Journal of Hazardous Materials 190 (2011) 515–519

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

A metabonomic analysis on health effects of drinking water on male mice (*Mus musculus*)

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ARTICLE INFO

Article history:
Received 31 January 2011
Received in revised form 17 March 2011
Accepted 21 March 2011
Available online 29 March 2011

Keywords:
Metabonomics
Drinking water
Mice
NMR

ABSTRACT

Health effects of drinking water on the male mice (*Mus musculus*) were investigated by metabonomics after exposure to the Taihu drinking water for 90 days. Metabonomics data combined with the results of conventional serum biochemistry tests and hepatic histopathology showed that the drinking water induced adverse health effects on the male mice. It was found that the serum levels of pyruvate, glutamine, arginine, lysine, N-acetyl glycoproteins, choline and citrate were significantly decreased in the treatment group. These results indicated that Taihu drinking water may induce damages on mice liver via perturbations of energy metabolism, amino acid metabolism and apoptosis. These observations yielded novel insights regarding the environmental health risk of Taihu drinking water.

1. Introduction

In China, rapid urbanization and industrialization lead to contamination of source water, with a result that health risks of drinking water become public concern. Taihu Lake is the third largest freshwater lake in East China, which is an important drinking water source for Wuxi City. Many organic pollutants have been detected in Taihu Lake, including polycyclic aromatic hydrocarbons (PAHs) [1,2] and organochlorine pesticides (OCPs) [3,4]. These pollutants can induce various adverse health effects on organism, such as hepatotoxicity, nephrotoxicity and even cancer [5,6].

"Omic" techniques such as transcriptomics and genomics are increasingly being used to determine the health effects of environmental pollutants on organisms and identify early warning biomarkers of exposure [7–9]. However, the results obtained by these methods have many uncertainties, since the final phenotypes are often affected by many intermediates which are generated as a result of transcriptomic or genomic changes induced by environmental contaminants [10,11]. Metabonomics has been used to determine the effects of environmental pollutants on organisms including fish [12–14], earthworm [15,16] and shellfish [17,18]. However, *in situ* exposure experiments on mammal are still relatively unexplored. The present study was designed to evaluate effects of drinking water exposure and identification of potential biomarkers for early warning of environmental health impacts on mice. Mice were exposed to drinking water for 90 days, and metabonomic changes and serum biochemical as well as hepatic histopathological parameters were determined.

2. Materials and methods

2.1. Drinking water sample

Drinking water given to the mice was sampled from tap water in Wuxi City supplied by Taihu Lake. The organic components in Taihu drinking water were measured by DSQ II Single Quadruple GC/MS (ThermoQuest, San Jose, CA, USA) with selected ion monitoring (SIM) mode. Metal ions were detected by ICP-OES (ICP-J-A1100, Jarell-Ash Inc., USA).

2.2. Animals and treatments

A total of 16 male *Mus musculus* mice weighing 18 ± 1 g were purchased from Animal Center of Academy of MMS Laboratory. The mice were maintained in a 12-h light/12 h dark cycle at 25 °C and 50% humidity, with free access to food and water. After two week acclimatization, the mice were randomly divided into two groups: the control group (n = 8, treated with distilled water) and the drinking water-treated group (n = 8, treated with Taihu drinking water). The mice were weighed each week and the amount of water consumed was recorded. After exposed to Taihu drinking water for 90 days, all the mice were slaughtered and the serum was collected by centrifugation and frozen at −80 °C until analysis.

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doi:10.1016/j.jhazmat.2011.03.077

2.3. Serum biochemistry test

Biochemical indices of serum samples were analyzed using Olympus 2700 analyzer (Olympus Co., Japan) including albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), globulin (GLB), high density lipid-cholesterol (HDL-C), lactate dehydrogenase (LDH), low density lipid-cholesterol (LDL-C), cholesterol (CHO), and triglyceride (TG) [19–21].

2.4. Hepatic histopathology

The mouse liver was removed, washed in normal saline, fixed in 10% formalin for at least 12 h, and sectioned at 5-μm thickness. Tissue slides were subsequently stained with haematoxylin and eosin (H&E), and observed under an optical microscope [22,23].

2.5. Sample preparation for NMR

The stored serum samples were thawed at room temperature. 75 μL of buffer solution (0.2 M Na_2HPO_4/0.2 M NaH_2PO_4) and 75 μL of D_2O were added to serum sample at a final volume of 500 μL. The mixed sample was transferred into 5-mm NMR tubes.

2.6. ^1H NMR spectra

The ^1H NMR spectra of serum samples were acquired on a Bruker AV600 spectrometer (Bruker, Germany) at 298 K. Water signals, proteins and lipoprotein resonances were suppressed by Carr–Purcell–Merbom–Gill (CPMG) spin-echo pulse sequence (RD-90°-(τ-180°-τ)-ACQ) with a total spin-echo delay ($2n\tau$) of 40 ms. 32 free induction decays (FIDs) were collected into 64,000 data points over a 8992.8 Hz spectral width with a relaxation delay of 5 s and an acquisition time of 3.64 s. The Fourier transformation algorithm was applied to the exponential line broadening of 0.3 Hz and all the spectra were referenced to the $-CH_3$ resonance of creatine at δ3.04. The metabolite resonances were identified according to previous studies [24,25].

2.7. NMR spectral data processing

After manually phased and corrected for baseline distortion using MestRec 4.9 software (http://www.mestrec.com/), each spectrum was segmented into 2000 regions of equal width 0.005 ppm, corresponding to the regions between δ0–10.0 ppm. The area for each region was calculated and the integral values were presented on an intensity distribution curve describing the whole spectrum. Regions between 5.0 and 4.5 ppm containing water resonances were removed. The remaining spectral regions were scaled to total spectral area.

2.8. Pattern recognition

The obtained data sets were centered and scaled to unit variance, and then were introduced into SIMCA-P 11.5 software (Umetric, Umeå, Sweden) for multivariate analysis. The NMR data were first analyzed by principal component analysis (PCA). The partial least-squares discriminant analysis (PLS-DA) model was established to discriminate the patterns of the metabolic alterations between the groups. To optimize the clustering of samples and select potential metabolic biomarkers, PLS-DA model with orthogonal signal correction (OSC) prefiltering was therefore used to remove variance unrelated to clustering. The data processing and model validation method used in this study was previously described in detail [26,27]. The manners of the metabolic variances were identified by the score plots and the metabolites associated with the group separations indicated by the coefficients in the coefficients plots.

Table 1
Concentrations of organic components (ng/L) and metal irons (μg/L) in Taihu drinking water.[a]

Component	Concentration	Component	Concentration
Acenaphthylene	1.49 ± 0.53	Pyrene	45.08 ± 0.49
Anthracene	54.14 ± 2.14	Al	0.021 ± 0.001
Benzo(a)anthracene	0.70 ± 0.33	Ba	0.093 ± 0.001
Benzo(b)fluoranthene	1.33	Ca	44.03 ± 0.44
Benzo(k)fluoranthene	0.83	Cu	<0.002
Chrysene-d12	1.34 ± 0.74	Fe	0.021 ± 0.001
Fluorene	559.04	K	6.26 ± 0.07
Phenanthrene	3.45 ± 0.29	Mg	9.74 ± 0.08

[a] Values are presented as means and standard deviations.

3. Results and discussion

3.1. Characteristics of Taihu drinking water

A total of nine polycyclic aromatic hydrocarbons (PAHs) were detected in the drinking water (Table 1). The total concentration of these PAHs was 667.4 ng/L much higher than the water quality standard of U.S. EPA (<200 ng/L). The drinking water may be of potential health risk with these organic pollutants existing. Metal ions were all lower than the standard of EPA (http://water.epa.gov/drink/contaminants).

3.2. Water intake and body weight

No death was observed during the whole experimental period. Water consumption was 83.3 and 85.6 ml day^{-1} in the treated group and control group, respectively. No significant differences were observed between the water intakes of the two groups ($p > 0.05$). In addition, drinking water treatment caused no significant variations in body weight during the whole exposure period. Wu et al. also found that no significant changes in water intake and body weight of mice were induced after exposure to drinking water for 90 days [28].

3.3. Serum biochemistry and histopathology

Table 2 shows the biochemistry parameters of the treatment group and control in day 90. The results indicated that no significant differences ($p > 0.05$) were observed in both groups and this is consistent with the studies of Wu et al. [28]. The results of histopathology test are shown in Fig. 1. Although, the control mice displayed a little cell swelling, the mice exposed to drinking water showed a wide area of hepatocellular hypertrophy.

Table 2
Effect of Taihu drinking water on selected biochemistry parameters.[a]

Parameters	Control	Taihu drinking water
Albumin (g/L)	29.3 ± 1.2	29.8 ± 2.5
Alkaline phosphatase (U/L)	54.1 ± 12.1	60.0 ± 13.7
Alanine aminotransferase (U/L)	79.8 ± 15.2	84.8 ± 14.9
Aspartate aminotransferase (U/L)	126.8 ± 23.5	166.1 ± 31.2
Creatinine (μmol/L)	22.4 ± 1.9	23. 8 ± 1.6
Globulin (g/L)	26.8 ± 2.1	24.7 ± 1.9
High density lipid-cholesterol (mmol/L)	1.9 ± 0.2	1.7 ± 0.2
Lactate dehydrogenase (U/L)	789.1 ± 62.2	776.7 ± 56.2
Low density lipid-cholesterol (mmol/L)	0.6 ± 0.1	0.5 ± 0.1
Cholesterol (mmol/L)	2.9 ± 0.3	2.7 ± 0.4
Triglyceride (mmol/L)	3.1 ± 0.7	2.7 ± 0.9

[a] Values are presented as means and standard deviations.

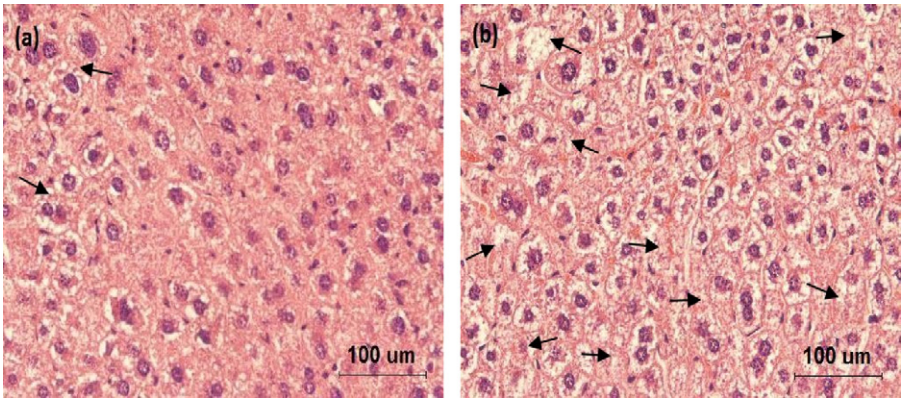

Fig. 1. Transverse tissue slice of mice livers in the control group (a) and drinking water-treated group (b). Arrow, cell swelling.

3.4. ^{1}H NMR spectra and pattern recognition

Fig. 2 shows representative ^{1}H NMR spectra of drinking water group and control group. The NMR bins from 1.7 ppm to 3.3 ppm showed significantly different peak intensities between the two groups ($p < 0.05$). Hence, these bins were selected for PCA and PLS-DA calculation.

Unsupervised PCA model was used in this study to determine the patterns of metabolic changes in serum related to the effects of Taihu drinking water (Fig. 3). In the PCA score plot, each point represents individual serum sample of mouse. A clear separation between control and treated samples was evident along PC1, and this model explained 61.16% of the variability among the samples.

To focus on the treatment related differences, supervised PLS-DA modeling was therefore performed on the spectra of serum samples from control and drinking water groups. Fig. 4a shows the score plot of PLS-DA based on the first two components. In the plot, control samples clustered together in the low left while the treated samples locate in the top right. The validation of the model was evaluated by the default leave-one-out procedure with the parameters of R2X, R2Y and Q2. In this PLS-DA model, the R2X and R2Y contributed 0.514 and 0.774 to the matrix, respectively (Fig. 6). More than half of the original data could be explained by the model. However, the Q2 of the model was 0.435, and the PLS-DA model needed a further modification for validation.

In order to remove the changed signals which were not related to the samples clustering, OSC filtering was performed before PLS-DA in this study. Fig. 4b shows that the samples between control and treatment groups were successfully separated. This model was more applicable and had a predictability value than that of PLS-DA without OSC (R2X = 0.535, R2Y = 0.998 and Q2 = 0.988) (Fig. 6).

In this study, coefficient plots of PLS-DA and OSC-PLS-DA were also calculated to determine the dominant metabolites influencing the differentiation between control and treatment group (Fig. 5). The results show that OSC filtration can remove the interference signals for the classification. Coefficient plots were used to locate key bins in the NMR spectra. Important bins of interest were chosen on the basis of centered and scaled coefficients (CoeffCS). Bins of interest in multivariate data were selected with a CoeffCS more than 1.5 [29]. Many metabolites responsible for differentiation were reduced after exposure, including pyruvate (δ2.38), glutamine (δ2.46), arginine (δ1.89), lysine (δ1.72), N-acetyl glycoproteins (δ2.04), choline (δ3.21) and citrate (δ2.55 and δ2.69) (Table 3). Positive CoeffCS indicate an increase of the metabolites and negative CoeffCS represent a decrease of the metabolites after drinking water treatment (Fig. 5).

CoeffCS of both pyruvate and citrate were negative indicating low level in serum. Pyruvate and citrate are important intermediates for tricarboxylic acid (TCA) cycle which is crucial for energy metabolism [30,31]. Decreased citrate is always related to hepatotoxicity induced by xenobiotic such as polychlorinated biophenyl (PCB) [32], Ce(NO_3)(3) [33] and tributyl phosphate [34]. The decreased levels of pyruvate and citrate may be induced by

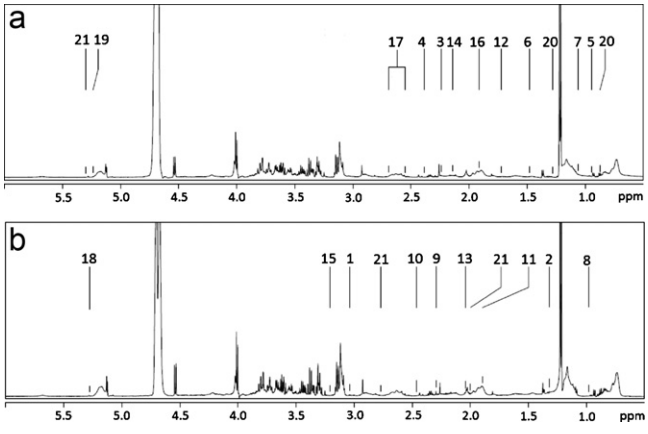

Fig. 2. Representative one-dimensional ^{1}H NMR spectra of serum samples from (a) control group and (b) drinking water group. Key: 1. creatine, 2. lactate, 3. acetone, 4. pyruvate, 5. leucine, 6. alanine, 7. valine, 8. isoleucine, 9. acetoacetate, 10. glutamine, 11. arginine, 12. lysine, 13. N-acetyl glycoproteins, 14. methionine, 15. choline, 16. acetate, 17. citrate, 18. TMAO, 19. α-glucose, 20. LDL/VLDL, 21. lipids.

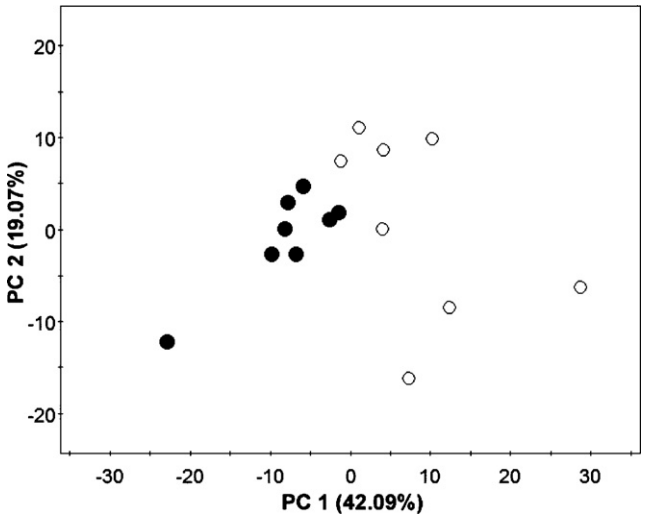

Fig. 3. PCA score plot showing samples between control and Taihu drinking water group (● control, ○ drinking water group).

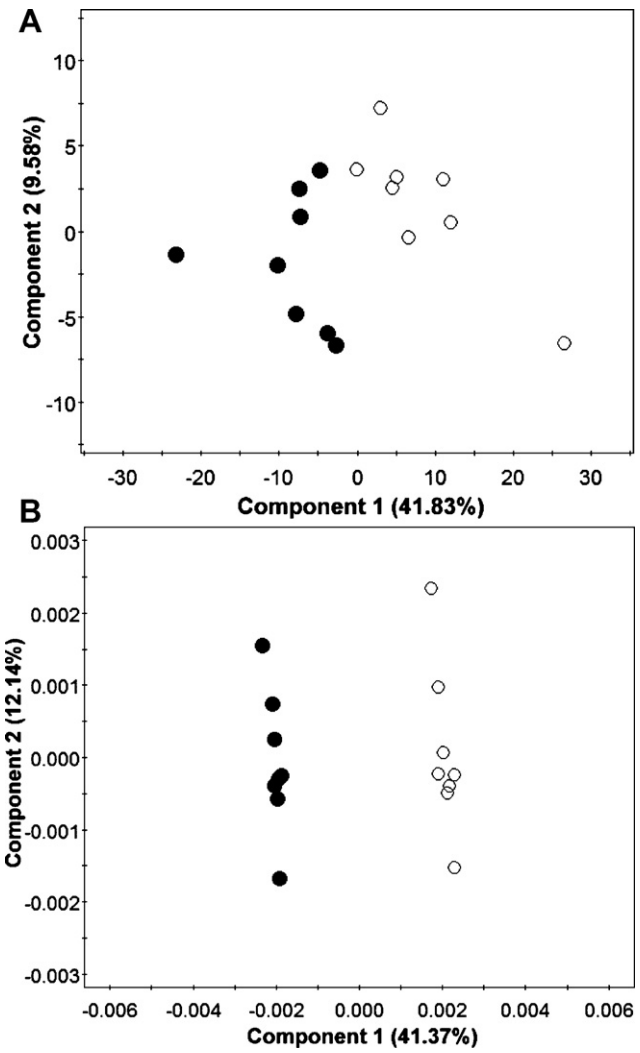

Fig. 4. Pattern recognition analyses of serum ^{1}H NMR spectra (a) PLS-DA score plot and (b) OSC-PLS-DA score plot (● control, ○ drinking water group).

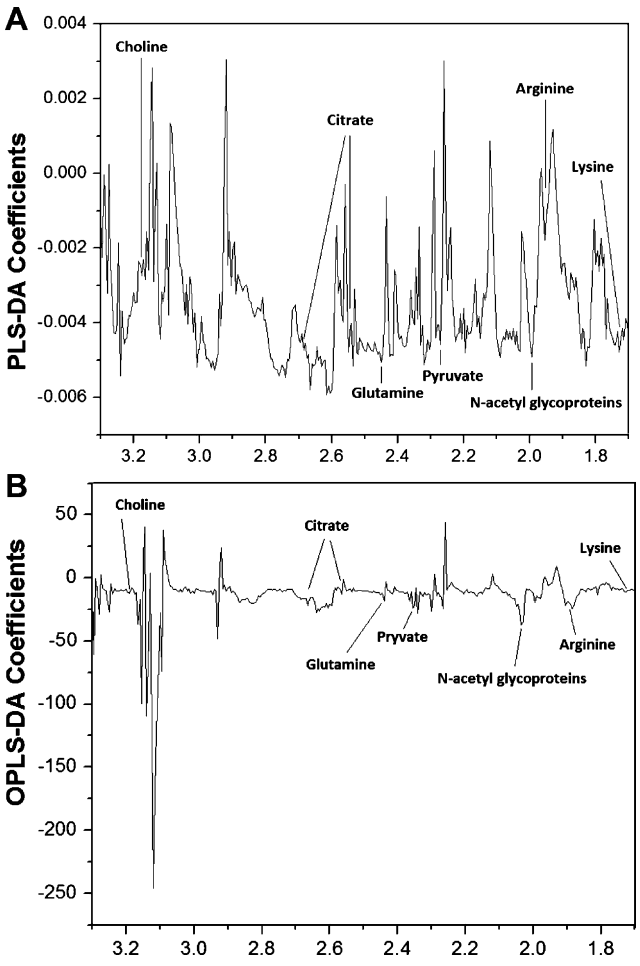

Fig. 5. Coefficient plots of PLS-DA model from control and treatment samples before and after OSC. (a) Coefficient plot of PLS-DA before OSC; (b) coefficient plot of PLS-DA after OSC.

the organic pollutants in the Taihu drinking water. In addition, the reduced glutamine (CoeffCS = −11.64) can result in inhibition of citric acid cycle followed by metabolic disorders of energy and amino acid [35,36].

The CoeffCS of arginine and lysine were −20.51 and −10.93, respectively. Previous metabonomics study indicated that liver cancer could induce disorder of arginine metabolism [37]. Furthermore, PAH could induce significant effects on metabonome of earthworm and lysine was identified as one of the potential response indicators of PAH exposure [16,38,39]. Decreased arginine and lysine indicated that normal amino acid metabolism was disordered which may be induced by the PAHs existing in the drinking water.

Table 3
Primary metabolites most contribute to classification.

Metabolite	Chemical shift (ppm)[a]	CoeffCS [1][b]
Pyruvate	2.38 (s)	−12.49
Glutamine	2.46 (m)	−11.64
Arginine	1.89 (m)	−20.51
Lysine	1.72 (m)	−10.93
N-acetyl glycoproteins	2.04 (s)	−26.51
Choline	3.21 (s)	−10.47
Citrate	2.55 (d), 2.69 (d)	−10.53, −15.10

[a] Key: s: singlet; d: doublet; m: multiplet.
[b] The centered and scaled coefficients (CoeffCS) > 1.5 is used for the selection of bins of interest in multivariate data.

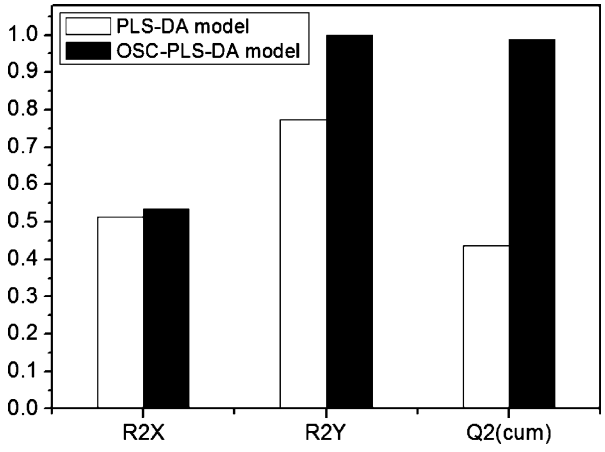

Fig. 6. Validation of PLS-DA model and OPLS-DA model. Validation of the model was conducted by the default leave-one-out procedure. R2X and R2Y represent the percentages of original X and Y data sets used to construct PLS-DA model. Higher R2X and R2Y indicate that more original data are represented. Q2 reflects the predictive capacity of the model, and higher Q2 means better predictability of the model is.

Taihu drinking water was found to induce decreased serum choline (CoeffCS = −10.47) of the male mice, which may be due to the apoptosis of hepatocyte [40,41]. This is in consistence with the molecular toxicity induced by drinking water, which was detected by gene chip in our previous study [28].

4. Conclusions

Taihu drinking water was found to have adverse health effects on mice, including perturbations of energy metabolism, amino acid metabolism and apoptosis. To better understand the mechanism of these effects, in-depth investigations should be performed to analyze effects induced by the individual compounds present in the Taihu drinking water. In addition, this study shows that metabonomics is useful in evaluating the health risk of drinking water.

Acknowledgements

This work was financially supported by Major State Water Pollution Control and Treatment Technique Program of China [2008ZX07528-005] and Social Development Plan of Science and Technology for Jiangsu Province of China [BE2009668].

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