantly changed the starting concentrations, showing that, in mouse plasma, baphicacanthin A was stable for at least 6 h at 25 °C, 24 h in an autosampler, and over three freeze–thaw cycles.

3.6. Pharmacokinetics of baphicacanthin A

Fig. 4 displays the temporal profiles for the plasma concentration of baphicacanthin A after intravenous and oral administration, and *Table* 5 details the relevant pharmacokinetic parameters. After intravenous

Table 4. Stability of baphicacanthin A

Nominal concentration (ng/mL)	Back-calculated concentration (ng/mL)	Precision (%)	Accuracy (%)
Bench-top stability (at 25 °C for 6 h)			
1.5	1.33 ± 0.15	11.09	88.54
30	28.13 ± 1.46	5.18	93.77
150	144.66 ± 4.52	3.12	96.44
Freeze-thaw stability (3 cycles, from -80 °C for 12 h to 25 °C for 6 h as one cycle)			
1.5	1.31 ± 0.16	12.42	87.17
30	31.70 ± 0.40	1.27	105.66
150	165.40 ± 2.98	1.80	110.27
Autosampler stability (at 6 °C for 24 h)			
1.5	1.31 ± 0.13	10.12	87.47
30	27.67 ± 0.36	1.31	92.24
150	145.22 ± 1.87	1.29	96.81

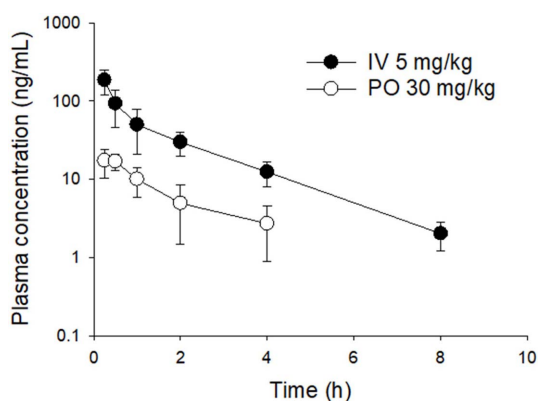Data represented as mean ± SD (n=3).

Fig. 4. Plasma concentration-time profiles of intravenously administered (5 mg/kg, ●) and orally administered baphicacanthin A (30 mg/kg, ○). Data are expressed as mean ± SD (n = 4).

Table 5. Pharmacokinetic parameters of baphicacanthin A in mice following intravenous or oral administration of baphicacanthin A

Parameters	IV (5 mg/kg)	PO (30 mg/kg)
C_0 (ng/mL)	529 ± 438	
C_{max} (ng/mL)	-	20.47 ± 5.73
T_{max} (h)	-	0.38 ± 0.14
AUC_{last} (ng·h/mL)	270.94 ± 47.61	33.20 ± 10.54
AUC_∞ (ng·h/mL)	294.87 ± 48.74	44.08 ± 12.74
$T_{1/2}$ (h)	1.85 ± 0.71	1.62 ± 1.14
MRT (h)	1.34 ± 0.61	2.34 ± 1.87
CL (L/h/kg)	17.33 ± 3.00	-
V_d (L/kg)	22.55 ± 8.09	-
BA (%)	-	2.49

C_0: initial plasma concentration estimated plasma concentration at time zero; AUC_{last} or AUC_∞: Area under plasma concentration-time curve from zero to last time or infinity; $T_{1/2}$: elimination half-life; MRT: mean residence time; CL: clearance (Dose/AUC); V_d: volume of distribution (MRT·CL)
BA: Bioavailability (Dose normalized AUC_{PO}/dose normalized AUC_{IV} × 100 %)
Data are expressed as mean ± SD (n = 4).

injection, the plasma concentration of baphicacanthin A demonstrated biphasic elimination kinetics, with a distribution half-life of 0.51±0.39 h and an elimination half-life ($T_{1/2}$) of 1.85±0.71 h. Besides, baphicacanthin A had a large V_d and high CL. These results suggest that baphicacanthin A distributes quickly to the peripheral compartments (*Table* 5).

After oral administration, the plasma concentration of baphicacanthin A reached C_{max} around the initial sampling times (i.e., T_{max} of 0.38±0.14 h), indicating rapid gastrointestinal absorption. However, despite this rapid intestinal absorption and the high oral dose

(30 mg/kg), baphicacanthin A had very low C_{max} (20.47±5.73 ng/mL) and oral bioavailability (2.49 %).

Next, we measured the aqueous solubility of baphicacanthin A (*Table* 6). Its solubility (182.51± 0.20 μg/mL) was much lower than the oral administration formulation's concentration (6 mg/mL as a suspension). The lipophilic nature of baphicacanthin A (a high LogP value of 2.61 and a high protein

Table 6. Physicochemical properties of baphicacanthin A

Parameters	Mean±SD (n = 3)
Log P[a]	2.61
Solubility (μg/mL)	182.51 ± 0.20
Protein binding (%)	95.07 ± 0.20

[a]LogP value was obtained from Chemdraw database

binding of 95.07 % when measured using 150 ng/mL baphicacanthin A) could allow the rapid absorption of the dissolved fraction; however, the two administration routes yielded similar elimination half-lives (p = 0.74, Student's *t*-test), indicating that the undissolved baphicacanthin A in the intestine could not be absorbed. Low solubility and rapid absorption at absorption site of baphicacanthin A could contribute to the low oral bioavailability of this compound. Similarly, aminophenoxazinone derivatives, that are structural analogs of baphicacanthin A and possess antitumor or antiviral activities, also showed low solubility (0.3 − 30 μg/mL in phosphate buffer (pH 7.4)) and lipophilicity (LogP of 1.5 − 1.86).[20] Oral bioavailability of the most active aminophenoxazinone derivative was 14.5 % in mice although it showed high permeability more than 10^{-5} cm/s in a parallel artificial membrane permeability assay (PAMPA).[20] In addition to their low solubility, phase II metabolism of aminophenoxazinone derivatives such as glucuronidation and sulfation may also contribute to the low oral bioavailability because the glucuronide metabolites of aminophenoxazinone derivative are mainly recovered from the feces rather than from the urine.[11,21,22]

4. Conclusions

We developed and validated a sensitive analytical method for baphicacanthin A in mouse plasma samples using an LC-MS/MS system. Besides, we used this method to document the pharmacokinetic characteristics and oral bioavailability of baphicacanthin A in mice following its intravenous and oral administration. Although the antioxidative and antiviral activities of baphicacanthin A remain unknown, its pharmacokinetic parameters are not particularly promising (low oral

bioavailability and high clearance and volume of distribution values). Thus, future pharmacological and pharmacokinetic studies on this compound will need to include structural or formulation optimization.

Acknowledgements

References

1. M. Le Roes-Hill, C. Goodwin and S. Burton, *Trends in Biotechnology*, **27**(4), 248-258 (2009).

2. C. Eggert, U. Temp, J. F. Dean and K. E. Eriksson, *FEBS Letters*, **376**(3), 202-206 (1995).

3. A. Bolognese, G. Correale, M. Manfra, A. Lavecchia, O. Mazzoni, E. Novellino, V. Barone, A. Pani, E. Tramontano, P. La Colla, C. Murgioni, I. Serra, G. Setzu and R. Loddo, *Journal of Medicinal Chemistry*, **45**(24), 5205-5216 (2002).

4. N. Lohani, H. N. Singh, and R. R. Moganty, *International Journal of Biological Macromolecules*, **87**, 433-442 (2016).

5. S. Katsamakas, A. L. Zografos and V. Sarli, *Current Medicinal Chemistry*, **23**(26), 2972-2999 (2016).

6. S. Ahn, J. A. Yoon and Y. T. Han, *Bulletin of the Korean Chemical Society*, **40**(7), 740-742 (2019).

7. Q.-T. Feng, G.-Y. Zhu, W.-N. Gao, Z. Yang, N. Zhong, J.-R. Wang and Z.-H. Jiang, *Chemical and Pharmaceutical Bulletin*, **64**(10), 1505-1508 (2016).

8. T. Tanaka, T. Ikeda, M. Kaku, X. H. Zhu, M. Okawa, K. Yokomizo, M. Uyeda and T. Nohara, *Chemical and Pharmaceutical Bulletin*, **52**(10), 1242-1245 (2004).

9. B. Zhou, Z. Yang, Q. Feng, X. Liang, J. Li, M. Zanin, Z. Jiang and N. Zhong, *Journal of Ethnopharmacology*, **199**, 60-67 (2017).

10. Y. Cuan, W. Li, Y. Dou and G. Yang, *Natural Product Research*, in press (2021), DOI: 10.1080/14786419.2021.2011275.

11. K. B. Adhikari, H. N. Lærke, A. G. Mortensen and I. S. Fomsgaard, *Journal of Agricultural and Food Chemistry*, **60**(46), 11518-11524 (2012).

12. K. B. Adhikari, B. B. Laursen, H. N. Lærke and I. S. Fomsgaard, *Journal of Agricultural and Food Chemistry*,

60(10), 2497-2506 (2012).

13. S. K. Steffensen, H. A. Pedersen, K. B. Adhikari, B. B. Laursen, C. Jensen, S. Høyer, M. Borre, H. H. Pedersen, M. Borre, D. Edwards and I. S. Fomsgaard, *Journal of Agricultural and Food Chemistry*, **64**(43), 8235-8245 (2016).

14. M. K. Choi, S. J. Nam, H. Y. Ji, M. J. Park, J. S. Choi and I. S. Song, *Pharmaceutics*, **12**(3), 268 (2020).

15. J. H. Jeon, J. Lee, M. K. Choi and I. S. Song, *Archives of Pharmacal Research*, **43**(12), 1335-1346 (2020).

16. J. H. Jeon, J. Lee, J. H. Park, C. H. Lee, M. K. Choi and I. S. Song, *Pharmaceutics*, **13**(9), 1496 (2021).

17. J. H. Jeon, J. Lee, C. H. Lee, M. K. Choi and I. S. Song, *Mass Spectrometry Letters*, **12**(1), 16-20 (2021).

18. M. Pang, J. Lee, J. H. Jeon, I. S. Song, Y. T. Han and M. K. Choi, *Mass Spectrometry Letters*, **12**(4), 200-205 (2021).

19. S. Chandran and R. S. Singh, *Die Pharmazie*, **62**(1), 4-14 (2007).

20. L. Tanner, J. C. Evans, R. Seldon, A. Jordaan, D. F. Warner, R. K. Haynes, C. J. Parkinson and L. Wiesner, *Antimicrobial Agents and Chemotherapy*, **63**(11), e01010-19 (2019).

21. K. B. Adhikari, B. B. Laursen, P. L. Gregersen, H. J. Schnoor, M. Witten, L. K. Poulsen, B. M. Jensen and I. S. Fomsgaard, *Molecular Nutrition & Food Research*, **57**(10), 1847-1858 (2013).

22. B. M. Jensen, K. B. Adhikari, H. J. Schnoor, N. Juel-Berg, I. S. Fomsgaard and L. K. Poulsen, *European Journal of Nutrition*, **56**(1), 387-397 (2017).

Authors' Positions

So Yeon Jeon   : Graduate Student
San Kim   : Graduate Student
Jin-Hyang Park   : Graduate Student
Im-Sook Song   : Professor
Young Taek Han   : Professor
Min-Koo Choi   : Professor