Journal of Chromatography B, 873 (2008) 151–158

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Effect of a traditional Chinese medicine preparation Xindi soft capsule on rat model of acute blood stasis: A urinary metabonomics study based on liquid chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 20 January 2008
Accepted 8 August 2008
Available online 19 August 2008

Keywords:
Sea buckthorn
Metabonomics
Urine
UPLC Q-TOF MS
Biomarker
Acute blood stasis

ABSTRACT

Xindi soft capsule is a traditional Chinese medicine preparation which consists of sea buckthorn flavonoids and sea buckthorn berry oil. In this study, a urinary metabonomics method based on the ultra-performance liquid chromatography combined with quadrupole time-of-flight tandem mass spectrometry (UPLC Q-TOF MS) was used to evaluate the efficacy and study the mechanism of traditional Chinese medicine preparation to blood stasis. With pattern recognition analysis (principal component analysis and partial least squares-discriminate analysis) of urinary metabolites, a clear separation of acute blood stasis model group and healthy control group was achieved, the dose groups were located between acute blood stasis model group and healthy control group showing a tendency of recovering to healthy control group, high dose and middle dose were more effective than low dose. Some significantly changed metabolites like cholic acid, phenylalanine and kynurenic acid have been found and identified and used to explain the mechanism. The work shows that the metabonomics method is a valuable tool in the research mechanism of traditional Chinese medicine.

1. Introduction

Sea buckthorn, a hardy bush, belongs to the *Elaeagnaceae* family and naturally distributed over Asia and Europe [1]. Its berries have been used as a raw material for functional foods and medicine, and are a centuries-old traditional Chinese medicines [2,3]. The composition of combined pulp and seed oil of sea buckthorn berry is unusual including nutritionally important fatty acids and sterols, and a high anti-oxidative vitamins, flavonoids and carotenoids [4,5]. The beneficial effects of sea buckthorn have been reported such as scavenging free radicals, lowering blood viscosity, reducing red blood cell aggregation index, enhancing cardiac function, treating cardiovascular disease, normalizing the plasma lipid levels, inhibiting thrombus formation and platelet aggregation [4-8].

Blood stasis is an important underlying pathology of many disease processes according to traditional Chinese medicine. Described in TCM theory as a slowing or pooling of the blood due to disruption of heart Qi, it is often understood in biomedical terms in terms of hematological disorders such as hemorrhage, congestion, thrombosis, local ischemia (microclots) and tissue changes [9].

Metabonomics is a new platform of systems biology, defined as the quantitative measurement of the dynamic multi-parametric metabolic responses of living systems to patho-physiological stimuli or genetic modifications [10], is based on the determination of global metabolite profiles in biological fluids and tissues with subsequent data analysis via a range of multi-variate statistical approaches [11]. As a powerful analytical platform, recently, the application of metabonomics has dramatically increased in the fields of pharmaceutical discovery and development [12-14], evaluation of drug efficacy and toxicity [15,16], therapy and novel potential biomarker discovery [17,18]. A number of analytical tools have currently been employed including ^{1}H NMR spectroscopy, direct MS–MS, HPLC–MS, CE–MS, and GC–MS [19]. Ultra-performance liquid chromatography (UPLC) coupled with MS leads to considerable decrease of the analysis time and increase of the sensitivity, has been considered to have a more bright future in the research of metabonomics [20,21].

In the present work, we studied a traditional Chinese medicine preparation which consists of sea buckthorn flavonoids and sea buckthorn berry oil based on the UPLC Q-TOF MS, the aims are to

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doi:10.1016/j.jchromb.2008.08.010

investigate the effect on acute blood stasis rat model and involved possible mechanism.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile was purchased from Merck (Merck, Germany). Formic acid (HPLC/SPECTRO grade) was purchased from Tedia (USA). Water was produced by Milli-Q ultra-pure water system (Millipore, Billerica, USA). Leucine–enkephalin was obtained from Sigma–Aldrich (MO, USA). Xindi soft capsule was obtained from Sichuan Meidakang pharmaceuticals company, it consists of sea buckthorn flavonoids and sea buckthorn berry oil. For rat administration, the material of Xindi soft capsule was dissolved in rap oil.

2.2. Animal studies and sample preparation

The protocol of the study was approved by the Ethics Committee of the Chengdu University of Traditional Chinese Medicine. The investigation was conducted in accordance with the ethical principles of animal use and care.

A total of 50 male Sprague–Dawley rats weighing 250 ± 20 g were used for this study. Animals were randomly divided into the following 5 groups: (1) healthy control group, (2) acute blood stasis model group, (3) low dose group of Xindi soft capsule, (4) middle dose group of Xindi soft capsule and (5) high dose group of Xindi soft capsule. Each rat was in an individual cage. Rats were administered by oral gavage one time each day for continuous seven days. The control group and model group were only administered by oral gavage 10 ml/kg (rat body weight) rap oil solvent, and the low, middle and high dose groups were administered by oral gavage the same amount of solution containing 37.5 mg/ml, 75 mg/ml and 150 mg/ml Xindi soft capsule material, respectively.

After the last time of Xindi soft capsule was administered by oral gavage, the acute blood stasis model and dose group rats were injected with adrenaline hydrochloride injection (0.4 mg/kg). After four hours, those rats were injected with the same injection again, waiting for 2 h, the rats were soaked in ice-water 5 min keeping their heads outside surface. Then rats were put in metabolic cages, and were fed freely. Each twelve hours of urine was acquired as a sample, four samples in two continuous days from each rat were collected and kept at −20 °C until analysis. A rat with middle dose group of Xindi soft capsule died during experiment. And several urine samples were not obtained, or their volumes were not enough to be analyzed. Finally, in each group, 32–36 samples in two days were collected and analyzed by UPLC Q-TOF MS.

Prior to analysis, the samples were thawed at room temperature, then centrifuged at 13,000 rpm for 15 min and the supernatant liquid was taken out. A 50-μl aliquot of the supernatant was diluted with 150 μl of distilled water and vortexed, the resulting solutions were transferred to an autosampler vial kept at 4 °C for analysis.

The animal models for hemorheology study were prepared by the same way, and then blood samples were collected. The parameters of hemorheology were detected with a LGR-80A auto blood viscometer (Zhongqindishi corp, Beijing, China).

2.3. Chromatography

The LC system used was an ACQUITY-Ultra Performance Liquid Chromatography system (Waters Corp., Milford, USA). The UPLC column used was a 100×2.1-mm ACQUITY-1.7 μm C18 column (Waters Corp., Milford, USA). The mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. The column was maintained at 40 °C and eluted with a concave non-linear gradient (Masslynx Guide: Waters 2690/2695 Pump Gradient Page) from 2% to 100% B in 16 min, kept for 3 min, at a flow rate 0.35 ml/min.

2.4. Mass spectrometry

Mass spectrometry was performed on a Q-TOF micro (Waters MS Technologies, Manchester, UK). The following parameters were used: capillary voltage, 3100 V; cone voltage, 30 V; collision energy, 4 eV; desolvation gas, 600 L/h; cone gas, 50 L/h; desolvation temperature, 300 °C; and the source temperature, 120 °C. The data acquisition rate was set to 0.4 s, with 0.1 s inter scan delay. All analyses were acquired using the lock spray to ensure accuracy and reproducibility, leucine–enkephalin was used as the lock mass (in positive ion mode $[M+H]^+ = 556.2771$) at a concentration of 2 ng/ml in methanol:water (50:50) + 1% acetic acid with a flow rate of 10 μl/min. The lock spray frequency was set at 20 s. Full-scan mass range of 80–700 m/z was acquired. Potential biomarkers were analyzed by LC–MS/MS. The collision energy was set at 25 eV, the other parameters were the same as the ones described above.

2.5. Data collection

The raw data were analyzed using the Micromass MarkerLynx Applications Manager version 4.0, this applications manager incorporates a peak deconvolution package that allows detection of the mass, retention time and intensity of the peaks eluting in each chromatogram. The data were combined into a single matrix by aligning peaks with the same mass–retention time pair together from each data file in the data set. The ion intensities for each peak detected were then normalized, with in each sample, to the sum of the peak intensities in that sample. The resulting data set was analyzed by principal components analysis (PCA) and partial least squares discriminate analysis (PLS-DA) using SIMCA-P software version 11.0 (Umetrics AB, Sweden).

3. Results and discussion

3.1. Influence of Xindi soft capsule on hemorheology of acute blood stasis model

Blood stasis in traditional Chinese medicine has been described as slowing or pooling of blood, which may cause pain or other symptoms. Hemorheology is a current technique to estimate blood stasis [22,23]. To study the effect of Xindi soft capsule on hemorrheology of acute blood stasis rats, blood viscosity, blood relative index, plasma viscosity, hematocrit and blood cell aggregation index (AI) were detected. The results are given in Table 1. The data showed that the differences of plasma viscosity, hematocrit and AI between the model group and the control group were all significant. Xindi soft capsule could obviously reduce blood viscosity, plasma viscosity, hematocrit and blood cell aggregation index (AI). The higher the dose amount, more remarkable the hemorheology change.

3.2. Analysis of the metabolic pattern in rat urine by UPLC Q-TOF MS

UPLC Q-TOF MS was thought as a powerful technique for the study of metabonomics. In our previous work [24], compared with the HPLC method, UPLC Q-TOF MS had been proved to be helpful to obtain more information of metabolites and to find metabolites with significant concentration change. In the meantime, based on Waters UPLC Q-TOF micro with ACQUITYTM 1.7 μm C18 column, the urinary metabolites were well analyzed with good repeatability and rich metabolite information [25]. Here the same instrument

Table 1
Influence of Xindi soft capsule on hemorheology (mean ± standard deviations, $n = 10$)

Group	Blood viscosity (mPa s)				Blood relative index		Plasma viscosity (mPa s)	Hematocrit (L/L)	AI
	1.00 s^{-1}	5.00 s^{-1}	30.00 s^{-1}	200.00 s^{-1}	High shear rate	Low shear rate			
Control	53.55 ± 6.18	20.90 ± 2.30*	10.61 ± 1.31	7.23 ± 0.77*	4.08 ± 0.44*	29.93 ± 3.60	1.82 ± 0.75*	0.60 ± 0.056*	7.34 ± 0.38*
Model	68.13 ± 19.39	25.01 ± 6.14	11.86 ± 2.38	7.80 ± 1.32	4.19 ± 0.68	36.62 ± 10.19	1.87 ± 0.66	0.64 ± 0.039	8.59 ± 1.29
Low dose	63.85 ± 13.39	23.61 ± 4.23	11.30 ± 1.66	7.44 ± 0.93*	4.06 ± 0.51*	34.79 ± 7.00	1.83 ± 0.56*	0.61 ± 0.042	8.51 ± 0.89
Middle dose	65.47 ± 14.49	24.82 ± 3.99	12.23 ± 1.22	8.25 ± 0.58	4.62 ± 0.33	36.68 ± 8.00	1.78 ± 0.20	0.58 ± 0.014*	7.91 ± 1.49
High dose	54.65 ± 7.38	21.39 ± 2.42*	10.86 ± 0.99	7.48 ± 0.56	4.18 ± 0.29	30.57 ± 3.89	1.79 ± 0.15*	0.58 ± 0.020*	7.23 ± 0.59*

AI: red blood cell aggregation index. *Compared with model $p < 0.05$. One-way ANOVA was used when the data were normal distribution. K independent sample tests were used when the data were not normal distribution.

was used to analyze rat urines but a 20-min gradient method was applied to chromatographic separation by increasing the column temperature to 40 °C and changing the buffer (Section 2.3). A representative base peak intensity (BPI) chromatogram is given in Fig. 1, the number of detected ions in the positive ion mode was approximately 9600.

To examine the stability of the method, a quality control sample was analyzed after each 10 urine samples were run. The data in total 17 determinations showed that the relative standard deviations of the retention time and the peak height of hippuric acid in the quality control sample were 0.23% and 9.5%, respectively. The used method had a good stability and repeatability.

3.3. Influence of Xindi soft capsule on the urinary metabolic pattern of acute blood stasis model

To know whether we can distinguish the model group and control group on the basis of the UPLC Q-TOF MS spectra and understand their metabolite difference, we first carried out a principal components analysis (PCA). The result shows that in the scores plot, the model group and control group can be clearly separated (Fig. 2). In 2 days after acute blood stasis was made, the acute blood stasis subjects can be distinguished from the normal subjects based on urine samples collected. The difference between acute blood stasis group and normal group is more remarkable than individual difference of the rat. It shows that the urinary metabolic pattern was significantly changed in the acute blood stasis group.

To determine whether Xindi soft capsule was possible to influence metabolic pattern of the acute blood stasis model subjects and to find the metabolites with a significant concentration change (to be simple, they are called as potential biomarkers), we constructed a model using partial least squares-discriminant analysis (PLS-DA), Fig. 3 shows the score and loading plots. To estimate the predictive ability of our model, we used 7-fold cross-validation, the parameters for the classification from the software were $R^2Y = 0.866$ and $Q^2Y = 0.505$, which are good to fitness and prediction, respectively. A response permutation test (Y scrambling) was used to assess the significance of the predictive ability and to exclude overfitting due to chance correlation [26]. The data shows our model had a R^2Y-intercept of 0.406 and a Q^2-intercept of −0.470. According to Eriksson et al. [26], the R^2Y-intercept should not exceed 0.4 and the Q^2-intercept should not exceed 0.05 for a valid model. It shows our model is reliable. It can be seen that from the PLS-DA score plot (Fig. 3a), a separation of the model group and control group was clearly achieved, while the dose group was mainly located between the model group and the control group. The subjects of middle group and high group obtained better separation from those of the model group than those of low dose group, they are much closer to the control group. Combined with the result of hemorheology this change of urinary metabolic pattern shows the model group is moving toward the control group and the blood stasis was being prevented and alleviated, exhibiting a tendency recovering to healthy control group after taking Xindi soft capsule.

The loading plots from the PLS-DA based on UPLC Q-TOF MS data are shown in Fig. 3b. The ions which are the furthest ones from the origin contribute significantly to the clustering of different groups. Mean peak heights of some typical ions in different groups are shown in Fig. 4. The concentrations of the ions with m/z 116.0 (Fig. 4a) and m/z 100.0 (Fig. 4b) were found increasing in the model group compared with those in the control group, their concentrations in the dose groups were decreased compared with those in the model group. The concentrations of the ions with m/z 355.3 (Fig. 4c) and m/z 162.0 (Fig. 4d) were found decreasing in the model group compared with those in the control group, their concentrations in the dose groups were increased and compared with

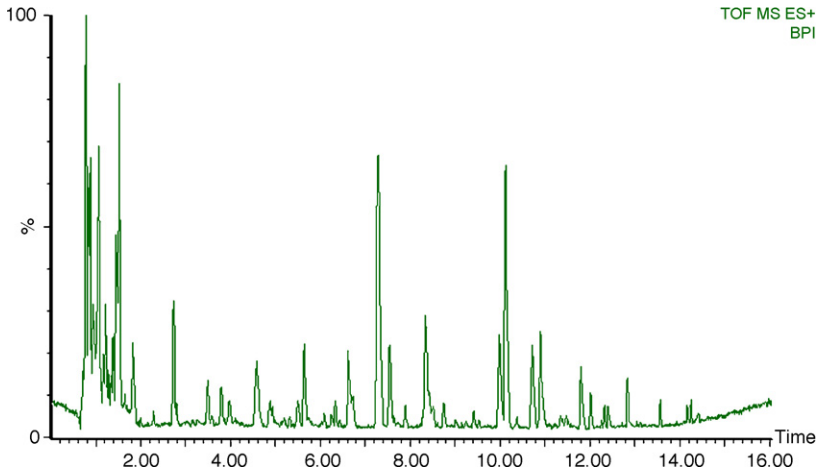

Fig. 1. Representative UPLC Q-TOF MS base peak intensity (BPI) chromatogram from a rat urine sample analyzed in positive ion mode.

those in the model group. Fig. 4a–d shows that after taking Xindi soft capsule the concentrations of some metabolites had tendency to come back from the model group to the control group. On the other hand, the concentration of the ion with *m*/*z* 132.0 (Fig. 4e) was found increasing in the model group and the dose groups than that in the control group, the reason of this change was not clear.

3.4. Identification of potential biomarkers

To identify potential biomarkers, the fragmentation pattern from MS/MS has to be used [25]. Here, a potential biomarker with *m*/*z* 355.3 at the retention time (tr) 14.03 min is taken as an example to illustrate the identification process. Mass spectrum at the retention time (tr) 14.03 min in the positive ion model is shown in Fig. 5a. The other ions like 373, 391 and 409 were also found. Each of them has the difference of water, may be they came from the same component. Compared with negative ion mass spectrum (given in Fig. 5b), *m*/*z* 407 was found at the same retention time. So the quasi-molecular ions were found to be 409 in ESI^+ and 407 in ESI^-, there must be three hydroxyl groups in the structure. To define its structure some databases like KEGG (http://www.genome.jp/kegg/) and Human Metabolome Database (http://www.hmdb.ca/) were searched with the molecular weight 408 Da, then some compounds without three hydroxyl groups were removed from the candidate list. In the meantime its fragmentation (Fig. 5c) from tandem MS was further investigated. The possible fragment mechanism was deduced (Fig. 5d). It was tentatively identified as cholic acid, according to the retention time and fragmentation pattern of standard sample, this important metabolite was confirmed to be cholic acid.

By using the same method described above, seven potential biomarkers were identified (Table 2), they are cholic acid (tr at

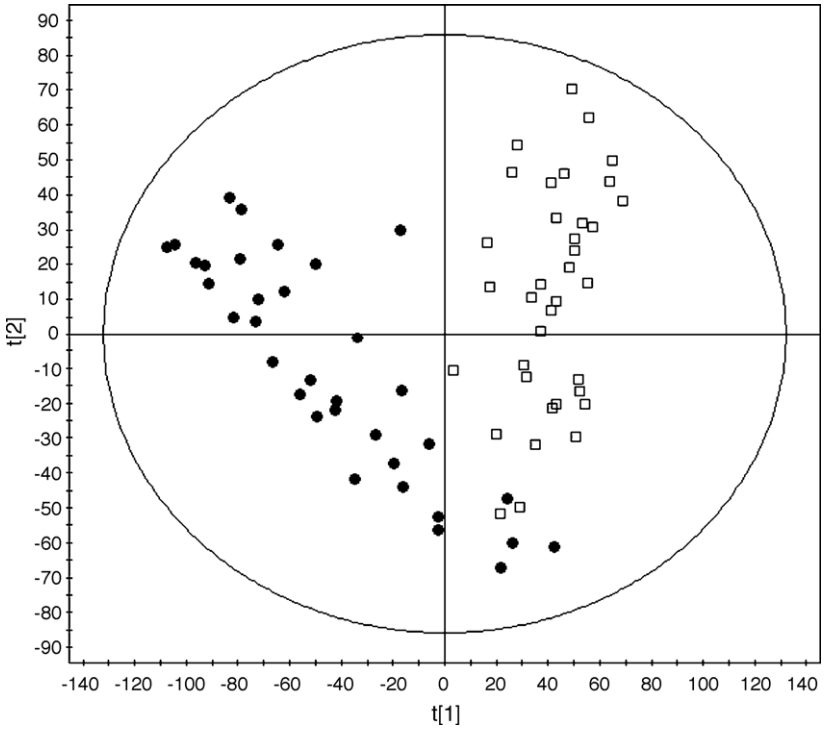

Fig. 2. PCA scores plots. (□) Control group and (●) model group.

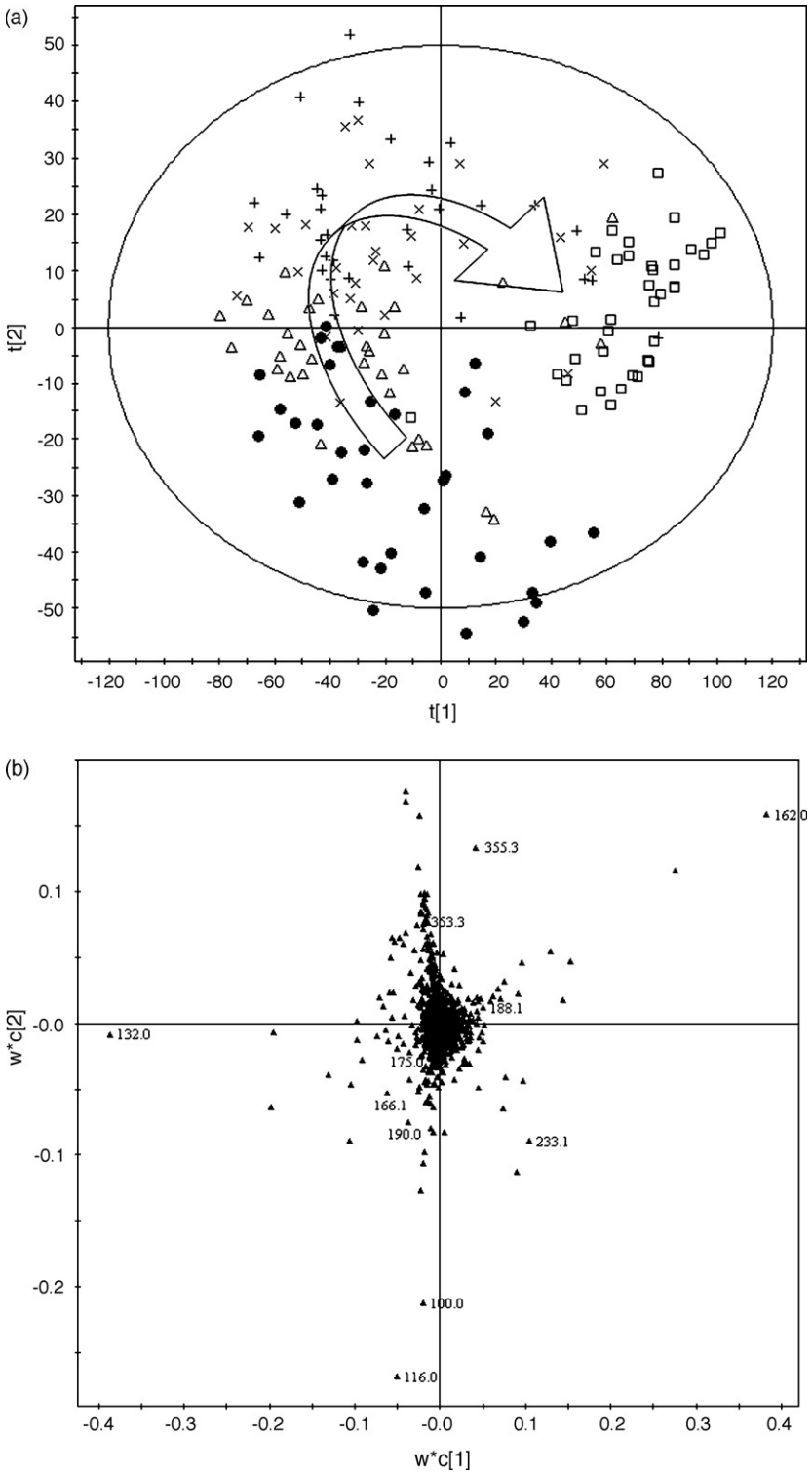

Fig. 3. (a) PLS-DA scores plots. (□) Control group, (●) model group, (△) low dose, (×) middle dose, (+) high dose. (b) PLS-DA loading plots.

14.03 min, *m*/*z* 355.3), dihydroxy cholanate (tr at 12.88 min, *m*/*z* 353.3), phenylalanine (tr at 2.58 min, *m*/*z* 166.1), kynurenic acid (tr at 5.68 min, *m*/*z* 190.0), tryptophan (tr at 6.65 min, *m*/*z* 188.1), arginine (tr at 0.71 min, *m*/*z* 175.0), *N*2-succinyl-L-ornithine (tr at 8.35 min, *m*/*z* 233.1).

Cholic acid is a major primary bile acid. They are synthesized in the liver and secreted in the gallbladder or in the intestine, conjugated mainly with taurine and glycine. Bile acids serve many important physiological functions including cholesterol homeostasis, lipid absorption, and generation of bile flow that help in the excretion and recirculation of drugs, vitamins, and endogenous and exogenous toxins [27,28]. In our study, cholic acid was significantly decreased in the model group than in control group ($p = 0.047$). In the dose groups, the concentration of urinary cholic acid was increased compared with the model group (Fig. 4c), dihydroxy cholanate is similar to cholic acid. That may be the reason

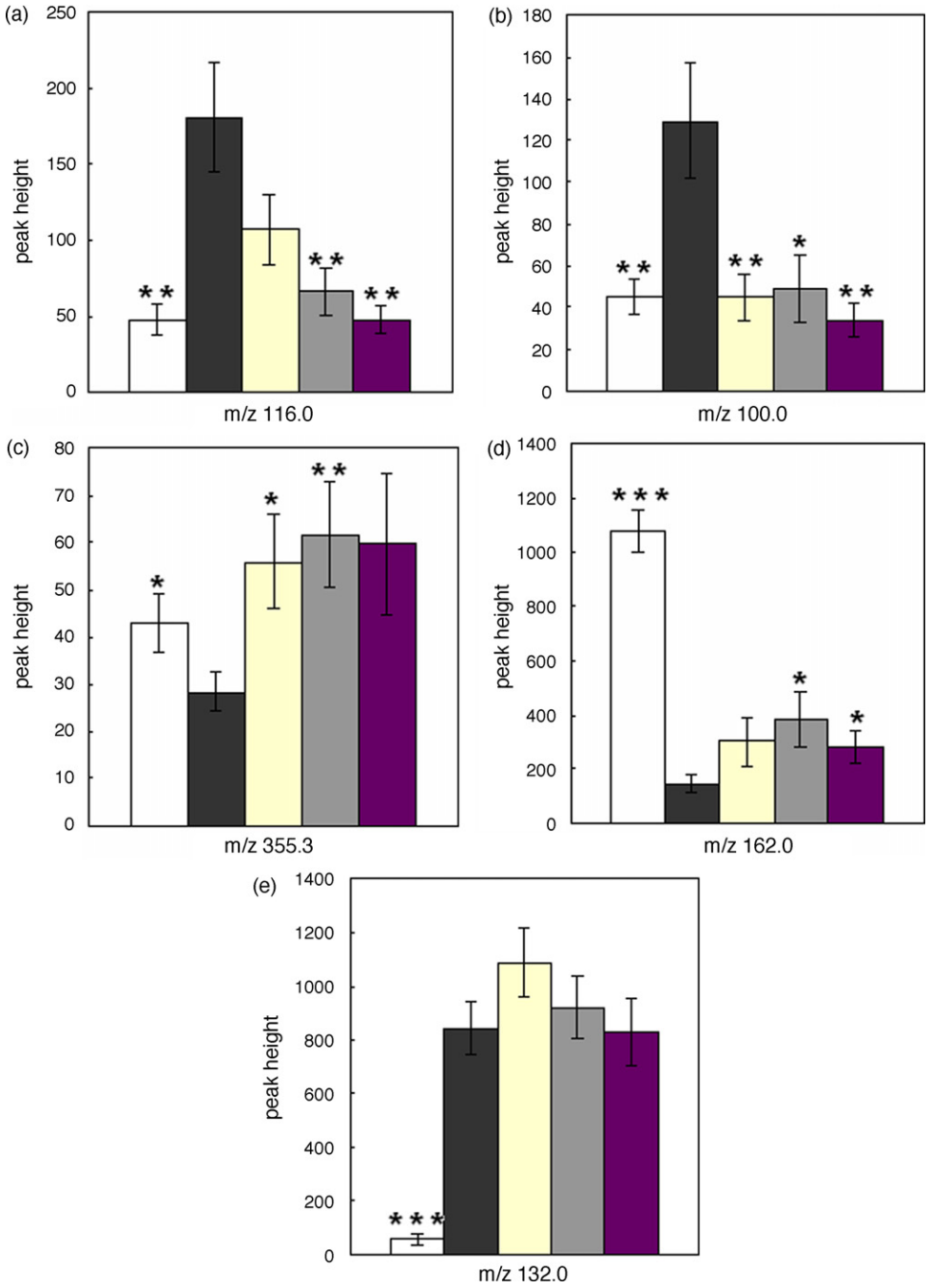

Fig. 4. Mean peak height (mean ± standard error) of some important ions. (□) control group, (■) model group, (■) low dose, (■) middle dose, (■) high dose. Student's *t*-test was used. *Compared with model $p < 0.05$, **compared with model $p < 0.01$, ***compared with model $p < 0.001$.

why it can depress blood viscosity and normalize the plasma lipid levels.

In the model group, phenylalanine and kynurenic acid were significantly increased, and they were found decreasing in the dose groups compared to the model group. The trends are similar to *m*/*z* 116.0 and *m*/*z* 100.0 (Fig. 4a and b). Phenylalanine is an essential amino acid, kynurenic acid is a metabolite of another essential amino acid tryptophan. Amino acids serve as substrates for protein synthesis, metabolic energy (oxidation through the carboxylic acid cycle), or gluconeogenesis and ketogenesis. Some amino acids are also substrates for neurochemical mediators [29]. Phenylalanine is the precursor of the amino acid tyrosine. Significant fate of tyrosine is a conversion to the catecholamines, e.g. dopamine, norepinephrine and epinephrine [30]. In our study, when the acute blood stasis models were made, the pathway of phenylalanine may be partly blocked, more phenylalanine was excreted to urine.

Tryptophan is metabolized via several pathways, and is the precursor for the biosynthesis of the neurotransmitter serotonin (5-hydroxytryptamine) a biochemical messenger and regulator, the kynurenine pathway is being paid increasing attention because it is involved in many diverse physiological and pathological processes [31,32]. Kynurenic acid is one of the few known endogenous excitatory amino acid receptor blockers with a broad spectrum

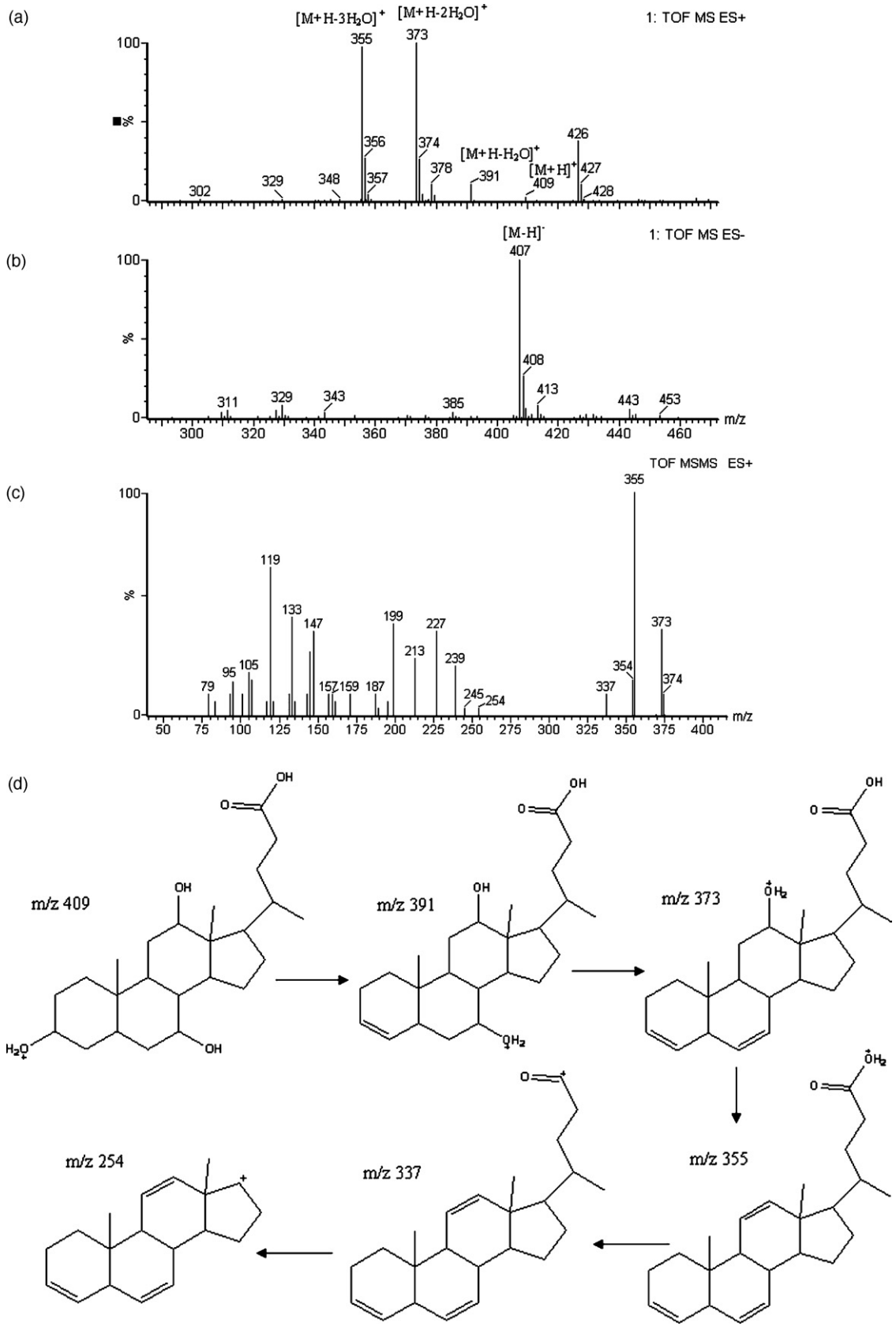

Fig. 5. Mass spectra at the retention time (tr) 14.03 min. (a) In positive ionization mode, (b) in negative ionization mode, (c) LC–ESI MS/MS spectrum, and (d) possible MS fragment mechanism.

Table 2
Identification results of potential biomarkers

Retention time (min)	Mass	Identification result	Pathway
14.03	355.3	Cholic acid	Bile acid biosynthsis
12.88	353.3	Dihydroxy cholanate	Bile acid biosynthsis
2.58	166.1	Phenylalanine	Phenylalanine metabolism, Phenylalanine, tyrosine and tryptophan biosynthsis
5.68	190.0	Kynurenic acid	Tryptophan metabolism
6.65	188.1	Tryptophan	Tryptophan metabolism, Phenylalanine, tyrosine and tryptophan biosynthsis
0.71	175.0	Arginine	Arginine and proline metabolism, Urea cycle and metabolism of amino groups
8.35	233.1	*N*2-Succinyl-L-ornithine	Arginine and proline metabolism, Urea cycle and metabolism of amino groups

of antagonistic properties in supraphysiological concentrations [33,34], has been proven to be neuroprotective [35] and decreasing pressor responses [36,37] in several experimental settings. In our study, tryptophan is also found different between the model group and the control group. So in the acute blood stasis model, the tryptophan pathway was possibly changed. The utilization of kynurenic acid may be lower than that in the normal, the ability of neuroprotective and decreasing pressor responses was also weakened. In the dose groups, the concentration of phenylalanine and kynurenic acid came back to normal. The results showed XinDi medicine has a good effect on blood stasis.

Arginine and *N*2-succinyl-L-ornithine contribute significantly to separate the model group and the control group, though there was no a significant change after the interventions of XinDi soft capsule. They are all in the pathway of urea cycle in which nitric oxide is synthesized. Nitric oxide plays a fundamental role in the vasculature because of its diverse influence on vascular protection including its well-reported antiproliferative, anti-inflammatory, anti-thrombotic and vasodilator effects [38,39].

4. Conclusions

A metabonomics method based on UPLC Q-TOF MS has been used to evaluate the efficacy of Xindi soft capsule on rat model of acute blood stasis. With pattern recognition analysis (PCA and PLS-DA), a clear separation of model group and control group was achieved. The dose groups were located between acute blood stasis model group and healthy control group. Combined with the result of hemorheology the change of urinary metabolic pattern shows the model group is moving toward the control group and the blood stasis was being prevented and alleviated. The effect of high group and middle group is more remarkable than that of low group. Some potential biomarkers like cholic acid, phenylalanine and kynurenic acid, etc., have been found and identified. The work shows that the metabonomics method is a valuable tool in the efficacy and mechanism research of TCM.

Acknowledgements

The study has been supported by the foundation from the State Ministry of Science and Technology of China (2006BAK02A12 and 2007DFA31060), foundation (No. 20425516) for Distinguished Young Scholars and the foundation (No. 20675082) from National Natural Science Foundation of China, and the Knowledge Innovation Program of the Chinese Academy of Sciences (K2006A14).

References

[1] V.B. Guliyev, M. Gul, A. Yildirim, J. Chromatogr. B 812 (2004) 291.
[2] T. Beveridge, T.S. Li, B.D. Oomah, A. Smith, J. Agric. Food Chem. 47 (1999) 3480.
[3] B. Yang, K.O. Kalimo, R.L. Tahvonen, L.M. Mattila, J.K. Katajisto, H.P. Kallio, J. Nutr. Biochem. 11 (2000) 338.
[4] A.K. Johansson, H. Korte, B. Yang, J.C. Stanley, H.P. Kallio, J. Nutr. Biochem. 11 (2000) 491.
[5] J.P. Suomela, M. Ahotupa, B. Yang, T. Vasankar, H. Kallio, J. Agric. Food Chem. 54 (2006) 7364.
[6] J.Y. Cheng, Q Tang, J.L. Yang, Chin. Trad. Patent Med. (Chinese) 28 (2006) 262.
[7] Y.Z. Feng, Y. Li, Z.J. Liao, Hippophae 19 (2006) 33.
[8] J. Cheng, K. Kondo, Y. Suzuki, Y. Ikeda, X. Meng, K. Umemura, Life Sci. 72 (2003) 2263.
[9] D. Bensky, A. Gamble, Chinese Herbal Medicine Materia Medica, rev. ed., Eastland Press, 1993, pp. 265–266.
[10] J.K. Nicholson, J.C. Lindon, E. Holmes, Xenobiotica 29 (1999) 1181.
[11] J.K. Nicholson, J. Connelly, J.C. Lindon, E. Holmes, Nat. Rev. Drug Discov. 1 (2002) 153.
[12] J.C. Lindon, E. Holmes, J.K. Nicholson, Pharm. Res. 23 (2006) 1075.
[13] J.C. Lindon, E. Holmes, J.K. Nicholson, Febs J. 274 (2007) 1140.
[14] D.G. Robertson, M.D. Reily, J.D. Baker, J. Proteome Res. 6 (2007) 526.
[15] J.L. Griffin, M.E. Bollard, Curr. Drug Metab. 5 (2004) 389.
[16] M. Chen, M. Su, L. Zhao, J. Jiang, P. Liu, J. Cheng, Y. Lai, Y. Liu, W. Jia, J. Proteome Res. 5 (2006) 995.
[17] P.Y. Yin, X.J. Zhao, Q.R. Li, J.S. Wang, J.S. Li, G.W. Xu, J. Proteome Res. 5 (2006) 2135.
[18] F.M. Li, X.M. Lu, H.P. Liu, M. Liu, Z.L. Xiong, Biomed. Chromatogr. 21 (2007) 397.
[19] W.B. Dunn, N.J. Bailey, H.E. Johnson, Analyst 130 (2005) 606.
[20] I.D. Wilson, J.K. Nicholson, J. Castro-Perez, J.H. Granger, K.A. Johnson, B.W. Smith, R.S. Plumb, J. Proteome Res. 4 (2005) 591.
[21] D.J. Crockford, J.C. Lindon, O. Cloarec, R.S. Plumb, S.J. Bruce, S. Zirah, P. Rainville, C.L. Stumpf, K. Johnson, E. Holmes, J.K. Nicholson, Anal. Chem. 78 (2006) 4398.
[22] F. Liao, Clin. Hemorheol. Microcirc. 23 (2000) 127.
[23] Y. Lu, Y.L. Hu, X.F. Kong, D.Y. Wang, J. Ethnopharmacol. 116 (2008) 313.
[24] X.J. Zhao, W.Z. Wang, J.S. Wang, J. Yang, G.W. Xu, J. Sep. Sci. 29 (2006) 2444.
[25] J. Chen, X.J. Zhao, J. Fritsche, P.Y. Yin, P. Schmitt-Kopplin, W. Wang, X. Lu, H.U. Haring, E.D. Schleicher, R. Lehmann, G.W. Xu, Anal. Chem. 80 (2008) 1280.
[26] L. Eriksson, E. Johansson, N. Kettaneh-Wold, S. Wold., Umetrics (2001).
[27] Z.R. Vlahcevic, W.M. Pandak, R.T. Stravitz, Gastroenterol. Clin. North Am. 28 (1999) 1.
[28] S. Perwaiz, B. Tuchweber, D. Mignault, T. Gilat, I.M. Yousef, J. Lipid Res. 42 (2001) 114.
[29] B.K. Lopansri, N.M. Anstey, G.J. Stoddard, E.D. Mwaikambo, C.S. Boutlis, E. Tjitra, H. Maniboey, M.R. Hobbs, M.C. Levesque, J.B. Weinberg, D.L. Granger, Infect. Immun. 74 (2006) 3355.
[30] J.D. Fernstrom, M.H. Fernstrom, J. Nutr. 137 (2007) 1539.
[31] D. Pawlak, Y. Takada, T. Urano, A. Takada, Brain Res. Bull. 52 (2000) 197.
[32] W.E. Dale, Y. Dang, O.R. Brown, Free Radic. Biol. Med. 29 (2000) 191.
[33] Z. Hartai, P. Klivenyi, T. Janaky, B. Penke, L. Dux, L. Vecsei, Acta Neurol. Scand. 112 (2005) 93.
[34] K. Sas, H. Robotka, J. Toldi, L. Vecsei, J. Neurol. Sci. 257 (2007) 221.
[35] A.M. Myint, Y.K. Kim, R. Verkerk, S. Scharpe, H. Steinbusch, B. Leonard, J. Affect Disord. 98 (2007) 143.
[36] R. De Matteo, G.A. Head, D.N. Mayorov, Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (2006) 257.
[37] L.G. da Silva Jr., R.C. Menezes, D.C. Villela, M.A. Fontes, Neuroscience 139 (2006) 1129.
[38] F. Feillet, J.V. Leonard, J. Inherit. Metab. Dis. 21 (Suppl. 1) (1998) 101.
[39] M.J. Romero, D.H. Platt, R.B. Caldwell, R.W. Caldwell, Cardiovasc. Drug Rev. 24 (2006) 275.