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Article

Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry for the Analysis of Complex Compounds in Serum and Its Application in Accurate Detection of Early Arsenic Exposure

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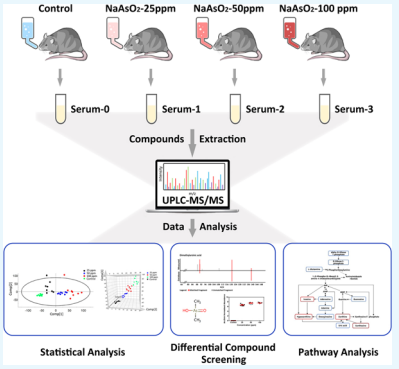
ABSTRACT: With the acceleration of industrialization, environmental arsenic pollution is threatening human health. However, by the time clinical symptoms appear, arsenic toxicity has usually caused irreversible damage to the body, so it is important to establish a rapid and accurate screening method for early arsenic exposure. In this work, 32 female C57BL/6 mice were exposed to different concentrations of inorganic arsenic in drinking water for a week. By analyzing the changes in serum, more than 20 compounds were detected to increase or decrease with the increase of arsenic intake. The abnormal increase in inosine, xanthine, xanthosine, and hypoxanthine and the abnormal purine pathway were found at the same time. Dimethylarsenic acid, an important inorganic arsenic metabolite in the body, was also found in serum. Combined with statistical analysis, early arsenic exposure can be easily and quickly detected, and the potential health risks of short-term exposure can be revealed simultaneously.



INTRODUCTION

Arsenic is a naturally occurring element in the earth's crust, which is mostly in the form of trivalent or pentavalent compounds in the natural world. Exposure to arsenic may cause many diseases, such as diabetes,[1−3] cardiovascular diseases,[4−7] and kinds of cancers.[8−12] International Agency for Research on Cancer (IARC) has identified arsenic and its compounds as explicit human carcinogens.[13,14] Excessive arsenic content in water resources is one of the important ways for human arsenic exposure. With the rapid development of industries, a large amount of high-arsenic wastewater was discharged into rivers and lakes; arsenic contamination in drinking water has been a global health problem for the past few decades.[15−18] Moreover, arsenic is also emitted into the environment by burning arsenic coal,[19,20] leading to excessive exposure of arsenic to the inhabitants of relevant areas.

Many research studies have attempted to reveal the toxicity of arsenic to human, especially trivalent inorganic arsenic. Inorganic arsenic and its compounds were generally considered to be more toxic than organic arsenic.[21−26] Modern studies have shown that exposure to inorganic arsenic could lead to DNA damage[27−30] and protein denaturation.[31−33] Although many research studies aimed at revealing the mechanism of arsenic damage to body, early detection of arsenic exposure at low doses was still difficult. When clinical symptoms of arsenic exposure have occurred, the body has usually already suffered some serious damage. Therefore, accurate detection of early exposure to low-concentration inorganic arsenic has great significance for early warning of arsenic damage risk and monitoring of environmental arsenic pollution.

Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is a useful method for separation and analysis of complex mixtures, and it is widely used in analysis of complex biological samples such as blood and urine. In this work, UPLC-MS/MS was used to detect changes in serum compounds in mice after short-term arsenic exposure. More than 20 serum compounds were altered and purine pathways were found to be abnormal after arsenic exposure. Although serum arsenic content remains at an extremely low level when the drinking arsenic intake increases, dimethylarsenic acid, an important in vivo metabolite of sodium arsenite, was still detected. Combined with statistical analysis, early arsenic exposure can be easily and quickly detected, and the potential health risks of short-term arsenic exposure can be revealed simultaneously.

RESULTS AND DISCUSSION

Physiological Status after Arsenic Exposure. After a week of sodium arsenite exposure, mice in the control group

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Table 1. Differential Compounds in Serum after Arsenic Exposure

formula	compound name	change	max fold change	score	Anova (p)	q value
$C_{15}H_{29}NO_4$	(±)-octanoylcarnitine	↓	4.324	56	0.003	0.008
$C_{15}H_{15}NO_8$	3-indole carboxylic acid glucuronide	↑	15.057	53.9	0.001	3.77×10^{-4}
$C_{21}H_{32}O_2$	5-alpha-pregnane-3,20-dione	↓	9.282	50.2	3.65×10^{-6}	3.50×10^{-5}
$C_{27}H_{44}O_3$	23*S*,25-dihydroxyvitamin D3	↓	5.113	53	0.001	2.38×10^{-3}
$C_{21}H_{30}O_5$	cortisol	↓	5.832	50.6	0.001	4.60×10^{-3}
$C_{15}H_{15}NO_8$	2,8-dihydroxyquinoline-beta-D-glucuronide	↑	10.355	54.3	7.89×10^{-5}	4.98×10^{-4}
$C_{14}H_{15}NO_8$	dihyroxy-1*H*-indole glucuronide I	↑	4.260	56.1	8.98×10^{-5}	2.69×10^{-4}
$C_{12}H_{14}O_7$	phenol glucuronide	↑	3.497	56.7	8.29×10^{-5}	2.53×10^{-4}
$C_{15}H_{17}NO_8$	6-hydroxy-5-methoxyindole glucuronide	↑	15.429	55.9	1.70×10^{-4}	4.53×10^{-4}
$C_{15}H_{15}NO_8$	3,4,5-trihydroxy-6-(1*H*-indole-3-carbonyloxy)oxane-2-carboxylic acid	↑	9.610	57.5	2.24×10^{-4}	5.66×10^{-4}
$C_{12}H_{16}O_{12}$	*α*-L-*threo*-4-hex-4-enopyranuronosyl-D-galacturonic acid	↑	7.539	54.8	0.001	2.05×10^{-4}
$C_{10}H_{12}N_4O_6$	xanthosine	↑	3.671	57.8	9.09×10^{-5}	4.04×10^{-5}
$C_{10}H_{12}N_4O_5$	inosine	↑	170.765	57	5.30×10^{-5}	5.85×10^{-5}
$C_{10}H_{12}N_4O_4$	deoxyinosine	↑	9.678	52.9	2.41×10^{-3}	5.54×10^{-4}
$C_5H_4N_4O$	hypoxanthine	↑	49.236	57.8	4.06×10^{-5}	2.14×10^{-4}
$C_5H_4N_4O_2$	xanthine	↑	614.780	56.6	1.60×10^{-8}	1.10×10^{-6}
$C_5H_{11}NO_3S$	L-methionine *S*-oxide	↑	9.094	54.3	0	0
$C_8H_8O_4S$	4-vinylphenol sulfate	↓	4.797	52.4	3.04×10^{-11}	3.53×10^{-10}
$C_6H_8N_2O_2$	pi-methylimidazoleacetic acid	↑	3.975	51	0.009	3.92×10^{-3}
$C_6H_6N_2O_2$	urocanic acid	↑	3.103	57.7	0.001	4.69×10^{-4}
$C_{21}H_{22}O_{13}S$	({6-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2*H*-1-benzopyran-8-yl]-3,4,5-trihydroxyoxan-2-yl}methoxy)sulfonic acid	↑	11.536	51	7.84×10^{-8}	9.70×10^{-7}
$C_{12}H_{14}O_8$	uralenneoside	↑	3.955	54	1.77×10^{-4}	4.68×10^{-4}
$C_{21}H_{18}O_{10}$	daidzein 7-*O*-glucuronide	↑	8.908	54.1	6.82×10^{-6}	3.39×10^{-5}
$C_2H_7AsO_2$	dimethylarsenic	↑	infinity	54.8	0	0
$C_9H_{13}N_5O_3$	1-hydroxy-2-oxopropyl tetrahydropterin	↓	123.289	53.1	5.24×10^{-8}	2.05×10^{-7}
$C_{21}H_{30}O_4$	7′-carboxy-alpha-tocotrienol	↓	52.270	50.9	7.28×10^{-6}	6.59×10^{-5}
$C_9H_{10}O_7S$	3-(3,5-dihydroxyphenyl)-1-propanoic acid sulfate	↑	5.857	54.4	1.28×10^{-9}	4.03×10^{-8}
$C_{21}H_{18}O_{11}$	baicalin	↑	9.076	53.3	7.52×10^{-8}	9.43×10^{-7}

and all experimental groups showed the same physiological status and behavior. The mice in three experimental groups showed no appearance changes such as hair loss, and no abnormal conditions such as convulsion, lethargy, and motor behavior changes. Moreover, the addition of different concentrations of arsenic did not change the drinking behavior of the mice; all mice in experimental and control groups drank roughly the same amount of water. Therefore, short-term exposure to a low concentration of arsenic generally does not show obvious discomfort symptoms, resulting in the difficulty of early detection of arsenic exposure.

Arsenic Exposure Perturbed Serum Components. UPLC-MS/MS-based arsenic exposure groups and control group samples, in total, revealed more than 10,000 features, which were contributed by a large number of complex compounds in the serum. After a series of screening, 301 different compounds were identified in serum (the corresponding compound names, molecular formulas, and other information are shown in Table S1, in the Supporting Information). Twenty-eight compounds showed significant concentration changes (increase or decrease) with the increase of arsenic exposure concentration (Table 1), which were considered as potential signals to reveal the harm of short-term

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ACS Omega 2021, 6, 28326−28333

arsenic intake. Significantly, although the arsenic concentration in serum was extremely low (lower than 0.6 ppm, Table 2),

Table 2. Total Arsenic Concentration in Serum of Different Groups

initial arsenic concentration in water (ppm)	total arsenic in serum (ppb)
0 (control)	5.047 ± 0.375
25	122.6 ± 5.681
50	176.6 ± 11.67
100	540.6 ± 23.76

dimethylarsenic acid, which stemmed from sodium arsenite metabolism in the body, was still found in serum, and its concentration significantly increased with the growth of arsenic exposure (Figure 4).

The Principle of Statistical Analysis for UPLC-MS/MS Data. Serum contains a large number of small molecule compounds, which generate huge mass spectral data with useless noise. Thus, a big challenge in analysis of a group mass spectral data is how to effectively extract information from this big data set. UPLC-MS/MS results of serum samples can be viewed as a matrix, which contain a large number of observational variables, compound information, and the corresponding strength of their signals (Figure 1). The

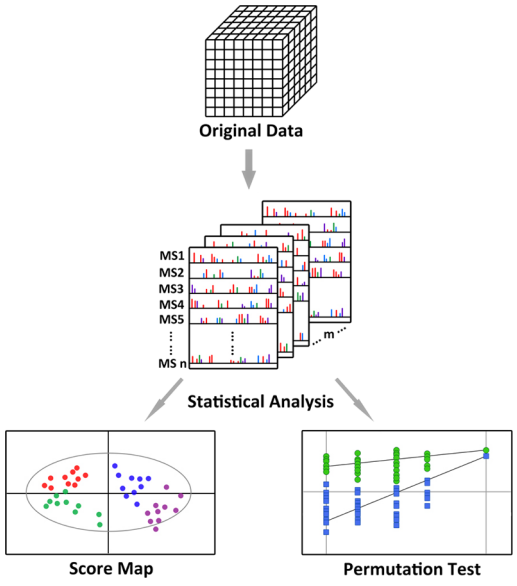

Figure 1. Multivariate statistical analysis for UPLC-MS/MS data.

purpose of multivariate statistical analysis is to reduce the dimensionality of high-dimensional data and display maximum information of the original data with the least number of variables. Meanwhile, multivariate statistical analysis can eliminate the noise and extract useful information from the original data efficiently and accurately.

PCA is probably the most widespread multivariate chemometric technique, and it could transform a large number of related variables into a group of orthogonal variables that best represent data characteristics through linear transformation. Using these orthogonal variables to describe the sample could reduce the dimension of original data and at the same time, remove useless noise. PCA is an unsupervised analysis mode, and the grouping of each sample is unknown during calculations. PCA can clearly display the repeatability within groups and the differences between groups, evaluate the repeatability of data, and find out the possible outliers.

However, due to the complexity of the serum chemical environment, unsupervised analysis is sometimes not good at distinguishing subtle differences between samples. OPLS-DA is a supervised analysis mode; the grouping relationship of samples is known during data analysis. In this way, the characteristic variables of each group can be better selected and distinguished, and the relationship between samples can be determined. Partial least-squares discriminant analysis (PLS-DA) used a partial least-squares regression method to reduce the dimension of the data and established a regression model to conduct discriminant analysis on the regression results. OPLS-DA is the correction of orthogonal transformation based on PLS-DA, which can filter out the noise unrelated to the classification information and improve the analytical ability and effectiveness of the model.

Score Plot and Permutation Test. The score is an important parameter in PCA and OPLS-DA analysis results. The score plot can clearly describe the distance between different samples and can be used to distinguish the similarity or difference of samples. In general, the closer the distance between the observed variables is, the higher similarities of samples are. On the contrary, the further the distance between the observed variables, the lower similarities of samples are.

A permutation test is a statistical inference method based on a large number of calculations and uses the complete (or random) arrangement of sample data. The permutation test obtains a statistical result by iterating on the predicted variables according to the known measured data variables. The reliability and overfitting degree of the model were represented by examining the y-intercept of the fitting line composed of the corresponding R^2 and Q^2 calculated values of all samples. The criteria for model validity are as follows: (1) all Q^2-values and R^2-values to the left are lower than the original points to the right; (2) the regression line of the Q^2-points intersects the vertical axis (on the left) at, or below zero.

Statistical Analysis for Serum Components. All MS/MS data of the identified compounds were sent to SIMCA 14.1 for statistical analysis. Figure 2A shows the score plot of PCA for all control and experimental groups after arsenic exposure. In score plots, each dot represents a serum sample of mice. Although most compound concentrations in serum do not have a significant change after arsenic exposure, PCA could well distinguish the control group from all experimental groups. Under low concentration and short exposure conditions (25 ppm, 1 week), the PCA method was still able to distinguish arsenic exposed mice from normal mice. However, when arsenic concentrations increased above 50 ppm, the unsupervised PCA method was unable to effectively distinguish between the two high-dose groups (50 ppm group and 100 ppm group). In a 3D score plot (Figure 2B), PCA still had difficulty in identifying the two high-dose groups. That is because the significant changes in serum compounds were few and a large number of compounds were not significantly changed. The large number of non-differential compounds caused the two high-dose groups (50 ppm and 100 ppm) to not be effectively distinguished by PCA.

To solve the problems in PCA, OPLS-DA was used for further difference identification. As a supervised analysis mode, OPLS-DA could better determine the relationship between samples during data analysis. In Figure 2C,D, the score plots of OPLS-DA clearly display that the three arsenic exposure

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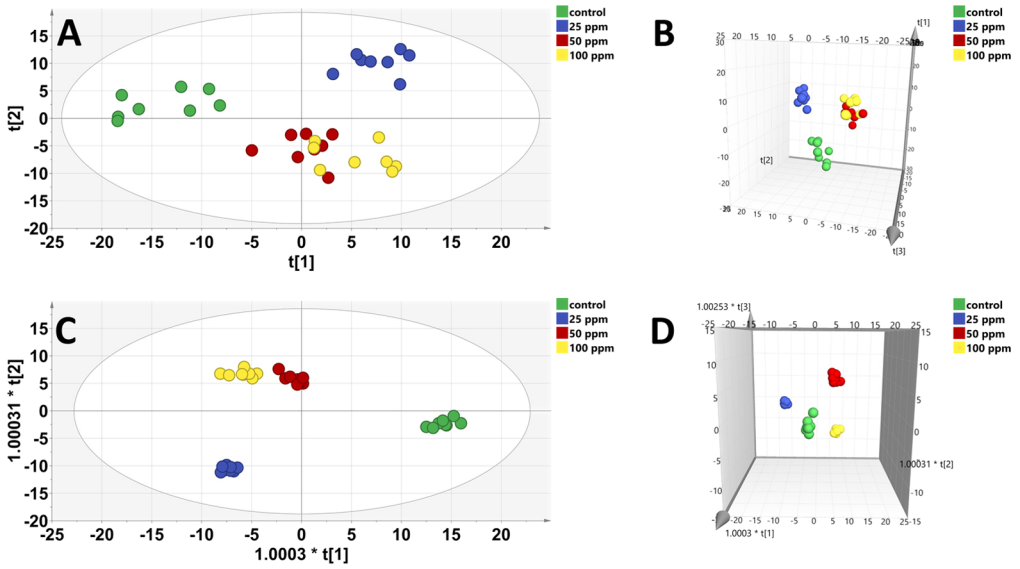

Figure 2. Results of statistical analysis. (A) PCA score plot in a 2D plane, (B) the PCA score plot in a 3D space, (C) OPLS-DA score plot in a 2D plane, and (D) the OPLS-DA score plot in a 3D space.

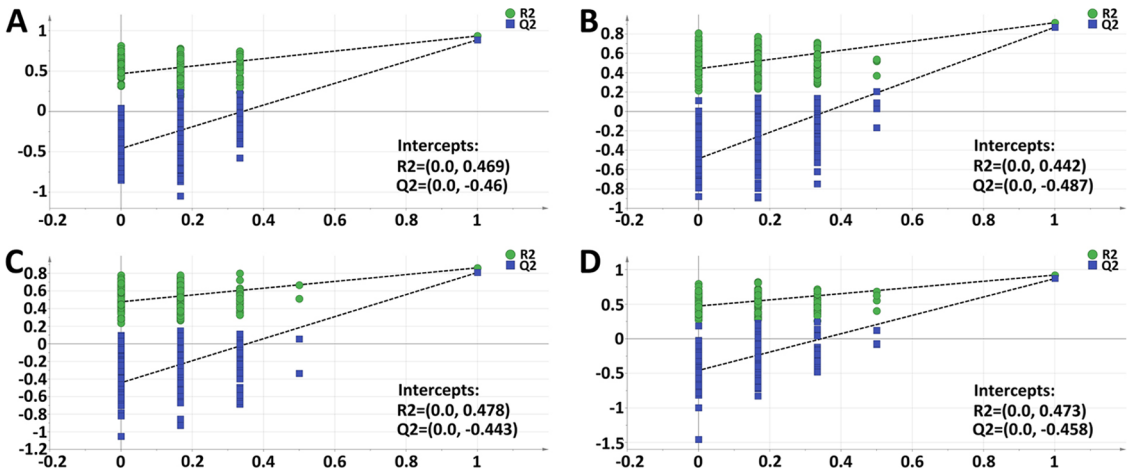

Figure 3. Permutation test of all OPLS-DA results: (A) control group, (B) 25 ppm group, (C) 50 ppm group, and (D) the 100 ppm group.

groups and one control group can be effectively distinguished in both the 2D plane and 3D space. The permutation test was then used to examine the reliability of the OPLS-DA regression model, and the permutation number of each variable was 200 times. For every group, the *y*-intercepts of the fitting lines of R^2 and Q^2 are significantly lower than the values of R^2 and Q^2 under the corresponding principal component (the high point on the right of the line, Figure 3). The *y*-intercepts of R^2-point and Q^2-point regression lines for each group were lower than 0.5 and −0.4, respectively. The results of the permutation test confirm the validity of the OPLS-DA model.

Combined with subsequent analysis of the pathway and the discovery of arsenic methylation products in serum, the relevant methods can be used to screen the risk of early low-concentration arsenic exposure.

Arsenic Residue in the Serum. In order to investigate the change of arsenic concentration in serum, ICP-MS was used to detect the total arsenic content in serum of each mouse in the first, and the results are displayed in Table 2. The results of the control group showed that the total arsenic concentration in the blank serum samples was less than 6 ppb. After arsenic ingestion in drinking water, the total arsenic content in serum changed obviously. All three experimental groups showed significant differences compared with the control group ($P < 0.0001$). However, although the serum arsenic concentration of the high-dose group was 100 times higher than that of the control group, the total serum arsenic level was still much lower than 1 ppm. As body metabolism makes the blood chemical environment relatively stable, arsenic is almost never retained in serum. Thus, if the sensitivity of the instrument is not high enough or the control sample is lacking, arsenic exposure risks can be difficult to detect only by routine metal testing.

Dimethylarsenic Acid in Serum. Current mainstream studies believe that inorganic arsenic is excreted from the body after ingestion through a series of methylation processes and almost does not persist in the blood. In our work, ICP-MS results also showed a low level of the total arsenic concentration in serum after drinking intake (Table 2). However, after the UPLC-MS/MS test, dimethylarsenic acid was detected in serum samples of all arsenic exposure groups (Figure 4), and its content increased with the increase of arsenic concentration in drinking water. Dimethylarsenic acid is an inorganic arsenic metabolite that is generally considered

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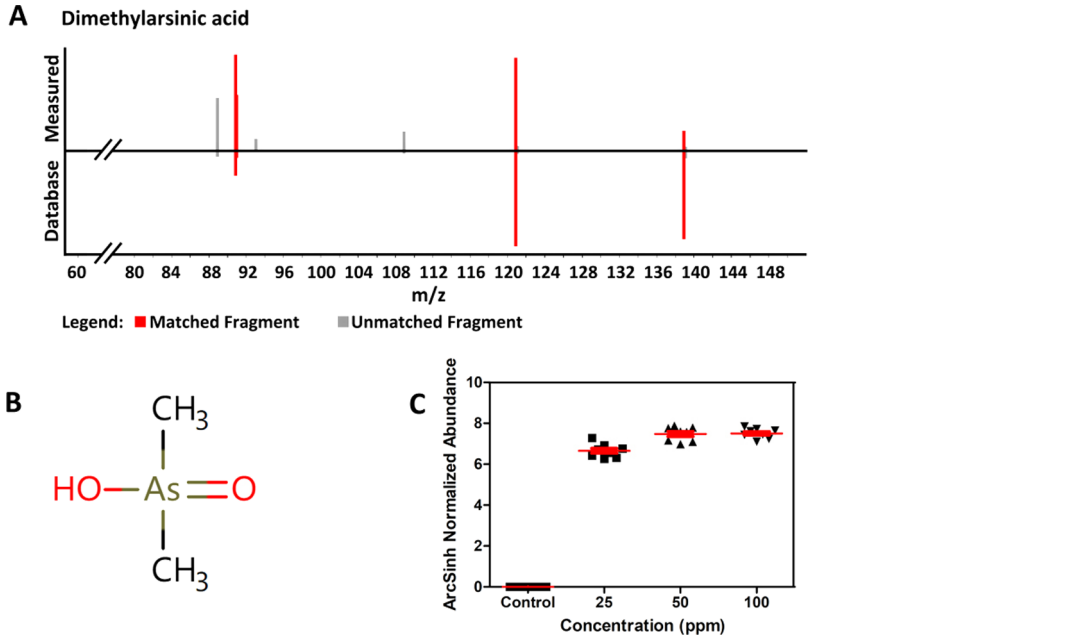

Figure 4. Dimethylarsenic acid in serum; (A) the detected MS spectrum of dimethylarsenic acid (matched with the METLIN database), (B) the structural formula of dimethylarsenic acid, (C) changes in serum dimethylarsenic acid content after different concentrations of arsenic intake.

to be excreted in urine and feces but not retained in the body.[34] By high-precision mass spectrometry, ultralow concentration compounds can be captured from a complex serum environment.

Arsenic Exposure Affects Purine Metabolism. To determine the health effects of arsenic exposure in mice, the information of all altered compounds was upload to MetaboAnalyst (https://www.metaboanalyst.ca/) for further pathway analysis, and an abnormal purine pathway was found. Eleven identified compounds were located in the middle and downstream of the purine pathway (Figure 5). Inosine, xanthine, xanthosine, and hypoxanthine were the compounds that showed a significant upward trend with the increase of arsenic intake (Figures 6 and 7). Meanwhile, these four compounds were accurately identified by MS/MS experiments of standard chemicals, and the corresponding results are shown in the Supporting Information (Figure S2). Although the mice showed no obvious physiological changes and behavioral abnormalities after 1 week of arsenic exposure, the disorder of purine metabolism is an important warning to the potential harm of short-term inorganic arsenic exposure. In the body, purine metabolism is related to a variety of cell functions, energy conservation and transport, formation of coenzymes and of active intermediates of phospholipids, and carbohydrate metabolism.[35] Thus, any system may be affected when purine metabolism disorders are present. There are many diseases that can affect purine metabolism, and the possibility of arsenic exposure should be guarded when no abnormality is found in traditional disease tests.

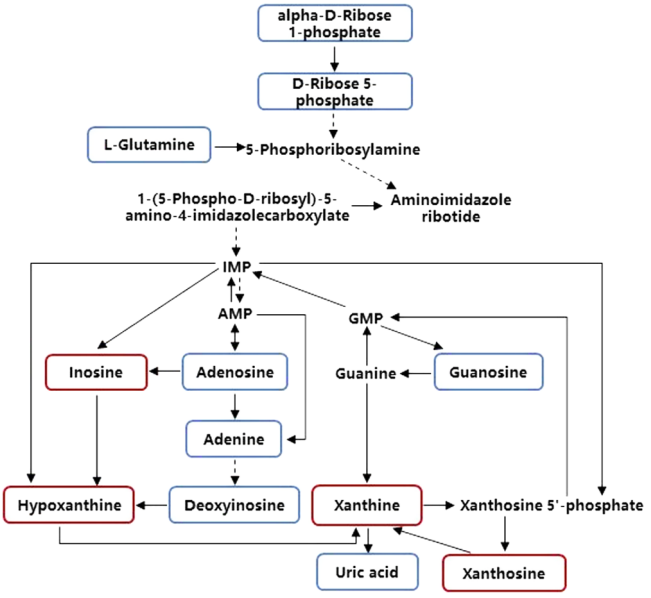

Figure 5. Diagram of the purine pathway. The red box indicates that the content of identified compounds increases with the increase of arsenic exposure concentration; the blue box indicates that the content of identified compounds does not change significantly with the increase of arsenic exposure concentration; those with no box represent compounds not detected by UPLC-MS/MS in serum.

CONCLUSIONS

In this work, 32 female mice were exposed to different concentrations of inorganic arsenic in drinking water for a week. UPLC-MS/MS was used to detect the changes of compounds in serum after arsenic exposure. The concentrations of inosine, xanthine, xanthosine, and hypoxanthine in serum were detected to increase with the increase of arsenic concentration in drinking water, indicating abnormal purine metabolism. Meanwhile, although the total arsenic concentration in serum was extremely low, dimethylarsenic acid, a methylation intermediate metabolized by inorganic arsenic in vivo, was detected in serum. By PCA and OPLS-DA methods, mice in different arsenic exposure groups and the control group could be clearly distinguished. In addition, statistical analysis was able to distinguish arsenic-exposed mice from normal mice at low concentrations of arsenic for short periods of time (25 ppm, 1 week). The relevant studies of this work are

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ACS Omega 2021, 6, 28326−28333

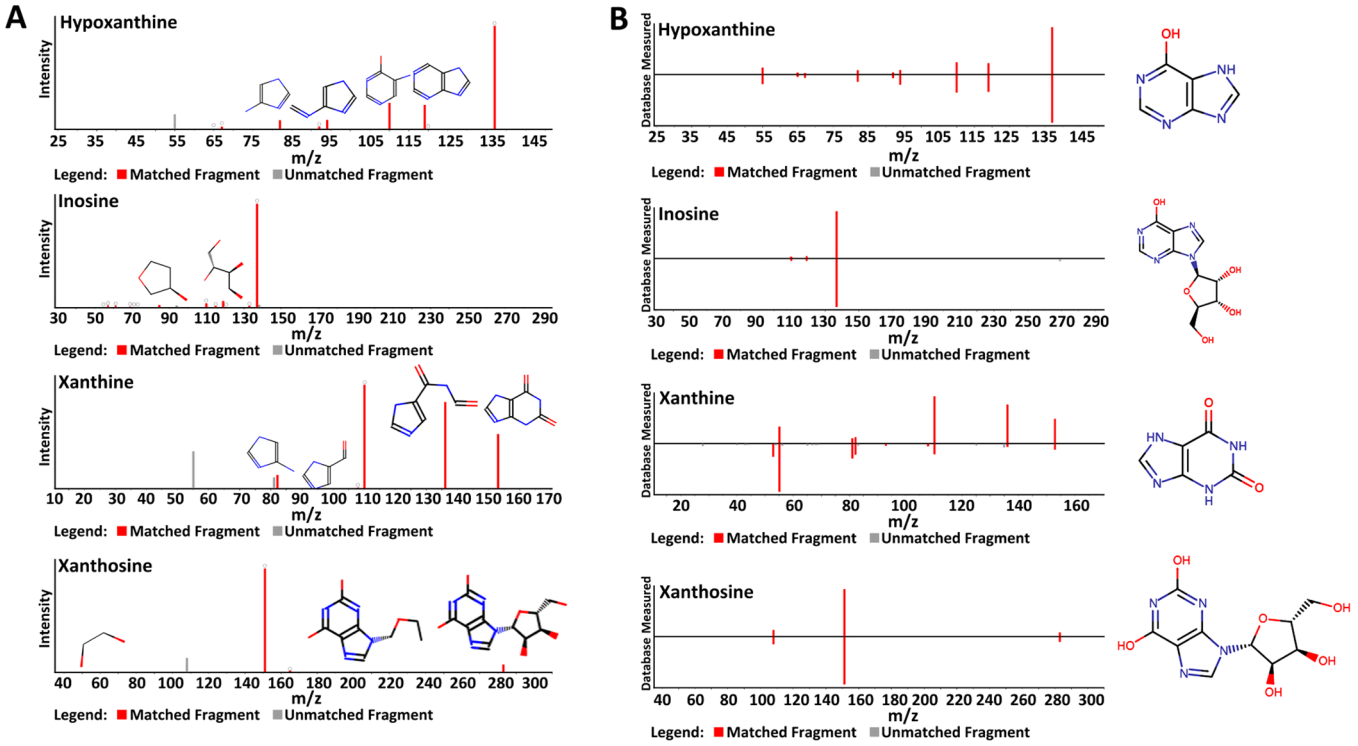

Figure 6. MS spectra and the corresponding structural formulas of inosine, xanthine, xanthosine, and hypoxanthine, (A) matched with the HMDB database and (B) matched with the METLIN database.

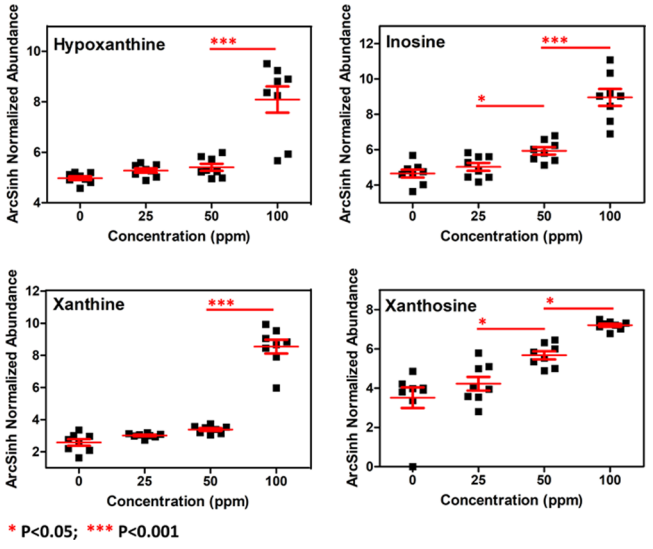

Figure 7. Contents of inosine, xanthine, xanthosine, and hypoxanthine in serum increased with the increase of arsenic intake.

helpful for accurate and rapid detection of arsenic exposure in the early stage and can reveal the potential health risks of arsenic exposure in the short term.

MATERIALS AND METHODS

Instruments, Materials, and Reagents. An LC-30 AD ultrahigh-performance liquid chromatography system (Shimadzu, Japan) coupled with a TripleTOF 6600 hybrid Q-TOF mass spectrometer (AB SCIEX, USA) was used to separate and detect serum samples. A Thermo iCAP Q inductively coupled plasma mass spectrometry system (ICP-MS, Thermo Fisher, USA) was used to investigate the total arsenic concentration in serum.

Progenesis QI (Waters, USA) was used for MS data analysis and serum compound identification. SIMCA 14.1 (MKS UMETRICS) was used for the corresponding statistical analysis.

Sodium arsenite ($NaAsO_2$) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Methanol, acetonitrile, formic acid, and nitric acid were purchased from Thermo Fisher Scientific Inc. $NaAsO_2$ was of analytical grade and the remaining chemicals were of mass pure grade, and all chemicals were used without further purification.

Animal Experiments. Previous studies have shown that female mice are more sensitive to the effects of inorganic arsenic than males.[34,36] In this work, 32 specific-pathogen-free grade (SPF) C57BL/6 female mice (18−22 g weight) were purchased from Charles River Experimental Animal Technology Co., Ltd (Beijing, China). These mice were randomly assigned to three arsenic-treated groups and one control group, and each group has eight mice. All mice were housed in polycarbonate cages on dry corncob bedding (4 mice/cage) under a barrier condition with a stable environment (22 °C, 40−70% humidity, and a 12 h light/dark cycle), and fed a standard pelleted rodent diet. After a week of acclimatization, the arsenic-treated groups were exposed to 25, 50, and 100 ppm arsenic in drinking water for a week, respectively, and the control group was fed with purified water. After a week of arsenic exposure, all mice were anesthetized with isoflurane, and the serum was sampled from mice eyes. The serum samples were stored at −80 °C for subsequent testing. All animal experiments in this work were approved by IACUC of the National Center for Biomedical Analysis; the permit number was IACUC-DWZX-2019-520.

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ACS Omega 2021, 6, 28326−28333

Serum Preparation. Briefly, 400 μL of methanol/acetonitrile solution (1:1, v:v) was added into 100 μL of serum to remove proteins. The mixture was vortexed for 30 s and incubated at −20 °C for 1 h and then centrifuged for 15 min at 15,000 rpm and 4 °C. The supernatant was collected and transferred to new centrifuge tubes and dried in a SpeedVac (FreeZone, Labconco, USA) at 4 °C. The dry samples were resuspended in 50% cold methanol solution that contains 1‰ formic acid for subsequent analysis.

ICP-MS Analysis. All serum samples were diluted 30 times by a gravimetric method using 2% nitric acid, and the total arsenic content of each sample was measured by ICP-MS. The power of ICP-MS was 1500 W, and the pump speed was 50 rpm. The auxiliary gas flow and atomized gas flow were 1.5 L/min, and the cooling air flow was 12 L/min.

UPLC-MS/MS Analysis. All serum samples were tested by an LC-30 AD ultrahigh-performance liquid chromatography system coupled with a hybrid Q-TOF mass spectrometer in both positive and negative ion modes. In order to increase the compounds' coverage, both a 2.1 × 100 mm ACQUITY 1.8 μm HSS T3 column (Waters Corporation, Milford, USA, Ireland) and a 2.1 × 100 mm ACQUITY 1.7 μm BEH amide column (Waters Corporation, Milford, USA) were used to perform the compounds' separation, respectively. For the UPLC separation (T3 column), the mobile phases were (A) water with 0.1% formic acid (FA) and (B) acetonitrile with 0.1% FA. The gradient was set as follows: 0−1.5 min, 1% B; 1.5−13.0 min, 1−99% B; 13.0−16.5 min, 99% B; 16.6−20.0 min, 1% B. For the hydrophilic interaction chromatography (HILIC) separation (BEH amide column), the mobile phases were (C) 10% water/acetonitrile with 10 mM ammonium acetate and 0.1% FA and (D) water with 10 mM ammonium acetate and 0.1% FA. The gradient was set as follows: 0−1.0 min, 5% D; 1.0−12.0 min, 5−55% D; 12.1−15.0 min, 55% D; 15.1−20.0 min, 5% D. The flow rate was 0.3 mL/min and sample injection volumes were 3 μL. The temperatures of the column and sample chamber were, respectively, maintained at 40 and 10 °C in both positive and negative ion modes. Calibration solution was inserted every six samples during the test to ensure the accurate analysis of samples (the related experimental methods and results are provided in the Supporting Information).

A TripleTOF 6600 mass spectrometer was operated in data-dependent analysis (DDA) mode in both positive and negative ion modes. The m/z scan range of the precursor ion in full-scan mode was set as 50−1250 Da. For the fragment ion, the m/z scan range was set as 50−1250 Da. Accumulation times of MS1 and MS2 were 150 and 30 ms, respectively. The CE voltage was in a series set at 15, 30, and 45 V testing parameters: gas1 and gas2, 50 Pa; curtain gas, 35 Pa; desolvation gas temperature, 500 °C; source temperature, 500 °C; ion spray voltage floating (ISVF), 5000 V (ESI+) or −4500 V (ESI−); declustering potential (DP), 80 V (ESI+) or −80 V (ESI−); collision energy, 30 ± 15 eV.

Identification of Chemical Compounds in Serum. MS/MS data were sent to Progenesis QI for peak picking, alignment, integration, and extraction of the peak intensities. METLIN and HMDB database were used for the first-step identification of compounds in serum. The MS spectra of pre-identified compounds (score > 50, mass error < 10 ppm, q value < 0.05, Anova (p) < 0.05) were matched against by a manual method to ensure the accuracy of the compounds' identification. A two-tailed Welch's t test with false discovery rate (FDR) correction was used for the statistical analysis of compound differences between control and treatment groups. The significantly perturbed compounds (q value < 0.05, Anova (p) < 0.05, max fold change > 3) were considered to be differential compounds that are triggered by arsenic exposure.

Statistical Analysis. Principal components analysis (PCA) and orthogonal partial least-squares discrimination analysis (OPLS-DA) were used to distinguish differences in mouse serum samples after exposure to different concentrations of arsenic. The permutation test was used to verify the reliability of the statistical model. All statistical analyses were performed by SIMCA 14.1.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04517.

Quality control results of the UPLC-MS/MS test, information of all identified compounds in serum after arsenic exposure, and the results of dentification of inosine, xanthine, xanthosine, and hypoxanthine by MS/MS experiments with standard chemicals (PDF)

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Notes

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