Appl Biol Chem (2018) 61(2):251–255
https://doi.org/10.1007/s13765-018-0353-9

Online ISSN 2468-0842
Print ISSN 2468-0834

NOTE

Analysis of the biodistribution of natural products in mice by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Received: 25 December 2017 / Accepted: 18 February 2018 / Published online: 2 March 2018

Abstract Natural products originating from plants have various beneficial pharmacological effects, such as anticancer, antimicrobial, and anti-inflammatory activities, while being nontoxic. Therefore, tremendous efforts have been invested in understanding their bioactivities in the body to facilitate therapeutic target validation. However, such research is still challenging for certain natural products, such as flavonoids, which are rapidly metabolized in and eliminated from the human body. To investigate the bioactivities of such products, particularly in certain tissues, it is necessary to understand their biodistribution in vivo. In this respect, reliable analytical methods with simple and efficient procedures for the in vivo evaluation of natural small molecules are urgently required. In particular, mass spectrometry (MS) can be effectively used to analyze small molecules after tissue extraction, as MS has various advantages including accuracy, simplicity, and high sensitivity. Herein, we report the biodistribution of a natural small molecule by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). After intravenously injecting gomisin H into a mouse as a model natural product, it was extracted from each organ and then analyzed by MALDI-TOF MS. The analysis showed that gomisin H accumulated mainly in the liver and relatively large amounts of the product existed in the kidney and brain compared to those in other tissues.

Keywords Biodistribution · Gomisin · Mass spectrometry · Natural products · Tissues

Introduction

Natural products are small chemical molecules produced by living organisms, such as plants, microbes, and animals [1]. Notably, more than twenty thousand natural products can be obtained from plants, including medicinal plant-derived chemical compounds called phytochemicals and their metabolites, which are widely and actively used as drugs in clinical medicine due to their diverse pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, antimicrobial, and anti-viral activities with little to no toxicity [2–6]. Therefore, a multitude of research efforts are being undertaken to analyze their bioactivity in the body to facilitate therapeutic target validation [2]. In particular, understanding the biodistribution of natural products in the body is essential for investigating their bioactivities in certain tissues; such knowledge would prove useful in enhancing the therapeutic benefits of phytochemicals [7]. However, such investigation is still challenging for certain natural products, such as flavonoids, which are rapidly metabolized in and eliminated from the body, and therefore, only a few methods have been reported. Optical fluorescence imaging [8, 9], high-performance liquid chromatography tandem mass spectrometry [10, 11], and radioisotope-labeling methods [12–14] are examples of conventional methods used to monitor the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13765-018-0353-9) contains supplementary material, which is available to authorized users.

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accumulated target natural products in vivo. However, the general use of these methods is hindered by their disadvantageous features, including false-positive signals, high backgrounds, toxic and complicated steps, and/or changes in the physical and chemical properties of the natural products after labeling. In this context, reliable analytical methods for the in vivo evaluation of natural small molecules via simple and efficient procedures are urgently needed. In this study, we report a method for investigating the biodistribution of natural products using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which has several advantages over conventional methods, including high sensitivity, multiplexing capability, and label-free format.

Materials and methods

Materials

Dimethyl sulfoxide, ethyl acetate, acetonitrile, and sodium dodecyl sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS) was obtained from Gibco BRL (Grand Island, NY, USA). Absolute ethanol was purchased from Merck (Darmstadt, Germany). Gomisin H and gomisin J were purchased from Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China).

Sample preparations and mass analysis

Briefly, gomisin H was intravenously injected to mice and the organ was extracted. The mouse tissues were homogenized and extracted with ethyl acetate, which were analyzed by MALDI-TOF MS in the presence of the internal standard. For details, see Supporting Information.

Results and discussion

Figure 1 schematically illustrates our method for the qualitative and quantitative analysis of a natural small molecule in mouse organs using MALDI-TOF MS to determine its biodistribution after intravenous injection. Each isolated organ (the brain, heart, lung, kidney, spleen, liver, and pancreas) was extracted with an organic solvent 15 min after injection and the natural small molecule extracted from the harvested organs was analyzed by MALDI-TOF MS in the presence of an IS, which has a chemical structure similar to that of the analyte but a different molecular weight. Therefore, the mass spectrum can be used to derive quantitative information on the analyte by comparing the peak intensities of the analyte and IS. Gomisin H derived from *Schisandra chinensis* was used as a model compound in the current study. It is reported to be a lignan with various pharmacological effects, including anti-inflammatory, anti-hepatotoxic, antipyretic, and anti-asthmatic properties [15, 16].

We first constructed a calibration curve for the quantification of gomisin H extracted from mouse organs. Gomisin H at various concentrations in the presence of gomisin J as the IS was analyzed by MALDI-TOF MS using AuNPs as the matrix. In general, the use of an organic matrix hampers the analysis of small molecules due to the interference of a matrix in the low-mass region, and therefore, inorganic nanomaterials have been widely used such as AuNPs, one of the most popular inorganic matrices for small molecule analysis. Peaks at *m/z* values of 440.9 $[M + Na]^+$ and 410.8 $[M + Na]^+$ corresponding to gomisin H and the IS, respectively, could be clearly observed in the mass spectra (Fig. 2(A)). The calibration curve indicates a good linearity in the gomisin H concentration range of 10–1000 μM. Such linearity proves the feasibility of our strategy for the quantitative analysis of

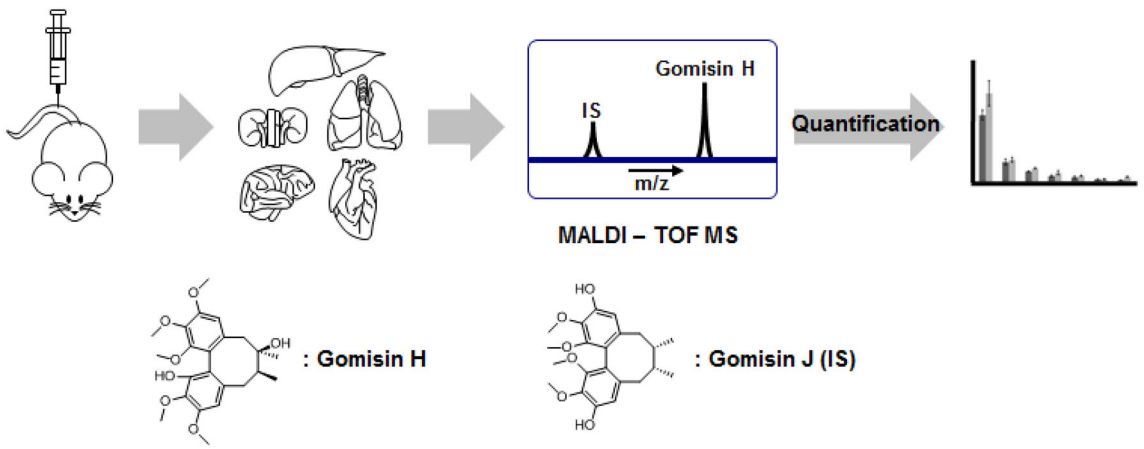


Fig. 1 Schematic representation of the investigative process used to analyze the biodistribution of gomisin H by MALDI-TOF MS

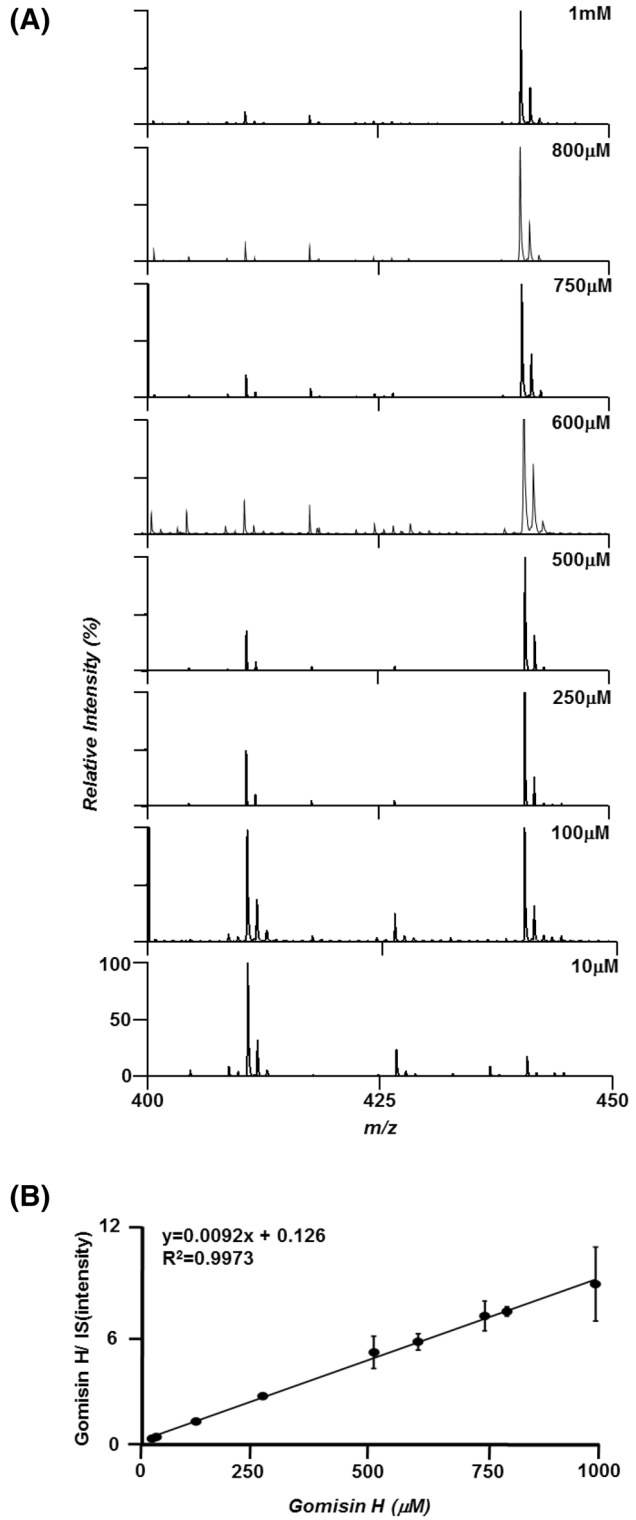


Fig. 2 (**A**) Representative mass spectra of gomisin H at various concentrations ranging from 10 to 1000 μM in the presence of gomisin J as the internal standard. (**B**) Calibration curve constructed by the linear regression of the ratio of signal intensities of gomisin H and the IS as a function of gomisin H concentration. The error bars represent standard deviations calculated from at least seven independent experiments

the target molecule using MALDI-TOF MS, which has an inherent limitation for quantification.

We next verified the fidelity of our strategy by examining the limit of detection (LOD) and quantification of gomisin H in spiked samples. Gomisin H sample was prepared at various concentrations and analyzed with MALDI-TOF MS which gave LOD of 10 aM (S/N > 3). We next measured MS intensity ratios between sample and IS (S/IS) against gomisin H spiked in liver tissue extracts of control mice. The quantification was performed using the constructed calibration curve. As shown in Table 1, recoveries of the known amount of gomisin H added in the liver extracts were obtained from 96 to 98%, and furthermore, we observed a coefficient of variance (CV) of 7.7 and 13.8%.

Next, we quantified the contents of gomisin H in the tissue extracts using the constructed calibration curve. Gomisin H in PBS containing 15% (v/v) each of Koliphor and DMSO was intravenously injected into ICR mice. In the control samples, PBS was injected into the mice. Fifteen minutes after the intravenous injection, the mice were killed and their organs were extracted. To evaluate the effects of organ extraction with and without a perfusion step, some of the mice were perfused with 0.9% saline to flush out blood. Subsequently, the mouse organs were collected, washed with PBS, weighed, and homogenized in PBS. The homogenates were then mixed with 10% SDS in PBS and EA, followed by sonication for 10 min. The supernatant was collected after centrifugation and extracted three times using equal volumes of EA. The combined organic layer was dried at room temperature. The dried extracts were dissolved in DMSO and analyzed using MALDI-TOF MS in the presence of the IS, gomisin J. Figure 3 shows the representative mass spectra of the tissue extracts with and without perfusion from a gomisin H-treated mouse (right panel) and a PBS-injected mouse (left panel). Peaks at *m*/*z* values of 410.8 $[M + Na]^+$ and 440.9 $[M + Na]^+$ corresponding to the IS (circles) and gomisin H (triangles), respectively, were clearly observed in the tissue extracts obtained from the gomisin H-treated mouse, whereas the tissues extracted from the PBS-injected control mouse exhibited the peak corresponding to IS only. This result proves that our strategy can be efficiently used to extract and analyze natural small molecules from complex biological samples, such as organs, without any complicated purification steps. Furthermore, in comparison with IS, the peak intensities of gomisin H without perfusion were higher than those obtained with perfusion; this observation can be attributed to the existence of the small molecules in blood. This result also indicates that our method has high sensitivity toward the amount of small molecules in the blood encapsulated in the organs.

Table 1 Quantification of gomisin H in liver extracts at various concentrations

Sample conc. (μM)	Expected (S/IS)	Observed (SI/S)	Precision (CV, %)	Accuracy (%)
200	1.97	1.94 ± 0.15	7.7	98
400	3.81	3.69 ± 0.51	13.8	96

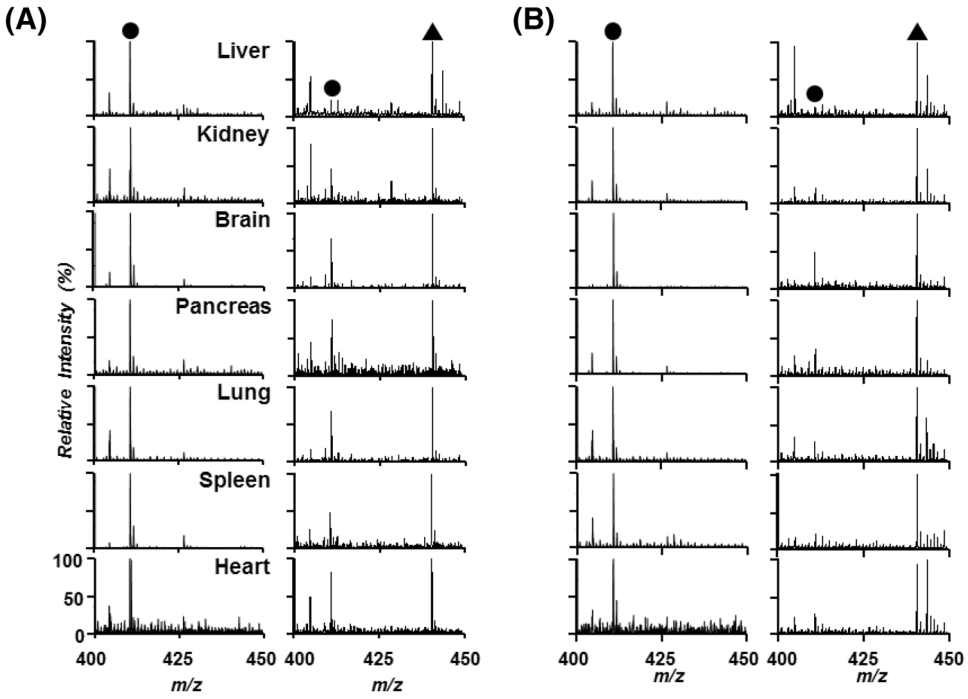
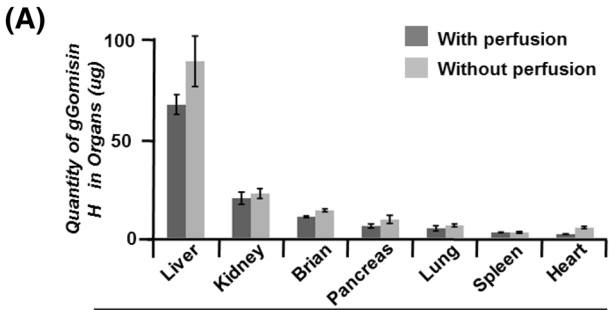


Fig. 3 Representative mass spectra of the extracts from various organs in the presence of IS with (**A**) and without (**B**) perfusion. The left-side spectra in (**A**) and (**B**) were from a PBS-injected control mouse, while the right-side spectra were from a Gomisin H-treated mouse. The peaks at *m/z* values of 410.8 $[M + Na]^+$ and 440.9 $[M + Na]^+$ correspond to the IS (circle) and gomisin H (triangle), respectively



(B)

Tissues	With perfusion (μg)	W/O perfusion (μg)
Liver	68.05±5.08	90.22±2.82
Kidney	20.30±3.07	22.54±2.53
Brain	10.79±0.39	13.93±0.76
Pancreas	6.00±1.01	9.27±2.11
Lung	4.64±1.33	6.26±0.67
Spleen	2.63±0.11	2.69±0.56
Heart	1.80±0.14	5.12±0.59

Fig. 4 Biodistribution of gomisin H in mouse organs. (**A**) Quantitative analysis of gomisin H in organs after perfusion for the removal of blood (dark bars) and without perfusion (bright bars). (**B**) Mean values of the gomisin H contents in mouse organs along with standard deviations ($n = 3$)

Later, we analyzed the biodistribution of gomisin H in mouse organs based on the quantitative results obtained using the calibration curve shown in Fig. 2(B). As shown in Fig. 4, the contents of gomisin H in the liver, kidney, brain, pancreas, lung, spleen, and heart samples after perfusion were (68.05 ± 5.08), (20.30 ± 3.07), (10.79 ± 0.39), (6.00 ± 1.01), (4.64 ± 1.33), (2.63 ± 0.11), and (1.80 ± 0.14) μg/g of organ, respectively. Without perfusion, the accumulated gomisin H contents were observed to slightly higher than those obtained after perfusion. The content of gomisin H in the liver was over 3 times greater than those in other organs. This is a reasonable observation because one of the main roles of the liver is the uptake and subsequent detoxification, metabolic transformation, and excretion of xenobiotics [17]. In addition, these results also illustrate the differences between the accumulated gomisin H contents in the organs with and without perfusion, stemming from the removal of gomisin H included in the blood. Notably, a significant decrease in the gomisin H content was observed in the liver, which might be attributed to the higher blood content in the liver compared to other organs.

In conclusion, we report a method for studying the biodistribution of natural products in mice using MALDI-

TOF MS. We successfully constructed a calibration curve, using which the accumulation of a natural product, gomisin H, in mouse organs was quantified; the small molecule contents were quantified by comparing the peak intensities of gomisin H and the IS. The results showed that a large proportion of the gomisin H intravenously injected into a mouse was accumulated in its liver due to its biological functions. Furthermore, we examined the effects of organ extraction with and without perfusion. We believe that our method can be practically used to study the in vivo biodistribution of natural compounds. In addition, the multiplexing capability of MS would enable our method to analyze pharmacokinetic drug–drug interactions.

Acknowledgments This research was supported by the National Research Foundation (NRF) of Korea (NRF-2016R1D1A1A09918111) funded by the Ministry of Education and the Agri-Bio Industry Technology Development Program [316028-3, Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET)].

References

1. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2:303–336
2. Benavente-Garcia O, Castillo J (2008) Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. J Agric Food Chem 56:6185–6205
3. Guo D-J, Cheng H-L, Chan S-W, Yu P-F (2008) Antioxidative activities and the total phenolic contents of tonic Chinese medicinal herbs. Inflammopharmacology 16:201–207
4. Lu Y, Li Y-Q, Liu Y-N, Lee K-H, Chen D-F (2013) Cytotoxic and potential anticancer constituents from the stems of *Schisandra pubescens*. Pharm Biol 51:1204–1207
5. Tapas AR, Sakarkar D, Kakde R (2008) Flavonoids as nutraceuticals: a review. Trop J Pharm Res 7:1089–1099
6. Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, Bartlett J, Shanmugam K, Münch G, Wu MJ (2011) Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem 59:12361–12367
7. D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R (2010) Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 11:1321–1342
8. Kang YY, Choi I, Chong Y, Yeo W-S, Mok H (2016) Complementary analysis of curcumin biodistribution using optical fluorescence imaging and mass spectrometry. Appl Biol Chem 59:291–295
9. Kang YY, Jung H, Yu G, Chong Y, Mok H (2016) Complexation of curcumin with 2-aminoethyl diphenyl borate and implications for spatiotemporal fluorescence monitoring. Int J Pharm 515:669–676
10. Sun H, Yin Q, Zhang A, Wang X (2012) UPLC–MS/MS performing pharmacokinetic and biodistribution studies of rhein. J Sep Sci 35:2063–2068
11. Xiong F, Wang H, Jiang Z, Huo M, Yan C, Zheng C, Gu N (2015) Integrated pharmacokinetics and biodistribution of multiple flavonoid C-glycosides components in rat after oral administration of Abrus mollis extract and correlations with bioeffects. J Ethnopharmacol 163:290–296
12. Choi MH, Rho JK, Kang JA, Shim HE, Nam YR, Yoon S, Kim HR, Choi DS, Park SH, Jang B-S (2016) Efficient radiolabeling of rutin with 125I and biodistribution study of radiolabeled rutin. J Radioanal Nui Chem 308:477–483
13. Hosseinimehr SJ, Ahmadi A, Taghvai R (2010) Preparation and biodistribution study of technetium-99m-labeled quercetin as a potential radical scavenging agent. J Radioanal Nucl Chem 284:563–566
14. Shimizu K, Asakawa T, Harada N, Fukumoto D, Tsukada H, Asai T, Yamada S, Kan T, Oku N (2014) Use of positron emission tomography for real-time imaging of biodistribution of green tea catechin. PLoS ONE 9:e85520
15. Oh S-Y, Kim YH, Bae DS, Um BH, Pan C-H, Kim CY, Lee HJ, Lee JK (2010) Anti-inflammatory effects of gomisin N, gomisin J, and schisandrin C isolated from the fruit of *Schisandra chinensis*. Biosci Biotechnol Biochem 74:285–291
16. Opletal L, Sovová H, Bártlová M (2004) Dibenzo [a, c] cyclooctadiene lignans of the genus Schisandra: importance, isolation and determination. J Chromatogr B 812:357–371
17. Mishra N, Yadav NP, Rai VK, Sinha P, Yadav KS, Jain S, Arora S (2013) Efficient hepatic delivery of drugs: novel strategies and their significance. BioMed Res Int 2013:382184