Sensitive analysis of arsenic species in urine by LC-MS/MS

*Takenori Yamauchi[1], Akihisa Hata[2], Yuko Yamano[1], Toshio Nakadate[1]

1 Department of Hygiene and Preventive Medicine Showa University, School of Medicine, Japan
2 Course of Clinical Laboratory Science, Department of Medical Risk and Crisis Management, Chiba Institute of Science, Japan

Abstract

Although it is familiar that arsenic is ingested routinely by food substance such as seaweed, species of arsenic metabolite in urine are not fully identified. In order to assess the toxicity of arsenic contained in food properly, it is necessary to identify unknown arsenic metabolites. One of the efficient approaches is omics approach with high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS/MS) using urine both before and after arsenic ingestion. However, analysis on arsenic species by LC-MS/MS is much less common than that by high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). Thus, analysis on available arsenic standard (i.e. arsenobetine, monomethyl arsonous acid, dimethylarsinic acid, trimethylarsine oxide, arsenocholine and dimethylmonothioarsinic acid) by HPLC-ESI-MS/MS was conducted as a pilot study. All of them without monomethyl arsonous acid was detected in a picogram or femtogram order. Furthermore, most of them can be quantified at least in a picogram order. However, a part of them such as dimethylmonothioarsinic acid was completely undetected or little detected when they were added to urine sample even in a ppm order. Thus, it indicated the presence of an inhibitor for ionization in urine, and also did the necessity of sample preparation before analysis. However, extremely high sensitivity of arsenic metabolites suggested the possibility of omics approach.

Keywords: Arsenic, Metabolites, LC-MS/MS, Omics, Pilot study

1. Introduction

Although the toxicity of arsenic is well known since early times, we ingested a food containing arsenic on a daily basis. Total amount of arsenic ingested per day was estimated to be 195 μg/day (Yamauchi, Takahashi et al. 1992), which was widely varied among individuals (Raml, Raber et al. 2009). Arsenic is contained in a variety of foodstuffs such as rice, wheat, vegetables, milk, meat, seafood and seaweed (Uneyama, Toda et al. 2007). Especially, seaweed contains a larger amount of both inorganic and organic arsenicals (García-Salgado, Raber et al. 2012). However, the toxicity of organic arsenicals such as arsenosugars and arsenolipids remains to be elucidated since the mechanism of metabolism and the toxicity of subsequent intermediate are not well documented.

Meanwhile, urine is generally used as a sample in in vivo metabolism study because of some reasons such as availability and ethical aspect. High performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is mainly used in analyzing arsenicals because it can prove the existence of arsenic. One limitation of HPLC-ICP-MS is that it cannot distinguish

Contact: Takenori Yamauchi, assistant professor, Department of Hygiene and Preventive Medicine Showa University
Address: 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, JAPAN
E-mail Address: yamauchi-t@med.showa-u.ac.jp, phone number: +81-3-3784-8137

substances if they are detected at almost the same retention time. Metabolites of arsenicals have the same or similar chemical constructions. Thus, it is not easy to determine that a peak detected by HPLC-ICP-MS is derived from only one metabolite of arsenical or not. In contrast, high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS/MS) has two advantages though it cannot prove the existence of arsenic. It can separate substances even if their retention time are completely the same. Secondly, it is possible that unknown metabolites are identified by differential analysis using urine sampled both before and after ingesting arsenicals.

Therefore, pilot study with arsenical standards and arsenicals added urine was conducted in order to acquire the basis of detection sensitivity.

2. Materials and Methods

2.1 Chemical Substances

Analytical standards of arsenobetine (AsBe) and monomethylarsonic acid (MMA) were purchased from WAKO. Those of dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO) and arsenocholine (AsCho) were from Tri Chemical Laboratory. Dimethylmonothioarsinic acid (DMMTA) was kindly provided by Dr. Hideki Wanibuchi at Osaka City University. Chemicals using for mobile phase; formic acid (FA), ammonium hydrogen carbonate and acetonitrile) were purchased from Wako, Sigma-Aldrich and Kanto chemical, respectively.

2.2 Sample Preparation

Five species of arsenical standards (i.e. AsBe, MMA, DMA, TMAO and AsCho) were dissolved into ultrapure water to contain 1.0 ppm each. Standard solution of 1 ppm DMMTA was separately produced. Then, six concentration of standard solutions (i.e. 100 ppb, 10 ppb, 1 ppb, 100 ppt, 10 ppt and 1 ppt) were produced via dilution. Urine free from ethical problem was centrifuged and the supernatant was ultrafiltrated with filter of which pore diamete 0.46 μm. Arsenical standard solution was added to collected urine to adjust concentration; 100 ppb, 10 ppb, 1 ppb, 100 ppt.

2.3 Analytical Instrument

In order to separate arsenicals, ACQUITY UPLC H-Class system (Waters) coupled with ACQUITY UPLC BEH Amide Column, 1.7 μm, 2.1 mm x 50 mm column (Waters) was used, and Xevo G2-XS QTof (Waters) was as a detector. Two patterns of mobile phase combination were adopted. One was 1% formic acid in water and acetonitrile, and the other was 20mM ammonium hydrogen carbonate at pH9.0 (adjusted with ammonia solution) and acetonitrile. Arsenicals were gradient-eluted at 0.4 mL/min using aquos phase: 5% and acetnitrile: 95% (5% to 60% aquos phase in 0.1 min, 60% aquos phase for 2.9 min). Column temperature was 40℃, and injection volume was 1 μL. Electrospray ionization was used in positive mode.

3. Results and Discussions

DMA, TMAO and AsBe was detected by HPLC-ESI-MS/MS even when the concentration was 1ppb (Fig. 1). Mass chromatogram indicated that quantity of them could be determined by HPLC-ESI-MS/MS.

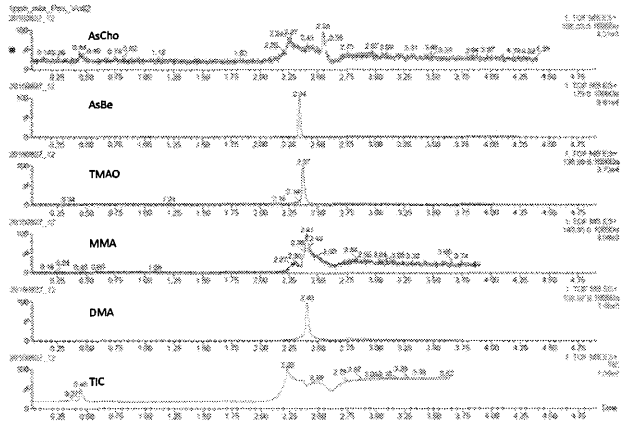

Fig. 1 Detection of 1 ppb standard solution

Although 1 pg of AsCho was below the limit of quantification, quantifiable chromatogram was available when 10 pg was injected (Fig. 2).

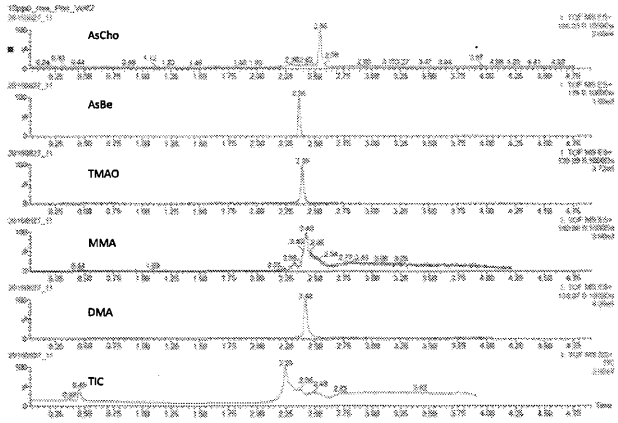

Fig. 2 Detection of 10 ppb standard solution

In the case of MMA, HPLC-ESI-MS/MS detected 1 pg of it. However, chromatogram was not enough to quantify MMA. Concentration of MMA, DMA, AsBe in human urine was reported to be in a ppb, ten ppb, ten ppb order, respectively (Suizu, Inoue et al. 2013). Thus, sufficient

sensitivity of HPLC-ESI-MS/MS was confirmed. In regard to MMA, although sensitivity of HPLC-ESI-MS/MS analysis was sufficient, analytical condition is not enough to quantify. Thus, it will be necessary to consider condition specific for MMA. TMAO in urine sample was detected in our study though it was not detected it according to the report cited above. Analysis on organic arsenicals with HPLC-ESI-MS/MS could be the essential method of arsenic analysis.

In the similar way, 1 pg of DMMTA was detedted and quantifiable chromatogram was available by HPLC-ESI-MS/MS analysis. However, it was not detected in urine sample to which it was added. Futhermore, it was not detedcted even when its concentration was 100 ppb (Fig. 3).

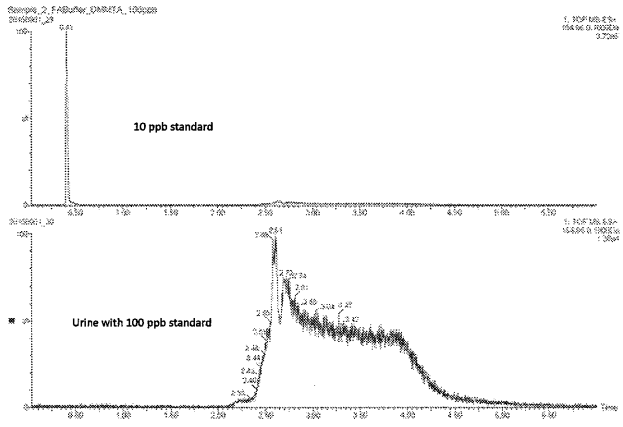

Fig. 3 Detection of DMMTA

It is not fully elucidated whether DMMTA is detected in urine of which one ingested arsenicals through foodstuffs or not. Our study indicated that processing urine sample was essential to study on DMMTA

4. REFERENCES

García-Salgado, S., G. Raber, R. Raml, C. Magnes and K. A. Francesconi (2012). "Arsenosugar phospholipids and arsenic hydrocarbons in two species of brown macroalgae." Environmental Chemistry 9(1): 63.

Raml, R., G. Raber, A. Rumpler, T. Bauernhofer, W. Goessler and K. A. Francesconi (2009). "Individual variability in the human metabolism of an arsenic-containing carbohydrate, 2',3'-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside, a naturally occurring arsenical in seafood." Chem Res Toxicol 22(9): 1534-1540.

Suizu, T., Y. Inoue, T. Iyo, T. Yosgida, Y. Saijo, T. Itoh, Y. Nakagi, Y. Sugioka, H. Okazaki, E. Hasegawa, Y. Hanawa and H. Yamauchi (2013). "Metabolism and the excretion of arsenic compounds following broccoli powder intake by six adult male volunteers." Japanese journal of clinical ecology 22(1): 59-65.

Uneyama, C., M. Toda, M. Yamamoto and K. Morikawa (2007). "Arsenic in various foods: cumulative data." Food Addit Contam 24(5): 447-534.

Yamauchi, H., K. Takahashi, M. Mashiko, J. Saitoh and Y. Yamamura (1992). "Intake of different chemical species of dietary arsenic by the japanese, and their blood and urinary arsenic levels." Applied Organometallic Chemistry 6(4): 383-388.