Int J Clin Exp Med 2016;9(11):21514-21520
www.ijcem.com /ISSN:1940-5901/IJCEM0027985

Original Article

Metabolic changes in rat lung after acute paraquat poisoning by gas chromatography-mass spectrometry

Shuanghu Wang[1*], Lijing Zhang[2*], Xianqin Wang[3], Zhiyi Wang[4], Congcong Wen[2], Jianshe Ma[2], Zhimou Gao[3], Lufeng Hu[5]

[1]The Laboratory of Clinical Pharmacy, The People's Hospital of Lishui, Lishui 323000, China; [2]Analytical and Testing Center, Wenzhou Medical University, Wenzhou, China; [3]College of Plant Protection, Anhui Agricultural University, Hefei, China; [4]The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China; [5]The First Affiliated Hospital, Wenzhou Medical University, Wenzhou, China. [*]Equal contributors.

Received December 2, 2015; Accepted July 4, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Paraquat is one of the most widely used herbicides in the world and is highly toxic to humans and animals. Respiratory failure as a result of lung injury is the most common cause of death from paraquat. In this study, we developed a lung metabolomic method by gas chromatography-mass spectrometry (GC-MS) to evaluate the effect of acute paraquat poisoning on rats. The acute paraquat poisoning group rats were given 36 mg/kg of paraquat by intragastric administration. Pattern recognition analysis, including both principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) revealed that acute paraquat poisoning induced metabolic perturbations. Compared to the control group, the level of (Z,Z)-9,12-octadecadienoic acid, arachidonic acid, 11-trans-octadecenoic acid in lung of acute paraquat poisoning group increased, while the level of butanedioic acid, phthalic acid, d-galactose decreased. The results indicate that metabolomic methods by GC-MS may be useful to elucidate acute paraquat poisoning through the exploration of biomarkers. According to the pathological changes of lung, there was obviously destroyed and collapsed alveolar structure in acute paraquat poisoning group.

Keywords: Metabolomics, GC/MS, paraquat, lung, poisoning, rat

Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) dichloride is a nonselective contact herbicide, which is used widely in many countries. The fatality rate of paraquat intoxication remains high due to the lack of an effective treatment [1]. The lung is a major target organ during paraquat poisoning, which is characterized by edema, hemorrhage, interstitial inflammation, and bronchial epithelial cell proliferation [2]. Respiratory failure as a result of lung injury is the most common cause of death from paraquat. The inflammatory reaction has been reported to be the main mechanism underlying paraquat -induced acute lung injury [3].

Metabolomics is widely used in life sciences and other fields such as safety evaluation, drug development, toxicity screening markers, disease diagnosis, gene function currently [4, 5]. In recent years, metabolomics has been widely applied to uncover biomarkers [6] and metabolic fingerprint in drug discovery and clinical toxicology [7], especially to investigating systematic metabolic responses to toxins [8] and the associated mechanisms [9]. The primary goal of this study is to study systematically the metabolic pathway changes induced by acute paraquat poisoning in rat lung.

Material and methods

Chemicals and animals

Trimethylchlorosilane (TMCS) and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich (Shanghai, China). Pyridine and methylhydroxylamine hydrochloride were purchased from Aladdin Industrial, Inc. (Shanghai, China). HPLC-grade n-heptane and acetonitrile were purchased

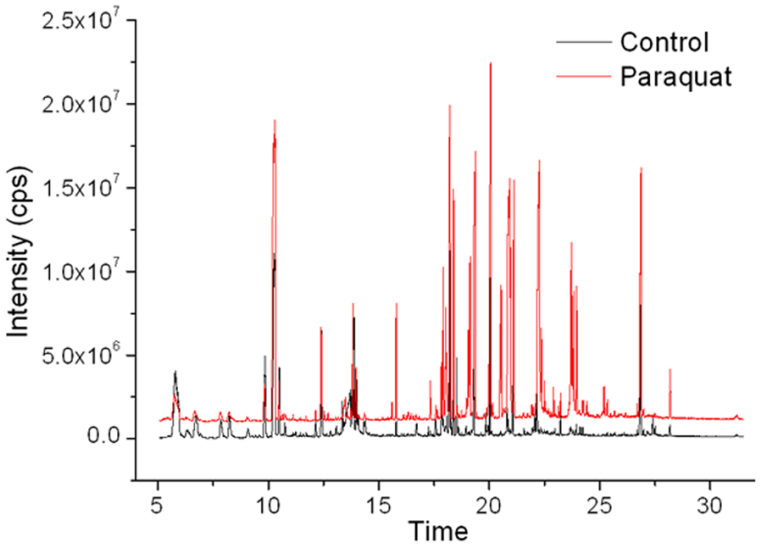

Figure 1. Typical GC-MS total ion chromatogram of in rat lung after acute paraquat poisoning.

Table 1. Summary of the changes in relative levels of metabolites in rat lung after acute paraquat poisoning

NO.	Renten time/min	Metabolite	VIP	Level
1	13.5232	Butanedioic acid	5.13616	↓,**
2	18.2481	d-Mannose	4.89435	-
3	5.75529	Propanoic acid	4.00141	-
4	20.8612	(Z,Z)-9,12-Octadecadienoic acid	3.8805	↑,**
5	22.2271	Arachidonic acid	3.659	↑,**
6	18.5105	Phthalic acid	3.54545	↓,**
7	21.735	beta.-D-Glucopyranuronic acid	3.27586	-
8	20.1483	d-Galactose	3.18554	↓,**
9	18.5771	d-Glucose	2.7115	-
10	10.3612	l-Threonine	2.63979	-
11	20.9092	11-trans-Octadecenoic acid	2.06262	↑,**

Note: Variable importance in the projection (VIP) was acquired from the PLS-DA model with a threshold of 2.0. Marks indicate the direction of the change, i.e. ↓ for decrease, ↑ for increase, - for no change. Compared acute paraquat poisoning group with control group, **P<0.01, as indicated by the statistical analysis T-test.

from Tedia Reagent Company (Shanghai, China). Sprague-Dawley rats (male, 220±20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd.

Instrumentation and conditions

Agilent 6890N-5975B GC/MS, HP-5MS (0.25 mm×30 m×0.25 mm), were purchased from Agilent Company (Santa Clara, California, USA). The GC oven was initially setted at 80°C and was kept at this temperature for 5 minutes. The temperature was then gradually increased to 260°C at a rate of 10°C/min, and then kept at 260°C for 10 minutes. Mass detection was conducted first in EI mode with electron energy of 70 eV, then in full-scan mode with m/z 50-550, and finally, by splitless mode injection [10, 11].

Sample preparation

The 300 μL of acetonitrile was added to 100 mg of lung tissue, the mixture was stored at -80°C for 20 min then grinded for 2 min by a SCIENTZ-48 Tissue Grinder, which the grinding parameter was 64 Hz and 1800 r/s. The tubes were vortex mixed for 1.0 min, then kept in an ice-bath for 15 minutes, and then 10,000 *g* were centrifuged for 10 minutes at 4°C. The 200 μL of the supernatant was transferred to a GC vial and evaporated to dryness under a stream of nitrogen gas. Methoximation was carried out at 70°C for 24 h after 50 μL of methylhydroxylamine hydrochloride (15 mg/mL in pyridine) was added. The 50 μL MSTFA (with 1% TMCS as the catalyst) was added and kept at 70°C for another hour, and then vortexed after adding 150 μL n-heptane [11].

Metabolomics study

Rats were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All forty rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. All experimental procedures were approved ethically by the Administration Committee of Experimental Animals of Wenzhou Medical University.

Forty rats (220±20 g) were randomly divided to acute paraquat poisoning group and control group. Acute paraquat poisoning group rats were given paraquat (36 mg/kg) by intragastric administration, control group were given saline.

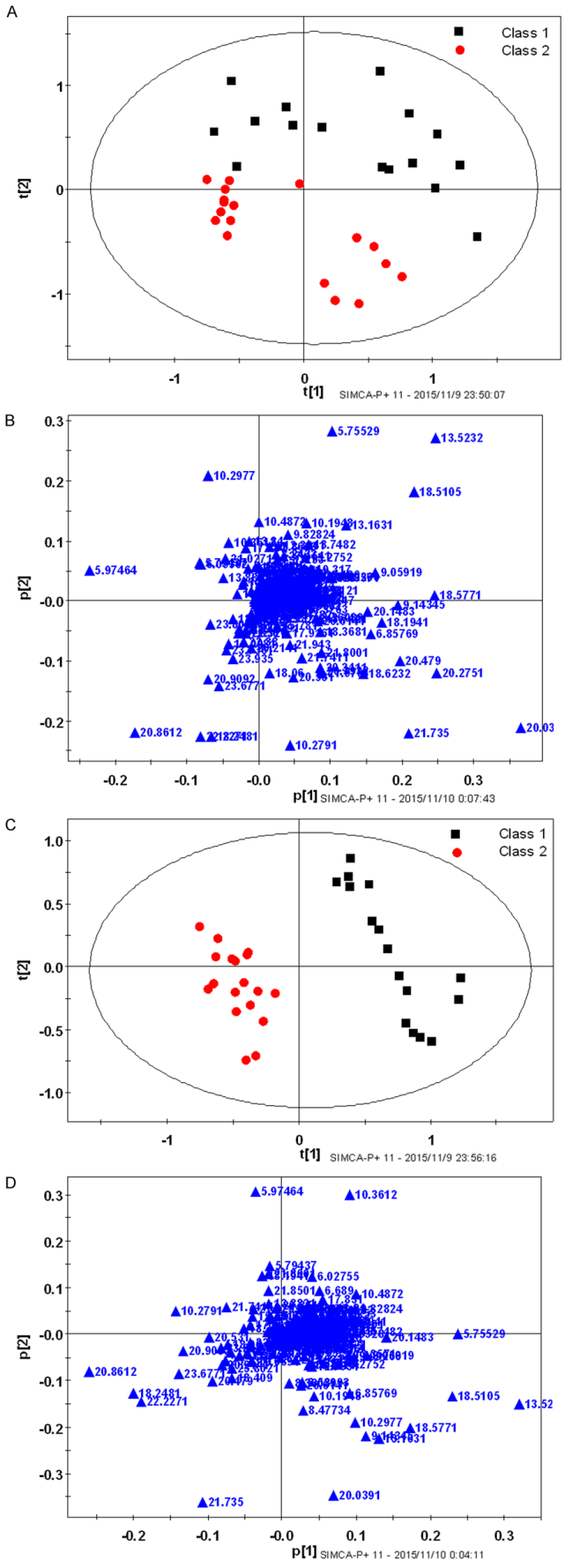

Figure 2. PCA score results of rat lung samples (A), the corresponding load diagram (B); PLS-DA score results of rat lung samples (C), the corresponding load diagram (D) after acute paraquat poisoning (36 mg/kg, Class 2), Control (Class 1).

Lung samples were collected from the rats from the control group and acute paraquat poisoning group at 8:00 am after 3 days, respectively. The lung was stored at -80°C.

Biochemical tests

The blood was collected from the tail vein for biochemical tests of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (Cr), uric acid (UA), albumin (ALB) from the control group and acute paraquat poisoning group at 8:00 am after 3 days. Serum samples were analyzed to measure ALT, AST, ALP, Cr, UA and ALB, which was used to evaluate the liver and kidney function.

Histopathology

After metabolomics experiment, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The lung were rapidly isolated and immersed in freshly prepared 4% w/v formaldehyde (0.1 M phosphate buffers, pH 7.2) for 48 h and then embedded in paraffin. Then 4-μm-thick histologic sections were prepared and stained with hematoxylin and eosin (HE). The morphological changes were observed under light microscope.

Data analysis

The GC/MS data was exported into Microsoft Excel, with the peaks normalized to the total sum of spectrum prior to multivariate analyses. The resulting data was processed through principal component analysis (PCA) and partial least squares discriminate analysis (PLS-DA) using SIMCA-P 11.5 software (Umetrics, Umea, Sweden).

Statistical analysis

Statistical analysis was carried out using SPSS software (Version 18.0, SPSS).

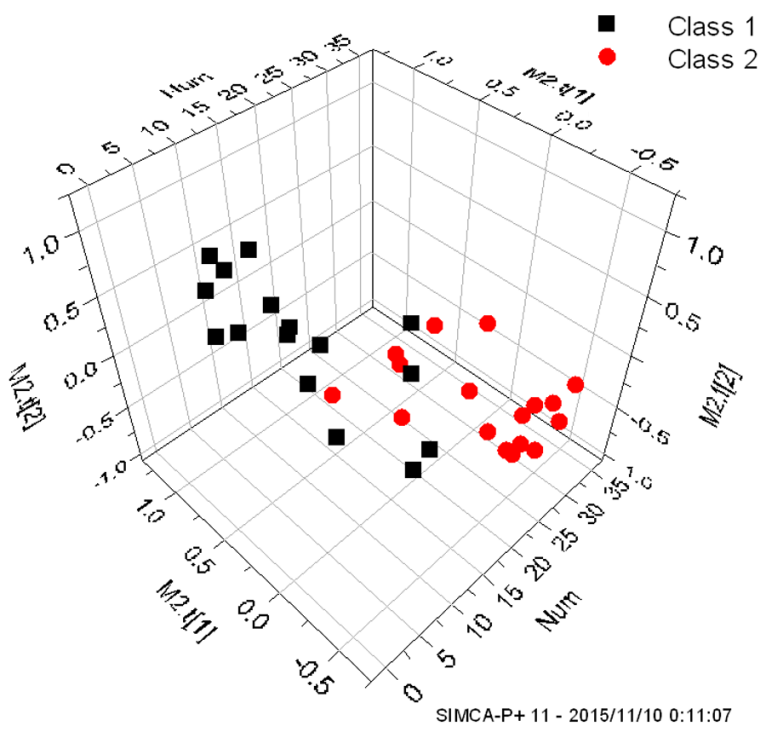

Figure 3. PLS-DA 3D score results of rat lung samples after acute paraquat poisoning (36 mg/kg, Class 2), Control (Class 1).

Independent samples T-test was applied in order to detect significant differences in all metabolites between two groups. A *P* value of <0.05 was considered statistically significant.

Results and discussion

Metabolomics study

Metabolomics is a newly emerging omics approach to the investigation of metabolic phenotype changes induced by environmental or endogenous factors [12-16]. It has shown promising results in healthcare fields, especially in disease diagnosis and drug-toxicity assessment, as reviewed recently [17, 18]. In drug-toxicity assessment, metabolomics is often concerned with finding toxicity-related biomarkers by investigating the changes in metabolic signatures induced by drug exposure [10, 19].

Figure 1 provides the typical metabolic profiles of lung acquired through GC-MS technique. Metabolic profile data pretreatment resulted in a final dataset consisting of sixty metabolic features from GC-MS analyses. The endogenous metabolites in the lung were identified using the NIST 2005 mass spectrometry database.

In this study, the changes of metabolites between acute paraquat poisoning groups and their control group were shown in **Table 1**.

In order to explore the metabolic profile changes after acute paraquat poisoning, we compared the GC-MS spectrum of PCA and PLS-DA of the paraquat with the rats in the control group (**Figure 2**). **Figure 2A** PCA and **Figure 2C** PLS-DA score chart showed that the first principal components of the rats in the acute paraquat poisoning group were distinguished from the rats in the control group, the corresponding load diagram were **Figure 2B** and **2D**. PLS-DA 3D (**Figure 3**) score chart showed that the rats in acute paraquat poisoning group were distinguished from the rats in the control group.

Biochemical tests

There is significant difference between control group and acute paraquat poisoning group for biochemical results, **Table 2**. ALT, AST, ALP, Cr, UA and ALB, which was used to evaluate the liver and kidney function. As shown in **Table 2**, the ALT, AST, ALP, Cr, UA of rats was significantly higher in acute paraquat poisoning group than that in the control group (P<0.05 or P<0.01). The results showed acute paraquat poisoning induced kidney and liver function damage.

Morphological changes of lung

The morphology of lung was significant changed in the acute paraquat poisoning group and control-group, according to hematoxylin and eosin staining method. In control group, the alveolar structure was intact, alveolar walls were thin and no macrophages infiltrated in the alveolar septa and alveolar cavities. While, there was obviously destroyed and collapsed alveolar structure in acute paraquat poisoning group. Besides, there was sign of the patch shaped fibrosis, interlobular septal thickening, and the lung interstitium increased in acute paraquat

Table 2. Biochemical results of control group and acute paraquat poisoning group rats (mean ± SD, n=8)

Group	Alanine aminotransferase (ALT)	Aspartate aminotransferase (AST)	Alkaline phosphatase (ALP)	Creatinine (Cr)	Uric Acid (UA)	Albumin (ALB)
Control	46	188	237	18	87	28
Paraquat	67**	286**	274**	20*	90*	24

Compared acute paraquat poisoning group with control group, *P<0.05 and **P<0.01, as indicated by the statistical analysis T-test.

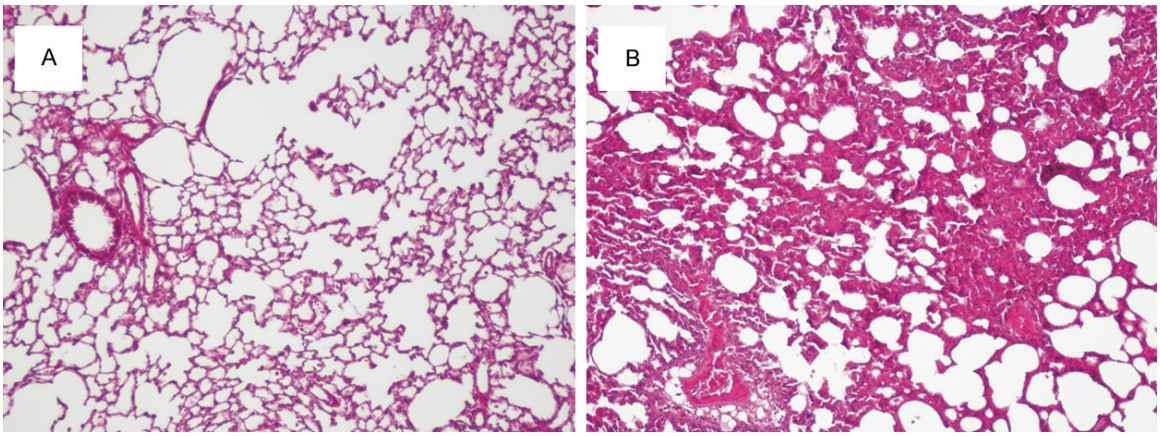

Figure 4. Morphological changes of lung in control-group (A) and acute paraquat poisoning group (B) (hematoxylin-eosin, ×100).

poisoning group. The morphological changes of lung were showed in **Figure 4**.

Changes in metabolite

Metabolomics comprises the measurement of endogenous metabolites, including amino acids, nucleic acid precursors, lipids, and degradation products of chemical intermediates in catabolism and biosynthesis. The advantage of metabolomics is that it provides the most functional measure of cellular status and can help to describe an organism's phenotype [20].

In this study, the changes of metabolites between acute paraquat poisoning groups and their control group were shown in **Table 1**. Compared to the control group, the level of (Z,Z)-9,12-octadecadienoic acid, arachidonic acid, 11-trans-octadecenoic acid in lung of acute paraquat poisoning group increased, while the level of butanedioic acid, phthalic acid, d-galactose decreased. These finding may be useful for new evidences in acute paraquat poisoning study. Additional prospective studies will be required to better understand these observations.

The HE-staining examination showed that the morphology of lung was significant changed in the rat of acute paraquat poisoning group. The alveolar structure was destroyed and collapsed, and there was significant pulmonary interstitial fibrosis, such as interstitial cells proliferated and interlobular septal thickened. This change restricted pulmonary ventilation and diffusive dysfunction which resulted in hypoxemia in blood circulation system and tissue metabolism. The tricarboxylic acid (TCA) cycle, or the Krebs cycle is the main energy metabolism way to metabolize carbohydrates, fats and proteins into carbon dioxide and chemical energy. Although oxygen does not participate directly in the TCA, the cycle operates only when O_2 is present. Therefore, in some hypoxemia conditions, the TCA will be inhibited.

The butanedioic acid is a intermediate products in TCA. It will be deceased when TCA is inhibited. When the TCA is inhibited, another energy metabolism way, anaerobic glycolysis will be activated. The anaerobic glycolysis is the anaerobic catabolism of glucose. The efficiency of energy productivity is lower than TCA.

Therefore it will consume more d-galactose transfer into anaerobic glycolysis to generate energy, which decreased the concentration of d-galactose. In this respect, there is consistency between results of HE and GC-MS.

Conclusion

These biomarkers ((Z,Z)-9,12-octadecadienoic acid, arachidonic acid, 11-trans-octadecenoic acid, butanedioic acid, phthalic acid, d-galactose) in rat lung were the additional evidence in the acute paraquat poisoning study. We demonstrated that metabolomic methods by GC-MS could provide a useful tool for exploring biomarkers to elucidate toxicity. According to the pathological changes of lung, there was obviously destroyed and collapsed alveolar structure in acute paraquat poisoning group.

Acknowledgements

This study was supported by grants from the Zhejiang Provincial Natural Science Foundation of China, No. LY14H230001 and LY15H150008; Students Science and Technology Innovation Activities and Xinmiao Talents Program of Zhejiang Province, No. 2015R413033; National Natural Science Foundation of China, No. 81401558.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhimou Gao, College of Plant Protection, Anhui Agricultural University, Hefei 230036, China. Tel: +86 551 65786322; Fax: +86 551 65786103; E-mail: gaozhimou@126.com; Dr. Lufeng Hu, The First Affiliated Hospital, Wenzhou Medical University, Wenzhou, China. Tel: +86 577 55579706; E-mail: hulufeng@163.com

References

[1] Liu ZN, Zhao M, Zheng Q, Zhao HY, Hou WJ and Bai SL. Inhibitory effects of rosiglitazone on paraquat-induced acute lung injury in rats. Acta Pharmacol Sin 2013; 34: 1317-1324.

[2] Venkatesan N. Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci 2000; 66: PL21-28.

[3] Orito K, Suzuki Y, Matsuda H, Shirai M and Akahori F. Chymase is activated in the pulmonary inflammation and fibrosis induced by paraquat in hamsters. Tohoku J Exp Med 2004; 203: 287-294.

[4] Griffin JL and Bollard ME. Metabonomics: its potential as a tool in toxicology for safety assessment and data integration. Curr Drug Metab 2004; 5: 389-398.

[5] Nicholson JK and Lindon JC. Systems biology: Metabonomics. Nature 2008; 455: 1054-1056.

[6] Boudonck KJ, Mitchell MW, Nemet L, Keresztes L, Nyska A, Shinar D and Rosenstock M. Discovery of metabolomics biomarkers for early detection of nephrotoxicity. Toxicol Pathol 2009; 37: 280-292.

[7] Beger RD, Sun J and Schnackenberg LK. Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity. Toxicol Appl Pharmacol 2010; 243: 154-166.

[8] Zhang L, Ye Y, An Y, Tian Y, Wang Y and Tang H. Systems responses of rats to aflatoxin B1 exposure revealed with metabonomic changes in multiple biological matrices. J Proteome Res 2011; 10: 614-623.

[9] Huang Q, Tan Y, Yin P, Ye G, Gao P, Lu X, Wang H and Xu G. Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. Cancer Res 2013; 73: 4992-5002.

[10] Wang X, Zhang M, Ma J, Zhang Y, Hong G, Sun F, Lin G and Hu L. Metabolic changes in paraquat poisoned patients and support vector machine model of discrimination. Biol Pharm Bull 2015; 38: 470-475.

[11] Zhang M, Deng M, Ma J and Wang X. An evaluation of acute hydrogen sulfide poisoning in rats through serum metabolomics based on gas chromatography-mass spectrometry. Chem Pharm Bull (Tokyo) 2014; 62: 505-507.

[12] Patti GJ, Yanes O and Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 2012; 13: 263-269.

[13] Deng M, Zhang M, Huang X, Ma J, Hu L, Lin G and Wang X. A gas chromatography-mass spectrometry based study on serum metabolomics in rats chronically poisoned with hydrogen sulfide. J Forensic Leg Med 2015; 32: 59-63.

[14] Deng M, Zhang M, Sun F, Ma J, Hu L, Yang X, Lin G and Wang X. A gas chromatography-mass spectrometry based study on urine metabolomics in rats chronically poisoned with hydrogen sulfide. Biomed Res Int 2015; 2015: 295241.

[15] Wen C, Zhang M, Ma J, Hu L, Wang X and Lin G. Urine metabolomics in rats after administration of ketamine. Drug Des Devel Ther 2015; 9: 717-722.

[16] Wen C, Zhang M, Zhang Y, Sun F, Ma J, Hu L, Lin G and Wang X. Brain metabolomics in rats after administration of ketamine. Biomed Chromatogr 2016; 30: 81-4.

[17] Monte AA, Heard KJ and Vasiliou V. Prediction of drug response and safety in clinical practice. J Med Toxicol 2012; 8: 43-51.
[18] Mamas M, Dunn WB, Neyses L and Goodacre R. The role of metabolites and metabolomics in clinically applicable biomarkers of disease. Arch Toxicol 2011; 85: 5-17.
[19] Kwon H, Park J, An Y, Sim J and Park S. A smartphone metabolomics platform and its application to the assessment of cisplatin-induced kidney toxicity. Anal Chim Acta 2014; 845: 15-22.
[20] Hong JH, Lee WC, Hsu YM, Liang HJ, Wan CH, Chien CL and Lin CY. Characterization of the biochemical effects of naphthalene on the mouse respiratory system using NMR-based metabolomics. J Appl Toxicol 2014; 34: 1379-1388.