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Metabonomics study on nephrotoxicity induced by intraperitoneal and intravenous cisplatin administration using rapid resolution liquid chromatography coupled with quadruple-time-of-flight mass spectrometry (RRLC-Q-TOF-MS)

Yubo Li,[§a] Xiuxiu Zhang,[§a] Huifang Zhou,[§b] Simiao Fan,[a] Yuming Wang,[a] Lu Zhang,[a] Liang Ju,[a] Xin Wu,[a] Huanyu Wu[a] and Yanjun Zhang[*a]

[a]Tianjin State Key Laboratory of Modern Chinese Medicine, School of Traditional Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, 312 Anshan west Road, Tianjin 300193, China.

[b]Department of experimental teaching, Tianjin University of Traditional Chinese Medicine, 88 Yuquan Road, Tianjin 300193, China.

[*]Author for correspondence:

e-mail: tianjin_tcm001@sina.com.

Tel and Fax number: +86-22-59596223.

[§]Yubo Li, Xiuxiu Zhang and Huifang Zhou contributed equally to the work as co-first authors.

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Abstract

Cisplatin is a well-known chemotherapeutic agent in cancer therapy. It is commonly administered intraperitoneally and intravenously in the clinic. The use of cisplatin is limited by its side effects, particularly its nephrotoxicity. In this study, Mass spectrometry-based metabonomics coupled with multivariate statistical analysis were used to find biomarkers of kidney injury and further applied to investigate on the disturbed metabolic pathways, which were induced by single intraperitoneal or intravenous injection of cisplatin to rats with the dosage of 6 mg/kg. The result found that sixteen biomarkers were changed because of drug administration. Among these sixteen biomarkers, eight biomarkers, including LPC(20:3), creatinine, LPC(14:0), LPC(18:3), LPC(22:5), arachidonic acid, proline and tryptophan, were found to be related to biochemical indicators of nephrotoxicity using Pearson correlation analysis. The identified biomarkers were mainly involved in valine, leucine and isoleucine biosynthesis, metabolism of sphingolipid, arginine and proline, glycerophospholipid, tryptophan, and arachidonic acid. In addition, the disturbed pathways were found to be time- and intraperitoneal or intravenous administration-dependent. The present result shows that mass spectrometry-based metabonomics approach could be applied to study on changes in metabolites and metabolic pathways associated with intraperitoneal or intravenous injection of cisplatin.

Abbreviations: DDP, Cisplatin; IP, Intraperitoneal; IV, Intravenous; BUN, Blood urea nitrogen; Scr, Serum creatinine; NMR, nuclear magnetic resonance; MS, mass spectrometry;

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RRLC-Q-TOF-MS, Rapid resolution liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry; IP-DDP-24h, the group of samples collected at 24 h after single intraperitoneal cisplatin administration; IP-DDP-72h, the group of samples collected at 72 h after single intraperitoneal cisplatin administration; IP-NS-24h, the groups of samples collected at 24 h after single intraperitoneal normal saline administration; IP-NS-72h, the groups of samples collected at 72 h after single intraperitoneal normal saline administration; IV-DDP-24h, the group of samples collected at 24 h after single intravenous cisplatin administration; IV-DDP-72h, the group of samples collected at 72 h after single intravenous cisplatin administration; IV-NS-24h, the groups of samples collected at 24 h after single intravenous normal saline administration; IV-NS-72h, the groups of samples collected at 72 h after single intravenous normal saline administration; ESI, electrospray ionisation; QC, Quality Control; PCA, principal component analysis; PLS-DA, partial least-squares-discriminant analysis; VIP, variable importance plot; LPC, lysophosphatidylcholine.

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Introduction

Cisplatin (DDP), a widely used chemotherapeutic agent, has long been used because of its effectiveness, broad range of anticancer activity, and the ability to induce chemoresistance.[1-3] However, the use of cisplatin is limited by its side effects, particularly its nephrotoxicity.[4-6] Cisplatin is administered intraperitoneally or intravenously in the clinic. Intraperitoneal (IP) cisplatin chemotherapy has been applied to resist ovarian, breast, and gastric cancers.[7,8] Intravenous (IV) cisplatin administration is applied to treat several types of cancers such as lung cancer as well as head and neck cancer.[9,10] Although some pharmacokinetics of cisplatin administration had been investigated,[11,12] The change on metabolic profiling induced by IP and IV injection of cisplatin remains unclear. Blood urea nitrogen (BUN) and serum creatinine (Scr) are the conventional markers for nephrotoxicity. However, increasing bodies of evidence indicates that these clinical parameters are limited and are lacking in sensitivity.[13-15] The level of these parameters is influenced by various factors such as age, dehydration status, protein intake, catabolism, and liver function.[14,15] Therefore, the discovery of more sensitive biomarkers and the establishment of a new approach for explaining characteristics in nephrotoxicity caused by two route of injecting cisplatin are necessary.

Metabonomics is a branch of systems biology that mainly includes genomics, proteomics, transcriptomics, and metabonomics.[16] Metabonomics is a rapidly developing subject applied in the study of numerous fields, such as toxicology, therapeutic efficacy, and natural products.[16-19] Metabonomics technology can detect and quantify a large amount of metabolites such as lipids,

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amino acids, peptides, organic acids, and vitamins.[18] These small and low-weight molecules are the final products in biological metabolite pathways and they have an important function in metabolism.[18] Metabonomics currently has an important function in predicting drug-induced toxicity because it can provide essential information on metabolic profiles in biofluid and organs derived from drug administration.[20,21] Moreover, the biomarkers identified by metabonomics can predict toxicity earlier than general clinical chemical methods, improving the development of toxic detection, which is beneficial to human health.

The foundation and core of metabonomics is high quality data that requires an advanced analysis platform, in which nuclear magnetic resonance (NMR) and mass spectrometry (MS) are commonly used.[22, 23] MS has been a powerful tool in metabonomics study with its high sensitivity, greater accuracy, and higher resolution. Rapid resolution liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (RRLC-Q-TOF-MS) provides high sensitivity with low detection limits, high resolution, good separation, and high accuracy and has become an important analysis platform for detecting metabolic changes in metabonomics.[24,25] In addition, RRLC-Q-TOF-MS can also provide ion fragment information through MS/MS data, which is significant for the identification of potential biomarkers.

In this study, we built a method based on metabonomics coupled with RRLC-Q-TOF-MS and multivariate statistical analysis to discover plasma biomarkers of kidney injury caused by IP and IV cisplatin administration. We also speculated on the the disturbed pathway. This study aims to find the characteristic of nephrotoxicity induced by two common used cisplatin administration at

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the metabolic level, trying to offer information for further investigation on mechanism of toxicity cisplatin.

Experimental

Reagents and materials

HPLC-grade acetonitrile was purchased from Oceanpak (Goteborg, Sweden). Distilled water was obtained from Wahaha Company (Hangzhou, China). Cisplatin was obtained from Jiangsu Hansoh Pharmaceutical Co., Ltd. (Lianyungang, China). The assay kits for BUN and Scr were obtained from the Biosino Bio-technology and Science Inc. (Beijing, China).

Animal treatment

The animal study was performed at the Institute of Radiation Medicine Chinese Academy of Medical Sciences (Tianjin, China). A total of 80 male Wistar rats weighing 200 g to 220 g were kept in SPF level lab. Animals were housed in one room under controlled light (12/12 h light/dark cycle), temperature (25 ± 1 degrees centigrade), and humidity (50 ± 5%). Before experimentation, all animals were acclimated for 1 wk, with access to free diet and clear water. Then, the animals were grouped randomly into four groups with 20 rats in each of the following groups: single IP normal saline administration (IP-NS) group, single IP cisplatin administration (IP-DDP) group, single IV normal saline administration (IV-NS) group, and single IV cisplatin administration (IV-DDP) group. DDP was first dissolved with normal saline (0.9% w/v) at a dosage of 6 mg/kg in the IP-DDP and IV-DDP groups. This study was approved by the Animal

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Ethics Committee of Tianjin University of Traditional Chinese Medicine under permit number TCM-2012-078-F01. All procedure was conducted in accordance with the Chinese national legislation and local guidelines.

Sample collection

Blood and kidney tissue were collected in this experiment. Prior to sample collecting, all animals were fasted for 12 hours and water was available during the whole procedure. Blood was collected from the intraocular angular vein after the rats were slightly anesthetized, and were collected at 24 and 72 h after administration in each group. After collecting blood, six animals were sacrificed, and the kidney tissues were immediately removed and stored in 10% formalin solution. Serum and plasma were separated through centrifugation at 3500 rpm for 15 min.

Serum was used to detect the level of BUN and Scr, which were measurement by automatic biochemical analyzer (BIOSINO, Ltd). Plasma was stored at minus 80 degrees centigrade prior to metabonomics analysis. The kidney were stained with hematoxylin and eosin (H&E) to observe the kidney pathological features. All the procedures were conducted in accordance with the Chinese national legislation and local guidelines. For convenient group labeling, IP-DDP-24h group was used to represent the group of samples collected at 24 h after single IP cisplatin administration. IP-DDP-72h group represented the group of samples collected 72 h after single IP cisplatin administration. IP-NS-24h and IP-NS-72h represented the groups of samples collected at 24 and 72 h, respectively, after single IP normal saline administration. IV-DDP-24h and IV-DDP-72h represented the groups of samples collected at 24 and 72 h, respectively, after

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single IV cisplatin administration. IV-NS-24 h and IV-NS-72 h represented the groups of samples collected at 24 and 72 h, respectively, after single IV normal saline administration.

Chromatographic acquisition

After plasma was thawed at room temperature, 600 μL acetonitrile was added to 200 μL of the plasma and the mixture was ultrasonicated in cold water for 10 min, vortexed for 1 min, then centrifuged at 13000 rpm for 15 min. Ten microliters of supernatant was injected onto the ACQUITY UPLC HSS C_{18} column (2.1 × 100 mm, 1.7 μm, Waters) maintained at 40 degrees centigrade at a flow rate of 0.3 mL/min. The separation system was Agilent RRLC (California, USA) with a binary solvent system, including mobile phase A, 0.1% formic acid in water and mobile phase B, 0.1% formic acid in acetonitrile. The gradient started with 99% A, then, 0-3 min, A: 99%-48%; 3-7 min, A: 48%-26%; 7-9 min, A: 26%-20%; 9-10 min, A: 20%-10%; 10-12 min, A: 10%-1%; 12-16 min, A: 1%-1%; 16-17min, A: 1%-99%; 17-20 min, A: 99%-99%. Seven minutes were set as posttime to stabilize liquid chromatographic system and systematic pressure. RRLC coupled with Q-TOF-MS equipped with electrospray ionisation (ESI) in positive mode was used. The MS parameters were as follows: drying gas temperature was 325 degrees centigrade, drying gas flow was 10 ml/min, desolvation gas flow was 600 L/h,, capillary voltage was 3.5 kV, nebulizer pressure was 350 psi, evaporative gas and auxiliary gas were high purity nitrogen, reference ions ($[M+H]^+$= 121.0509, 922.0098) were employed to ensure accuracy during chromatographic acquisition. The range of data acquisition was from 50 to 1000 Da. All the samples were injected randomly. Before injecting samples collected in different groups,

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Quality control (QC) samples, a mixture of plasma samples from each group, were first applied to detect the instrument precision and stability. if the total chromatographic system was not stable, the injection of plasma can not be permitted and the chromatographic acquisition could start until the whole system was at a good and stable condition. Moreover, QC samples were injected to test for the stability of samples and system during the whole acquisition.

Data process

After data acquisition, all the MS raw files was extracted by Agilent MassHunter Qualitative Analysis B.04.00 software with noise elimination level 5. Automated peak detection, peak alignment, and normalization were performed by Agilent MassHunter Mass Profiler software (version 4.0). The data was processed and transformed to an Excel format, containing whole information of the Mass, RT, peak area of the samples.

Further investigation on the multivariate statistical analysis, including principal component analysis (PCA) and partial least-squares-discriminant analysis (PLS-DA), was loaded into SIMCA-P + 11.5 software (Umetrics AB, Umea, Sweden). The data was scaled prior to multivariate data analysis. We used Pareto scaling in performing further analysis. The general trends and groupings of the total samples can be obtained, and remove outliers beyond 95% confidence level through PCA. PLS-DA, a supervised analysis technique, is a better method in removing undesirable information when building a model.[26] To ensure that the established analysis model actually reflected discrimination of metabolites induced by DDP, the feasibility of the analysis model was verified through cross validation by taking out one-seventh of the

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samples in each group.[27] The potential biomarkers were selected through S-plot, loading plot and variable importance plot (VIP). The selected potential biomarkers were further tested through independent sample t test using SPSS 17.0, and only biomarkers with $p<0.05$ were considered in the next step. The biomarkers were finally identified using standard or MS/MS fragment information coupled with the available database, such as HMDB (http://www.hmdb.ca/), KEGG (http://www.genome.jp/kegg/), and MassBank (http://www.massbank.jp/).

The Pearson correlation analysis was used to explore the relationship between identified biomarkers and the result of the biochemical analysis. Pearson correlation coefficient is a linear correlation coefficient used to reflect the linear correlation of the two variables. The correlation coefficient $r >0$ indicates that the two variables are positively correlated. The correlation coefficient $r <0$ indicates they are negatively correlated. The higher absolute value of r, the stronger correlation of two variables is. Pearson correlation analysis were conducted using SPSS 17.0 in this study.

The interaction, construction, and pathway analysis of potential biomarkers was performed through MetPA (http ://metpa.metabolomics.ca./MetPA/faces/Home.jsp). MetPA is a free, web-based tool aiming for metabonomics studies, which is convenient and easy-used for researchers to analyze the most relevant metabolic pathways affected by specific factor.

Results and discussion

Biochemical analysis

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The BUN and Scr levels were significantly elevated ($p<0.05$) in two DDP-72 h groups in both IP and IV cisplatin administration (Fig. 1A and 1B). The BUN level in two DDP-24 h groups in both routes of cisplatin administration statistically changed ($p<0.05$) (Fig. 1B), contrary to the Scr level, which had no marked change in the two DDP-24 h groups (Fig. 1A). The BUN and Scr contents did not change in the two control groups at different time points. BUN and Scr are generally conventional monitor of nephrotoxicity and has been used as a standard determiner of kidney injury for many years. When BUN and Scr level significantly elevate, it shows kidney has been injured. The results indicated that nephrotoxicity became obvious at 24 and 72 h after single IP and IV cisplatin administration.

Histopathological examination

Histopathological observation was used to study pathological manifestations and confirm direct kidney tissue injury. As shown in Fig. 2, mild edema was present in the renal tubular epithelial cells, and inflammatory cells were also observed in the kidney tissue from the IP-DDP-24h group. Compared to control group, evident expansion can be observed in the renal tubule from the IP-DDP-72 h group through microscopy. Edema, cell apoptosis, and inflammatory cells were also clearly observed in the kidney tissue from the IP-DDP-72 h group. No obvious damage was found in the kidney tissues from the control groups. The kidney tissue damage induced by IV cisplatin administration was similar to the damage caused by IP cisplatin administration (Fig. 2). Coupled with biochemical analysis, it can be concluded kidney damage had been induced by cisplatin.

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Metabolic profiling and data processing

Using RRLC-Q-TOF-MS technology, we obtained the plasma chromatograms of control and administration groups. Approximately 4500 ions were detected through RRLC-Q-TOF-MS. Ions were separated well within 20 min. Some discrimination was found in the typical total ion current chromatograms in positive mode between different groups (Fig. S1, ESI†), indicating that the plasma metabolite fingerprint was altered because of drug interference.

Multivariate statistical analysis was performed to better visualize the differences among the obtained complex data. Here, PCA and PLS-DA were used to filter out potential biomarkers between different groups. In PCA and PLS-DA plots, each point stands for a sample and each sample includes the information of metabolic profile. PCA is an unsupervised analysis technique, aiming to demonstrate the natural inter-relationship among different groups.[28] The plasma samples were divided into different regions in PCA score plot, indicating that the plasma endogenous substance changed because of IP and IV injecting cisplatin and this change showed time-dependency (Fig. 3A, 3B). To further distinguish between the DDP and control groups, PLS-DA was used to determine the potential biomarkers related to nephrotoxicity. Fig. 3C-3E respectively showed the score plot, S-plot and loading plot of PLS-DA model established in IP-DDP-24h group versus IP-NS-24h. The potential biomarkers were selected through S-plot, loadings plot, and variable importance plot. What's more, the different samples that appeared in different blocks indicated the presence of a time-dependent metabolic distinction in plasma collected at 24 and 72 h after single IV cisplatin administration (Fig. S2A, ESI†). Differences in

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the plasma metabolites between IP and IV cisplatin administration at the same time point were evident from PLS-DA analysis (Fig. S2B, ESI†). R^2 (cum) and Q^2 (cum) parameters usually indicate the fitness and prediction of the model. These two parameters are below 1 and can show a proper and good model when close to 1.[29] In this study, The established model were reasonable with high value of R^2 and Q^2 (Table S1, ESI†). Metabolites with VIP >1 and variations far away from the S-plot and the loading plot were chosen as potential biomarkers. According to the criterion of data processing established above, endogenous metabolites significantly changed were summarized for preferential study.

Identification of biomarkers

The multivariate statistical analysis facilitated the identification of specific metabolites from a large amount of plasma endogenous metabolites. The m/z value of the metabolites was determined, which was the relatively accurate molecular mass provided by the Q-TOF-MS analysis platform. We searched candidates from HMDB database utilizing m/z value of the metabolites. The candidates obtained from HMDB database included several exogenous substances that were ignored. Thus, endogenesis was chosen for the following study. Based on the result of searching on HMDB database, we identified the biomarkers by available standards and MS/MS fragment.

We took the ions at (t_R=11.58 min, m/z 468.3086) as an example to explain the identification. The molecular formula was supposed to be $C_{22}H_{46}NO_7P$ by searching on HMDB database

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utilizing m/z 468.3086, besides, the main fragment ions in positive MS/MS spectrum were found at m/z 450.3, 391.3, 285.2, 184.1, 125.0, 104.1, which could be the ions formed by the $[M+H]^+$ of lost $-H_2O$, $-C_3H_{10}NO$, $-C_5H_{13}NO_4P$, $-C_{17}H_{33}NO_2$, $-C_{20}H_{40}NO_3$, and $-C_{18}H_{38}NO_4P$ respectively. To confirm the structure of this ion, according to the Chemspider database, the metabolite was finally identified as lysophosphatidylcholine (14:0) [LPC (14:0)]. The mass spectrum of LPC (14:0) were shown in Fig. 4. The detailed information of identified differential metabolites in plasma was provided in Table 1.

Correlation analysis

We used the Pearson correlation analysis to explore the relationship between biomarkers and the result of the biochemical analysis. Results showed that the lysophosphatidylcholine(20:3) (LPC (20:3)) in plasma correlated with the indicators of kidney injury, with a positive correlation factor ($r = 0.95$, $p = 0.003$). An obvious positive correlation was also discovered between creatinine and the index of nephrotoxicity with Pearson correlation factor as high as 0.97. In addition, the biochemical indicators of nephrotoxicity, BUN and Scr, were negatively associated with the level of LPC(14:0), LPC(18:3), LPC(22:5), arachidonic acid, proline and tryptophan. Detailed information of the r coefficient and the p-value of significant correlation were shown in Table 2. The result of the Pearson correlation analysis also demonstrated that metabonomics was closely related to traditional biological examination in clinic and that metabonomics technology had become an important tool in safety assessment.

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Interpretation of selected biomarkers

The potential biomarkers related to nephrotoxicity caused by cisplatin administration were divided into two groups. The first group was lysophosphatidylcholine (LPC). Lysophosphatidylcholine is considered to have an essential function in the impairment of vasodilation and induction of apoptosis.[30,31] LPC also poses a threat to vascular endothelial function and is involved in inflammatory disease.[31] The change in the plasma LPC levels may be caused by cisplatin-induced nephrotoxicity.

Other biomarkers were treated as the second group. The elevated level of creatinine in plasma reflects the damage in the function of glomerular filtration.[32] Cisplatin may induce injury in the renal vasculature and result in decreased blood flow and ischemic injury of the kidneys, contributing to a decline in glomerular filtration rate.[2] Arachidonic acid mediates inflammation widely perceived to be a side effect of cisplatin administration.[2, 33-35] Cisplatin-induced inflammation result in the development of kidney damage and renal injury.[2] Proline participates in arginine and proline metabolism. Tryptophan is an essential amino acid and the precursor of serotonin.

Disturbed metabolic pathways

We applied MetPA to obtain detailed pathway information. In this study, the pathway impact value above 0.05 was filtered out as a potential target pathway, and those pathways were perceived to be related to nephrotoxicity. We found that valine, leucine, and isoleucine

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biosynthesis (1), tryptophan metabolism (2), sphingolipid metabolism (3), and arginine and proline metabolism (4) were affected in Wistar rats in the IP-DDP-24h group (Fig. 5A). Simultaneous to IV administration, arachidonic acid metabolism (5) and pathway 1 were found to have changed in our study (Fig. 5B). In addition, we found that pathways 1 to 3 and glycerophospholipid metabolism (6) were mainly responsible for the nephrotoxicity in the IP-DDP-72h group (Fig. 5C). Pathways 1-4 and 6 were found to be disturbed at 72 h after single IV cisplatin (Fig. 5D). It can be concluded that the disturbed pathways showed a time- and administration-dependent manner.

In our study, The change in the contents of the same biomarkers compared with their respective control groups in the two routes of cisplatin administration significantly differed. For example, the plasma content of LPC(20:3) was unchanged in the IP-DDP-24h group, but it increased by threefold of its control group in the IV-DDP group at the same time point; the plasma LPC (18:3) level in the IP-DDP-24h group was reduced by 0.3 times and by 0.6 times at the same time point in the IV-DDP group. In addition, the cisplatin-disturbed pathways in the same time point of different administration were not consistent and existed an administration-dependency. Based on former mentioned fact, it can be speculated that the nephrotoxicity induced by two routes of cisplatin administration may do have some distinction. Meanwhile, this distinction may be related to the different mechanism of nephrotoxicity induced by the two routes of cisplatin, which will be further investigated.

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Conclusions

This study showed that the established MS-based metabonomics approach could be applied to study on nephrotoxicity induced by IP and intravenous IV injection of cisplatin. Kidney injury model could be built through single IP or IV cisplatin administration with a dose of 6 mg/kg. Biomarkers of nephrotoxicity caused by two routs of cisplatin administration were identified respectively. Among selected sixteen biomarkers, eight metabolites, including LPC (20:3), creatinine, LPC(14:0), LPC(18:3), LPC(22:5), arachidonic acid, proline, and tryptophan, were chosen to be related to kidney injury. The pathways of valine, leucine, and isoleucine biosynthesis, as well as the metabolism of sphingolipid, arginine and proline, glycerophospholipid, tryptophan, and arachidonic acid, were disturbed after cisplatin administration. The disturbed pathways in the different groups exhibited time- and administration-dependence. It was also worthy to note that the nephrotoxicity caused by two routes of cisplatin had some distinction, which may reveal the difference in their mechanism of kidney injury. The present result shows MS-based metabonomics approach could be applied to study on changes in metabolic profiling induced by IP and IV injection of cisplatin, which may provide useful information for further mechanism research on cisplatin.

Acknowledgments

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Tables:

Table 1 Identified differential metabolites in plasma in different groups.

No.	t_R (min)	Metabolite	Obsd $[M+H]^+$	Calcd $[M+H]^+$	Error (ppm)	Formula	Molecular structure	content Change[a] (DDP/NS)	The groups detected this biomarker[d]	Pathway (KEGG)
1	0.89	Urea[b]	61.0398	61.0396	3.3	CH_4N_2O	61.0 $[M+H]^+$	↑	IP-DDP-24h group IP-DDP-72h group IV-DDP-72h group	Arginine and proline metabolism
2	0.92	Creatinine[b]	114.0661	114.0662	−0.9	$C_4H_7N_3O$	114.1 $[M+H]^+$	↑	IP-DDP-72h group IV-DDP-72h group	Arginine and proline metabolism
3	0.93	Proline[b]	116.0704	116.0706	−1.7	$C_5H_9NO_2$	116.1 $[M+H]^+$	↓	IP-DDP-24h group IV-DDP-72h group	Arginine and proline metabolism
4	0.89	Valine[b]	118.0859	118.0863	−3.4	$C_5H_{11}NO_2$	118.1 $[M+H]^+$	↓	IP-DDP-24h group IP-DDP-72h group	Valine, leucine and isoleucine biosynthesis

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									IV-DDP-24h group IV-DDP-72h group	
5	5.18	Tryptophan[c]	205.0968	205.0972	−2.0	$C_{11}H_{12}N_2O_2$	205.1 $[M+H]^+$ 188.1 $[M+H-NH_2]^+$	↓	IP-DDP-24h group IP-DDP-72h group IV-DDP-72h group	Tryptophan metabolism
6	9.08	Sphinganine[c]	302.3045	302.3054	−3.0	$C_{18}H_{39}NO_2$	302.3 $[M+H]^+$ 284.3 $[M+H-H_2O]^+$	↑	IP-DDP-24h group IP-DDP-72h group IV-DDP-72h group	Sphingolipid metabolism
7	11.55	Arachidonic acid[b]	305.2471	305.2475	−1.3	$C_{20}H_{32}O_2$	305.2 $[M+H]^+$	↓	IV-DDP-24h group	arachidonic acid metabolism
8	11.58	LPC(14:0)[c]	468.3086	468.3085	0.2	$C_{22}H_{46}NO_7P$	468.3 $[M+H]^+$ 450.3 $[M+H-H_2O]^+$ 391.3 $[M+H-C_3H_{10}NO]^+$ 285.2 $[M+H-C_5H_{13}NO_4P]^+$ 184.1 $[M+H-C_{17}H_{33}NO_2]^+$ 125.0 $[M+H-C_{20}H_{40}NO_3]^+$	↓	IP-DDP-24h group	Glycerophospholipid metabolism

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							104.1 $[M+H-C_{18}H_{38}NO_4P]^+$			
9	9.78	LPC(16:1)[c]	494.3243	494.3241	0.4	$C_{24}H_{48}NO_7P$	494.3 $[M+H]^+$ 476.3 $[M+H-H_2O]^+$ 184.1 $[M+H-C_{19}H_{35}NO_2]^+$ 125.0 $[M+H-C_{22}H_{42}NO_3]^+$ 104.0 $[M+H-C_{20}H_{40}NO_4P]^+$	↑	IP-DDP-24h group IP-DDP-72h group IV-DDP-72h group	Glycerophospholipid metabolism
10	11.66	LPC(17:0)[c]	510.3530	510.3554	−4.7	$C_{25}H_{52}NO_7P$	510.4 $[M+H]^+$ 492.4 $[M+H-H_2O]^+$ 433.4 $[M+H-C_3H_{10}NO]^+$ 285.2 $[M+H-C_5H_{13}NO_4P]^+$	↓	IP-DDP-72h group IV-DDP-72h group	Glycerophospholipid metabolism
11	9.46	LPC(18:3)[c]	518.3239	518.3241	−0.4	$C_{26}H_{48}NO_7P$	518.3 $[M+H]^+$ 500.3 $[M+H-H_2O]^+$ 184.1 $[M+H-C_{16}H_{32}NO_4P]^+$ 125.0 $[M+H-C_{24}H_{42}NO_3]^+$	↓	IP-DDP-24h group IP-DDP-72h group IV-DDP-24h group	Glycerophospholipid metabolism
12	11.05	LPC(18:2)[c]	520.3376	520.3398	−4.2	$C_{26}H_{50}NO_7P$	520.3 $[M+H]^+$	↑	IP-DDP-72h group	Glycerophospholipid metabolism

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							502.3 $[M+H-H_2O]^+$ 184.1 $[M+H-C_{21}H_{37}NO_2]^+$ 149.1 $[M+H-C_{22}H_{44}NO_3]^+$ 104.1 $[M+H-C_{22}H_{42}NO_4P]^+$		IV-DDP-24h group	
13	9.85	LPC(20:5)[c]	542.3223	542.3241	−3.3	$C_{28}H_{48}NO_7P$	542.3 $[M+H]^+$ 524.3 $[M+H-H_2O]^+$ 259.1 $[M+H-C_{17}H_{33}NO_2]^+$ 185.1 $[M+H-C_{19}H_{36}NO_3P]^+$ 126.0 $[M+H-C_{26}H_{42}NO_3]^+$	↓	IP-DDP-24h group	Glycerophospholipid metabolism
14	11.89	LPC(20:3)[c]	546.3529	546.3554	−4.6	$C_{28}H_{52}NO_7P$	546.4 $[M+H]^+$ 528.4 $[M+H-H_2O]^+$ 184.1 $[M+H-C_{18}H_{36}NO_4P]^+$ 125.1 $[M+H-C_{26}H_{46}NO_3]^+$	↑	IP-DDP-72h group IV-DDP-24h group IV-DDP-72h group	Glycerophospholipid metabolism
15	11.71	LPC(20:2)[c]	548.3714	548.3711	0.5	$C_{28}H_{54}NO_7P$	548.4 $[M+H]^+$ 471.3 $[M+H-C_3H_8O_2]^+$	↓	IP-DDP-72h group IV-DDP-72h group	Glycerophospholipid metabolism

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							370.2 $[M+H-C_8H_{19}NO_5P]^+$ 184.0 $[M+H-C_{23}H_{41}NO]^+$ 125.0 $[M+H-C_{26}H_{48}NO_3]^+$ 104.0 $[M+H-C_{24}H_{46}NO_4P]^+$			
16	11.62	LPC(22:5)[c]	570.3528	570.3554	−4.6	$C_{30}H_{52}NO_7P$	570.4 $[M+H]^+$ 552.3 $[M+H-H_2O]^+$ 184.1 $[M+H-C_{21}H_{41}NO_3P]^+$ 125.0 $[M+H-C_{28}H_{47}NO_3]^+$	↓	IP-DDP-24h group IP-DDP-72h group IV-DDP-24h group IV-DDP-72h group	Glycerophospholipid metabolism

[a] the change of biomarker's content between DDP group and its corresponding control group, ↑, content increased; ↓, content decreased.

[b] identified by standards.

[c] identified by MS/MS information.

[d] the groups in which biomarkers were identified, IP-DDP-24h group stands for the group of samples collected at 24 h after single intraperitoneal cisplatin administration; IP-DDP-72h group stands for the group of samples collected at 72 h after single intraperitoneal cisplatin administration;

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IV-DDP-24h group stands for the group of samples collected at 24 h after single intravenous cisplatin administration; IV-DDP-72h group stands for the group of samples collected at 72 h after single intravenous cisplatin administration.

Table 2 Detailed information of the r coefficient and the p-value of significant correlated biomarkers.

NO.	Biomarker	r value	p value
1	LPC (20:3)	0.950	0.003
2	Creatinine	0.970	0.001
3	LPC(14:0)	-0.731	0.009
4	LPC(18:3)	-0.633	0.012
5	LPC(22:5),	-0.878	0.004
6	Arachidonic acid	-0.756	0.030
7	Proline	-0.868	0.005
8	Tryptophan	-0.903	0.002

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Figures:

Fig. 1 Effect of cisplatin on the level of Scr and BUN. (A) Changes in Scr level. (B) Changes in BUN level. Data are the means ± s.e.m. $^{*}P < 0.05$ compared to their control group respectively.

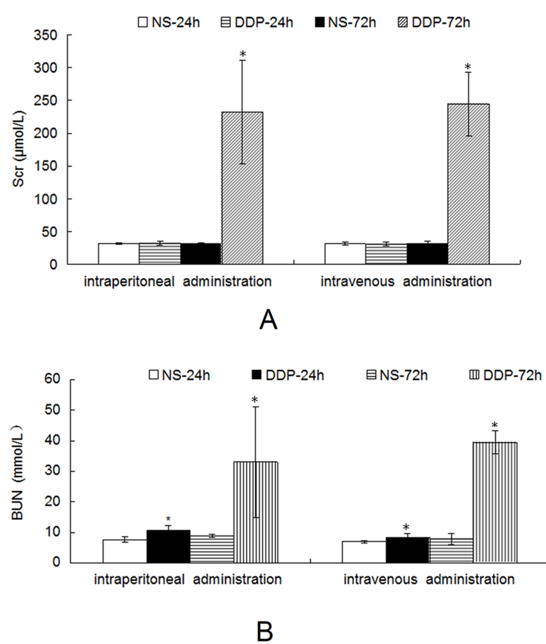

Fig. 2 Effect of cisplatin administration on the kidney tissue assessed by histopathology. Histological sections of the kidney were stained with H&E; 200×magnification.

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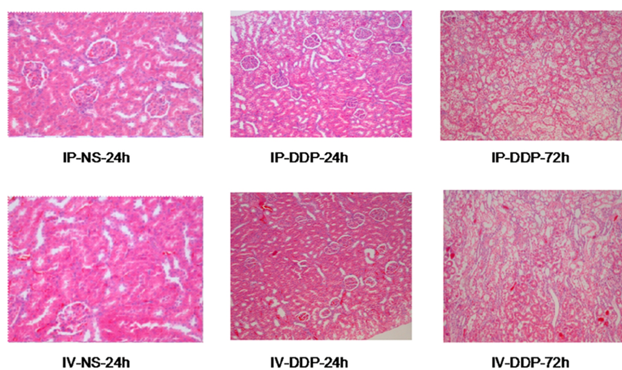

Fig. 3 The result of multivariate statistical analysis. (A) PCA score plot of IP-DDP-24h, IP-NS-24h, IP-DDP-72h and IP-NS-72h groups. (B) PCA score plot of IV-DDP-24h, IV-DDP-72h, IV-NS-24h and IV-NS-72h groups. (C) PLS-DA score plot of IP-DDP-24h and IP-NS-24h. (D) PLS-DA S-plot of IP-DDP-24h and IP-NS-24h. (E) PLS-DA loading plot of IP-DDP-24h and IP-NS-24h.

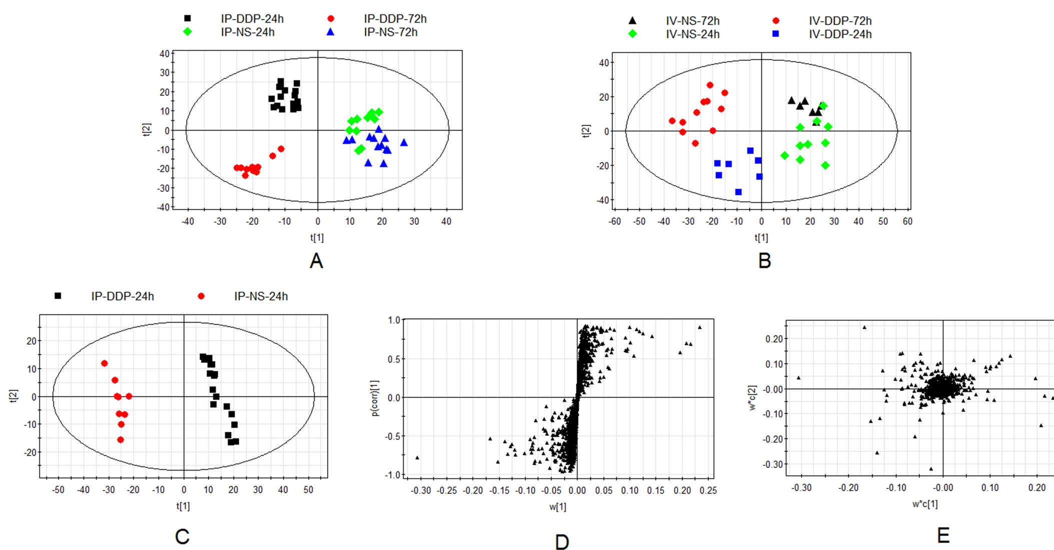

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Fig. 4 The MS/MS spectrum of LPC (14:0) showing its fragments in plasma samples obtained from RRLC-Q-TOF-MS/MS.

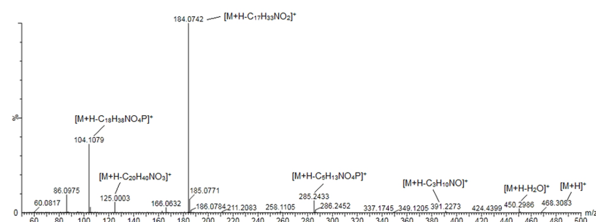
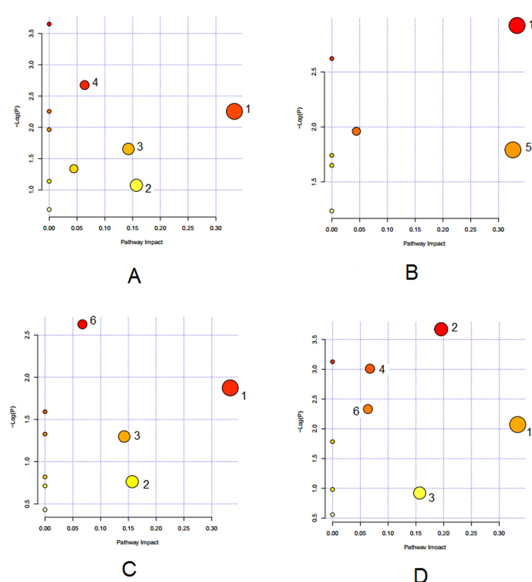


Fig. 5 Summary of pathway analysis with MetPA. (A) The interrupted metabolic pathways in IP-DDP-24h group. (B) The interrupted metabolic pathways in IV-DDP-24h group. (C) The interrupted metabolic pathways in IP-DDP-72h group. (D) The interrupted metabolic pathways in IV-DDP-72h group. 1: valine, leucine and isoleucine biosynthesis, 2: tryptophan metabolism, 3: sphingolipid metabolism, 4: arginine and proline metabolism, 5: arachidonic acid metabolism, 6: glycerophospholipid metabolism



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