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Study of plasma metabolic profiling and biomarkers of chronic unpredictable mild stress rats based on gas chromatography/mass spectrometry

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A metabolomic investigation of chronic unpredictable mild stress (CUMS) rats was carried out. Plasma obtained from Sprague-Dawley (SD) rats treated by CUMS was analyzed using gas chromatography/mass spectrometry. Thirty-seven metabolites were identified among the detected compounds. Subsequent data analysis using the t test and principal component analysis (PCA) revealed significant metabolic changes in the rats' plasma after CUMS treatment. Clear separation between the model and control group was achieved, and the level of twelve metabolites, including amino acids, sugar, organic acids and fatty acids, were significantly different between plasma samples from the controls and CUMS group. These observations suggested that the depressed state may be associated with perturbation of amino acid metabolism, energy metabolism and glycometabolism. The study suggested that the metabolomics approach could be used as a potential powerful tool to investigate the biochemical change in certain physiopathological conditions, such as depression, as an early diagnostic means. Copyright © 2010 John Wiley & Sons, Ltd.

Depression is a serious public mental disease. It is a major cause of disability, suicide and physical disorders.[1] It amounts to 12.3% of the global burden of disease, and is predicted to rise by up to 15% by 2020.[2] Chronic unpredictable mild stress (CUMS), a well-validated animal model, has been used widely for studying clinical depression as well as evaluating antidepressant effects of diverse drugs.[3,4] Much of the work has been done successfully in individual gene expression, protein structure and function, as well as biochemical studies on sympathetic nervous system, such as, hypothalamic–pituitary–adrenocortical-axis, noradrenergic and immunological systems.[5–8]

The emerging metabolomics offer a promising opportunity to generate a novel hypothesis for addressing the molecular mechanisms of diseases in whole organisms, ultimately towards a comprehensive understanding of physiopathological outcomes of an organism. Metabolomics can provide much valuable information on stimuli-induced biochemical perturbations, with metabolic profiles carrying mechanism-related information, and the approach can be regarded as complementary to genomics and proteomics approaches.[9–12] It has been increasingly used as a versatile tool for the discovery of molecular biomarkers in many areas such as diagnosing or prognosing clinical diseases, exploring the potential mechanism of diverse diseases and assessing therapeutic effects of drugs.[13–16] Nuclear magnetic resonance (NMR) spectrometry, liquid chromatography/mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS), all capable of generating multivariate metabolic data, are the three most commonly used techniques in the field of metabolomics. GC/MS has been proven to be a robust metabolomic tool due to its high resolution and selectivity. Combined with the easily accessible NIST database,[17] GC/MS has gained more application in different fields.[18] One of the advantages is that large numbers of substances can be selectively determined in a single analysis of very complex samples. All metabolomics studies result in complex data sets that require visualization software and multivariate analysis methods for interpretation, in which principal component analysis (PCA), allowing the identification of groups of variables that interrelated via phenomena that cannot be directed observed, is one of the such methods widely used.

Recently, metabolomic studies on the metabolic disorder of brain tissues[19] and urine[20,21] from the CUMS treated rats have been reported. In addition, plasma of elder adults with depression and without depression have also been reported.[22] However, the metabolic pattern was also influenced by the antidepressant medications and other

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drugs, such as statins, and it was difficult to distinguish between the effects of the disease and effects of drugs. The primary goal of this work is to characterize metabolic abnormalities in plasma from the rat model of CUMS. Gas chromatography/mass spectrometry (GC/MS) was applied to reveal the metabolic profiling of the plasma samples from both a control group and the CUMS group. Endogenous metabolites for discrimination between the CUMS and control groups have been found to be potential plasma biomarkers for depression. This work will not only expand our understanding of molecular mechanism for depression, but also provide a constructive protocol to determine the state of depression, which may reveal valuable information for the early diagnosis of depression.

EXPERIMENTAL

Reagents

Pyridine, acetonitrile, n-heptane, methoxylamine hydrochloride (*O*-methyl hydroxylamine) were analytical grade from China National Pharmaceutical Group Corporation (Shanghai, China). *N*-Methyl-*N*-(trimethylsilyl)trifluoracetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Pierce Chemical Company (USA). n-Tetracosane (Johnson Matthey Company) was used as an internal quality standard. Alanine, valine, isoleucine, proline, glycine, serine, threonine, glutamic acid, phenylalanine, fructose, galactose, glucose, tyrosine, tryptophan, lactic acid, urea, phosphoric acid, butanedioic acid, hexadecanoic acid, linoleate, and octadecanoic acid, purchased from Solarbio (Shanghai, China), were used as standard substances.

Animals

The experiments were approved by national legislations of China and local guidelines. A total of 12 male Sprague-Dawley (SD) rats (200 ± 20 g) from the Experimental Animal Center of The National Institute for the Control of Pharmaceutical and Biological Products were employed in this study.

Chronic unpredictable mild stress procedure

After 2 weeks habituation, all the rats were divided into the following two groups, CUMS group and control group ($n = 8$), according to the body weights and behavior scores in open-field experiments. The CUMS procedures include nine different kinds of stressors as follows: cage tilting for 24 h; damp sawdust for 24 h (200 mL of water per individual cage, which is enough to make the sawdust bedding wet); noises for 1 h (alternative periods of 60 dBA noise for 10 min and 10 min of silence); swimming in 4°C cold water for 5min; exposure to an experimental room at 50°C for 5min; 24 h of food deprivation and 24 h of water deprivation, respectively; tail clamp for 1 min; 15 unpredictable shocks (15 mA, one shock/5 s,10 s duration); restricted movement for 4 h. One stressor was applied per day and the whole stress procedure lasted for 4 weeks with a completely random order. The control rats were housed together, while the model rats were housed individually. All rats were maintained under standard laboratory conditions (24 ± 1°C, 45 ± 15% relative humidity, and 12 h/12 h light/dark cycle), and had free access to distilled water and food except for the period of water and food deprivation prior to the sucrose preference test.

Behavior test

Open-field test

The open-field test was performed as previously described[23] and was conducted between 8:00 am to 12:00 am in a quiet room ($\leq$60 dB). The open-field apparatus consisted of a square arena 100 cm × 100 cm, with a 40-cm-high side wall, the floor marked with a grid dividing it into 25 equal-size squares. Each animal was tested in the apparatus once. It was placed in the central square and observed for 5 min. Scores were calculated by the amount of time it spent rearing (defined as standing upright on its hind legs), and the number of crossing (grid lines it crossed with at least three paws) and deject numbers.

Sucrose preference test

The procedure was performed as described previously.[23] After a 24-h period of water and food deprivation, each rat was subjected to an individual metabolic cage in which two bottles containing water and 1% sucrose solution were placed. The ratio of the amount of sucrose solution to that of total solution ingested within 1 h represented the parameter of hedonic behavior.

Statistics analysis

Quantitative data were presented as mean ± standard deviation (SD). The significance of variation between groups in data of behavior changes was determined using independent-sample t-test by SPSS 11.5 for Windows.

Sample collection and preparation

Twenty-four hours after the final stress, all the rats were anesthetized with a peritoneal injection of ethyl carbamate, and blood samples were collected quickly in heparinized tubes from the femoral artery. Plasma was obtained by centrifugation at 3000 *g*, 4°C for 10 min and was frozen immediately at −80°C prior to analysis. Plasma was thawed at 4°C and vortex-mixed before use. The plasma samples (100 μL) were protein precipitated using 2.5 volumes of acetonitrile and the samples were then centrifugeds at 12 000 *g* for 10 min at 4°C. A volume of 150 μL of each supernatant was evaporated to dryness under vacuum prior to derivatization. All the dried samples were derivatized with 30 μL methoxylamine hydrochloride (15 mg/mL in pyridine) at 70°C for 60 min, followed by 50 μL MSTFA at 40°C for 90 min; 700 μL of heptane with 0.1 mg/mL of tetracosane as internal standard was added to each GC vial, and vortex-mixed for 2 min before GC analysis.

GC/MS method

GC/MS analysis was performed using a Polaris Q ion trap mass spectrometer (Thermo Fisher Scientific Inc., USA). Chromatography was performed on a DB-5MS capillary column (30 m × 250 μm i.d., 0.25 μm film thickness; 5% diphenyl cross-linked 95% dimethylpolysiloxane; Agilent J&W Scientific, Folsom, CA, USA). Helium carrier gas was used at a constant flow rate of 1 mL/min. Volumes of 1.0 μL of derivatized samples were injected into the GC/MS instrument. To acquire a good separation, the column temperature was initially maintained at 60°C for 3 min,

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and then increased from 60 to 140°C at a rate of 7°C/min for 4 min. Then, the column temperature was increased to180°C at 5°C/min for another 6 min. After that, the temperature was increased to 280°C at 5°C/min, and held for 2 min. The injection, interface, and source temperatures were set at 200°C, 280°C, and 200°C, respectively. After a solvent delay of 9 min, MS detection was implemented in electron ionization mode (electron energy of 70 eV) and full scan mode (*m/z* 50–650).

Identification of the endogenous metabolites

All collected plasma samples were analyzed, and low molecular weight metabolites were represented as the chromatographic peaks in the GC total ion current (TIC) chromatograms. Peaks with intensity higher than 10-fold of the signal-to-noise (S/N) ratio were recorded and integrated. EI-MS spectra of these peaks were interpreted using AMDIS (version 2.1, DTRA/NIST, USA) software, and identification of metabolites were based on the NIST library 2005, some of which were further confirmed using the commercial available standards by comparing their MS spectra and retention times.

Data analysis

All the GC/MS raw files were converted into NetCDF format via Xcalibur (Thermo Fisher Scientific Inc., Waltham, MA, USA), and subsequently processed by the XCMS toolbox[24] using the default settings of XCMS with the following exceptions: XCMS Set (full width at half maximum: fwhm = 4; 4; S/N cutoff value: snthresh = 10, max = 20), group (bw = 10). The resulting table was exported into Microsoft Excel, where normalization was performed prior to multivariate analysis. The resulting three-dimensional matrix, involving peak index (RT-m/z pair), sample names (observations), and normalized peak area percent, were introduced into SIMCA-P 11.0 software package (Umetrics AB, Umea, Sweden). The mean-centered and pareto-scaled data were analyzed in SIMCA-P 11.0, utilizing PCA to display natural separation among the two groups by visual inspection of score plots.

The intensities of the identified metabolites in the control and CUMS groups were compared using the independent-samples t-test. To minimize the number of missing values, only identified metabolites that were consistently detected in at least 80% of the samples were included in this t-test, a value of $P < 0.05$ was considered statistically significant. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG)[25] and Human Metabolome Database (HMDB)[26] database, the metabolic pathways which were affected by the CUMS stress were identified.

RESULTS AND DISCUSSION

Effect on open-field activity scores, body weight and sucrose preference

Open-field test, body weight and sucrose preference test were measured during the experimental period. In these tests, there are significant differences between the rats in the CUMS group and the control group (Fig. 1). Rats in the CUMS group showed a significant decrease in the number of rearing and crossing ($P < 0.01$), the sucrose preference test ($P < 0.05$), and also in the body weight ($P < 0.01$) compared with the control group, indicating the stress-related effects on the rats.

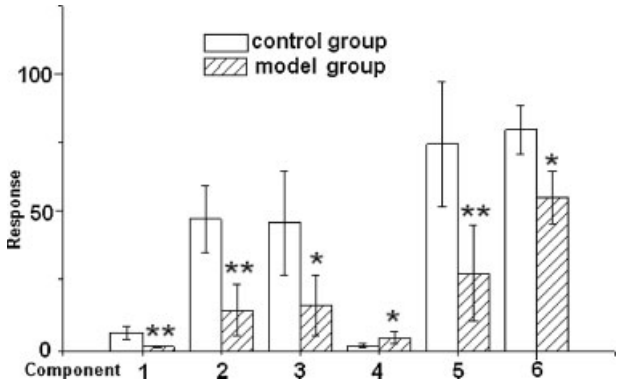

Figure 1. Changes in behavior in the control group and the model group, 1 rearing, 2 crossing, 3 grooming, 4 deject numbers, 5 body weight change, 6 sucrose preference. Data are represented as mean ± SD. n = 6 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group.

Method validation of GC/MS analysis

Precision of injection

Precision of injection was carried out by the continuous analysis of six injections of the same sample. Stable retention time of the peaks was observed, and the main peaks were presented without any retention time drift, the relative standard deviation (RSD) values of retention time were less than 0.1% and peak area was less than 10%. The stability of retention time was of great benefit for matching and extracting the commensal peaks and the precision of injection reflected the stability of GC/MS analysis, which was of great importance to guarantee the reliability of the acquired metabolomic data.

Repeatability of sample preparation

Sample preparation before GC/MS analysis should be performed carefully to ensure the stability of metabolomic analysis. Six parallel samples were prepared using the same preparation protocol to examine the repeatability of sample preparation, and the resulting data showed that the repeatability of sample preparation was satisfactory for metabolomic analysis. Almost all the corresponding peaks were presented at the same retention times, and RSD values of the peak area for the main peaks were less than 10%.

Stability

The stabilities of most of the 12 test compounds in the plasma were acceptable, with RSDs of less than 5% within 24 h (1 h, 2 h, 4 h, 8 h, 12 h, 24 h). Therefore, all of the samples from each batch were analyzed within 24 h, and maintenance of the equipment was carried out after every 24-h analysis.

GC/MS metabolic profiling of plasma

Plasma metabolic profiles of six CUMS rats and six control rats were obtained by GC/MS with the method as described above. Typical GC/MS total ion current (TIC) chromato-

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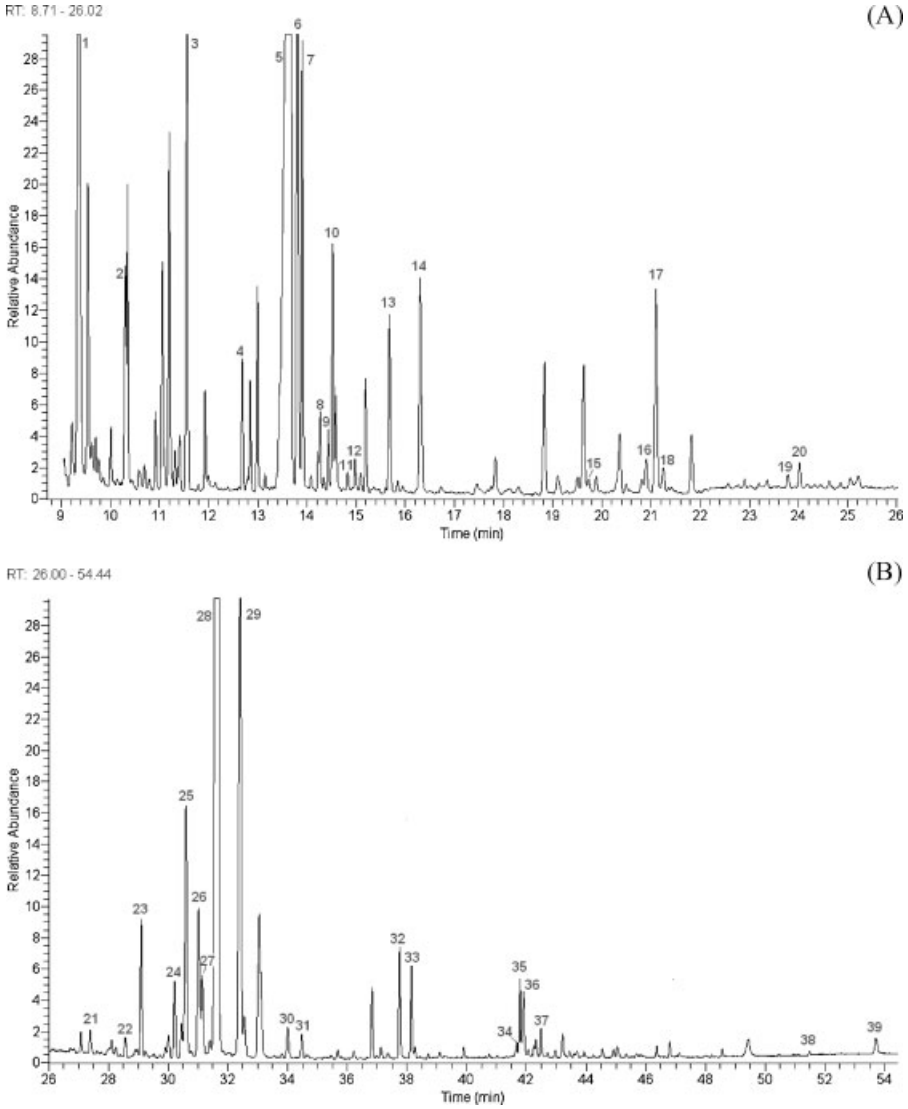

Figure 2. Chromatographic profiles of the derivatized plasma. Peaks: *1* lactic acid, *2* alanine, *3* 3-hydroxybutyric acid, *4* valine, *5* urea, *6* glycerol, *7* phosphoric acid, *8* isoleucine, *9* proline, *10* glycine, *11* butanedioic acid, *12* 2,3-dihydroxypropanoic acid, *13* serine, *14* threonine, *15* malic acid, *16* 4-hydroxyproline, *17* glutamine, *18* 2,3,4-trihydroxybutyric acid, *19* glutamic acid, *20* phenylalanine, *21* glycerophosphoric acid, *22* 1,4-benzenedicarboxylic acid, *23* citric acid, *24* deoxyglucose, *25* fructose, *26* fructose, *27* galactose, *28* glucose, *29* glucose, *30* tyrosine, *31* turanose, *32* hexadecanoic acid, *33* myo-inositol, *34* tryptophan, *35* linoleate, *36* oleate, *37* octadecanoic acid, *38* monostearin, *39* cholesterol.

grams are shown in Fig. 2. The identification of endogenous metabolites was based on comparison with the corresponding standards according to their retention times and mass spectra characteristics or searching the mass spectral database library NIST 2005.

The identification of amino acids was compared with the standards based on their retention time and EI fragments. An example is presented in Fig. 3 for the identification of valine. The identification of sugars, such as fructose, was challenging, since they are all isomers with the same molecular weights and exhibit very similar fragment ions in EI spectra. Therefore, they could not be identified exactly only by comparison with the NIST 2005 library. Authentic standards should be introduced to obtain a TIC and EI spectrum, with which the specified peak in the TICs of samples should be consistent. The identification of fructose is shown in Fig. 4, two peaks appeared in the TIC chromatogram due to the two isomers produced by oximation. The other kinds of metabolites were tentatively determined based on their EI-MS data by searching the NIST 2005 mass spectra library.

In all, 37 metabolites were identified in the plasma profiling in this study, including amino acids, fatty acids, sugars, and organic acids. The peaks in TIC profiles of the plasma samples represented the endogenous metabolites in plasma. Therefore, each TIC profile could be considered as a fingerprint of endogenous metabolites in plasma.

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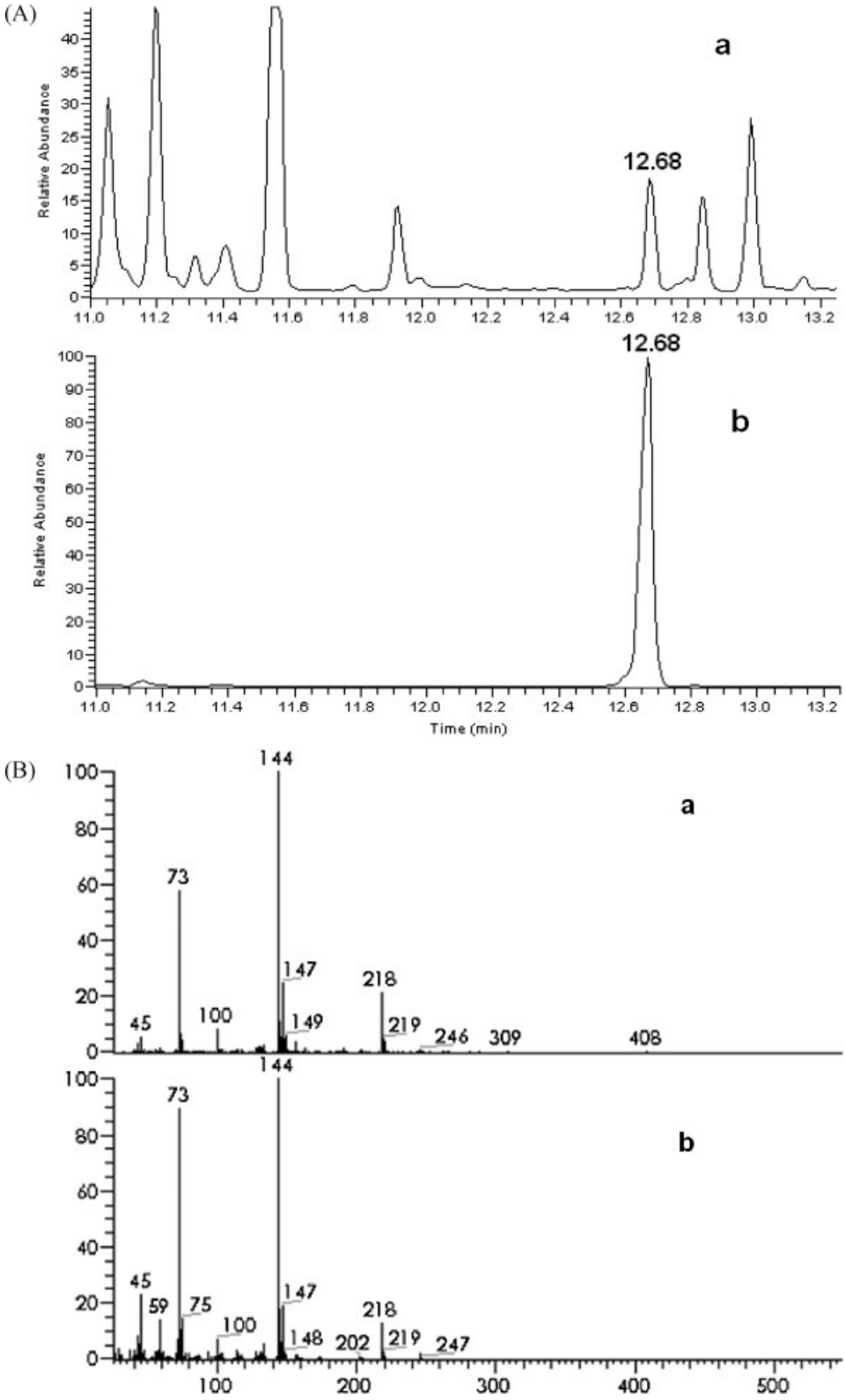

Figure 3. Identification of amino acid in plasma samples: (A) expanded regions of TIC profiles for (a) a plasma sample and (b) a valine standard. (B) EI-MS spectra for the peak (12.68) identified as valine from (a) plasma sample and (b) the authentic standard.

Changes in plasma metabolites of CUMS rats

Thirty-seven identified metabolites were analyzed by independent-samples t-test (Table 1). Among them, the concentrations of 12 metabolites, including 5 amino acids, 3 fatty acids, 2 organic acids and 2 sugars, were different between plasma samples from the control and CUMS groups. In particular, the concentrations of tyrosine, glutamic acid, tryptophan, hexadecanoic acid, linoleate and octadecanoic acid were significantly lower in the CUMS group than those in the control group, while the levels of glycine, butanedioic acid, 2,3-dihydroxypropanoic acid, glutamine, fructose and glucose were significantly increased in CUMS

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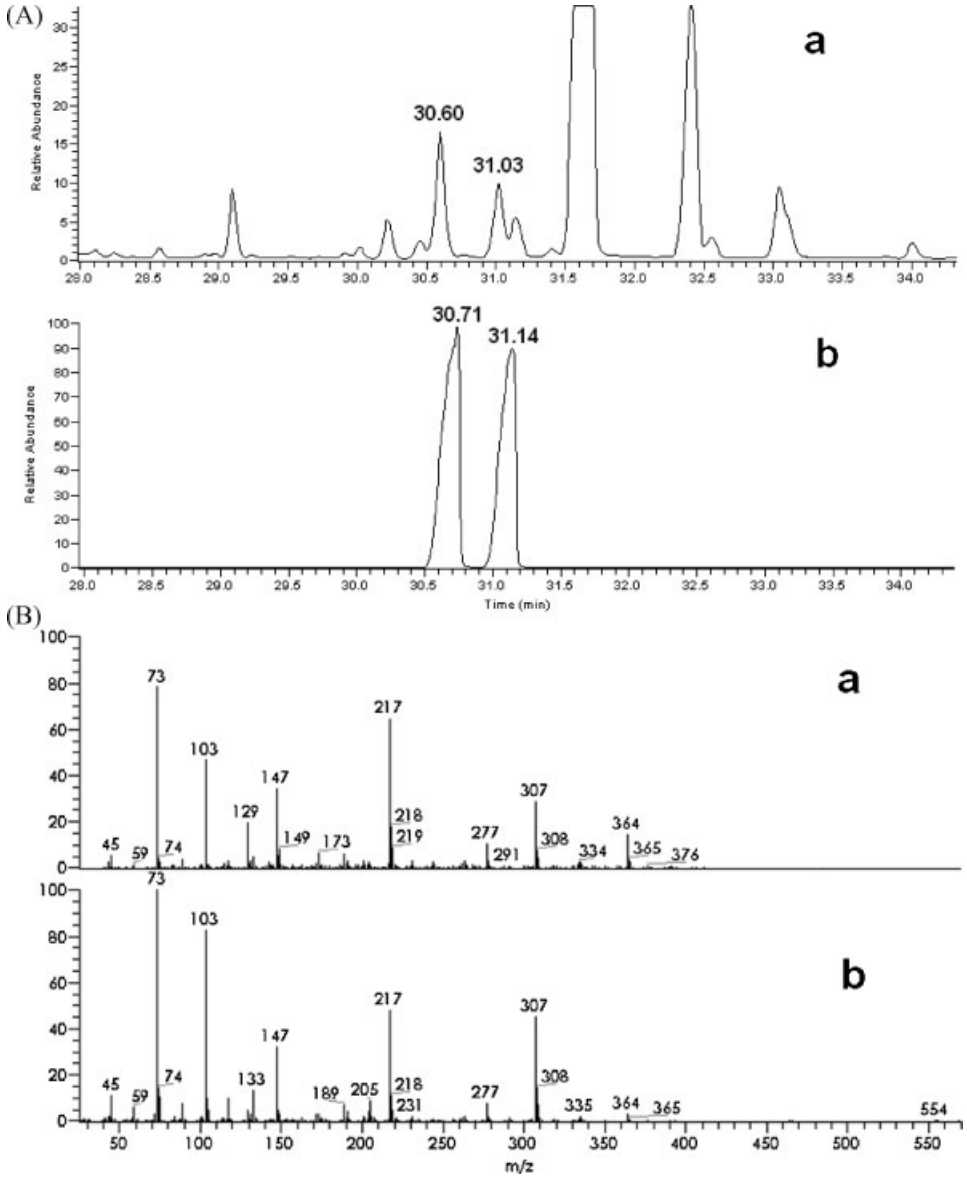

Figure 4. Identification of sugars in plasma samples: (A) expanded regions of TIC profiles for (a) a plasma sample and (b) a fructose standard. (B) EI-MS spectra for the peak (30.60) identified as fructose from (a) plasma sample and (b) the authentic standard.

group. These results strongly suggest that metabolic differences existed between the CUMS and control groups due to the CUMS treatment.

Pattern recognition analysis

PCA is a commonly available method for metabolomic data analysis. As an unsupervised method, PCA projects multivariate data to a low-dimensional plot to discover the similarities and differences within a dataset. In the present study, all the GC/MS data were further analyzed by PCA to visualize the plasma metabolic profiling difference between CUMS and control rats. The inherent metabolic difference among plasma and relation to stress factors can be reflected by a PCA scores plot (PC1 versus PC2) (Fig. 5). The X-axis represents the scores of PC1, which account for 60.4% of the total variance, while the Y-axis shows the scores of PC2, which account for 17.6% of the total variance. In this map, the CUMS group can be clearly separated from the control group along PC1. These findings suggested that the plasma metabolic pattern was significantly changed under the CUMS treatment.

Biochemical interpretation

Amino acid metabolism perturbation

Glycine, an inhibitory neurotransmitter,[19] was increased in the CUMS group ($P < 0.05$); while glutamic acid, the excitatory neurotransmitter in the mammalian nervous system,[19] was significantly decreased in the CUMS group. In addition, glutamine in the plasma of the CUMS group was significantly increased, suggesting that the glutamate and glutamine biosynthesis was affected after CUMS treatment. Tryptophan is metabolized via several pathways, and is the precursor for the biosynthesis of the neurotransmitter serotonin (5-hydroxytryptamine), a biochemical messenger and regulator. In this study, the concentration of tryptophan in the plasma was significantly lower ($P < 0.01$) in the CUMS group than the control group, which was in agreement with

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Table 1. Relative levels of metabolites detected by GC/MS in the plasma of rats following CUMS treatment

No.	Retention time (min)	Identification result	Relative intensity in control group means ± SD	Relative intensity in model group means ± SD	Independent-sample T test (*P* value)
1	9.36	lactic acid[a]	157.43 ± 25.01	164.35 ± 25.59	0.646
2	10.30	alanine[a]	6.35 ± 0.91	5.58 ± 1.33	0.274
3	11.55	3-hydroxybutyric acid[b]	15.76 ± 6.22	23.07 ± 9.87	0.156
4	12.68	valine[a]	4.46 ± 1.11	4.56 ± 1.13	0.880
5	13.60	urea[a]	164.15 ± 45.27	132.98 ± 18.00	0.164
6	13.80	glycerol[b]	132.95 ± 31.18	159.78 ± 50.15	0.339
7	13.91	phosphoric acid[a]	15.45 ± 6.36	16.81 ± 8.14	0.755
8	14.27	isoleucine[a]	3.19 ± 0.82	4.16 ± 1.57	0.210
9	14.43	proline[a]	2.77 ± 0.50	2.68 ± 0.28	0.707
10	14.51	glycine[a]	10.27 ± 0.36	11.12 ± 0.44	0.025*
11	14.83	butanedioic acid[a]	0.28 ± 0.08	0.48 ± 0.09	0.004*
12	14.98	2,3-dihydroxypropanoic acid[b]	0.89 ± 0.15	1.26 ± 0.30	0.023*
13	15.68	serine[a]	6.28 ± 1.24	6.01 ± 0.67	0.717
14	16.30	threonine[a]	8.44 ± 2.02	8.99 ± 1.03	0.567
15	19.73	malic acid[b]	0.66 ± 0.09	0.71 ± 0.13	0.568
16	20.90	4-hydroxyproline[b]	2.46 ± 0.55	2.17 ± 0.21	0.279
17	21.10	glutamine[a]	7.91 ± 2.25	11.61 ± 1.92	0.012*
18	21.25	2,3,4-trihydroxybutanal acid[b]	0.59 ± 0.1	0.60 ± 0.07	0.862
19	23.78	glutamic acid[a]	1.37 ± 0.19	1.07 ± 0.06	0.013*
20	24.02	phenylalanine[a]	1.36 ± 0.22	1.18 ± 0.29	0.272
21	27.39	glycerophosphoric acid[b]	0.49 ± 0.07	0.58 ± 0.12	0.180
22	28.57	1,4-benzenedicarboxylic acid[b]	0.23 ± 0.08	0.28 ± 0.06	0.227
23	29.10	citric acid[a]	5.34 ± 1.09	6.69 ± 0.50	0.084
24	30.21	deoxyglucose[b]	2.94 ± 0.61	2.85 ± 0.54	0.798
25	30.60	frucose[a]	2.51 ± 0.94	10.27 ± 1.62	0.000**
26	31.03	frucose[a]	1.34 ± 0.73	6.42 ± 1.26	0.000**
27	31.14	galactose[a]	4.86 ± 1.16	5.91 ± 0.75	0.092
28	31.66	glucose[a]	195.16 ± 24.23	291.52 ± 47.90	0.001**
29	32.41	glucose[a]	52.63 ± 18.47	77.38 ± 16.40	0.034*
30	34.01	tyrosine[a]	2.61 ± 0.61	1.42 ± 0.34	0.029*
31	34.48	turanose[b]	1.48 ± 0.60	2.07 ± 0.39	0.072
32	37.75	hexadecanoic acid[a]	4.70 ± 0.42	3.10 ± 1.18	0.036*
33	38.15	myo-inositol[b]	4.05 ± 0.76	4.09 ± 0.61	0.935
34	41.68	tryptophan[a]	1.37 ± 0.31	0.83 ± 0.22	0.007**
35	41.79	linoleate[a]	3.97 ± 0.60	2.74 ± 0.94	0.023*
36	41.91	oleate[a]	2.89 ± 0.64	4.05 ± 2.26	0.276
37	42.49	octadecanoic acid[a]	0.72 ± 0.11	0.54 ± 0.10	0.047*
38	51.49	monostearin[b]	0.05 ± 0.02	0.05 ± 0.01	0.992
39	53.71	cholesterol[b]	0.34 ± 0.09	0.26 ± 0.04	0.185

Relative intensities of metabolites in the control group and model group were expressed as mean ± SD (n = 6). *P <0.05, **P <0.01

[a] Metabolites identified by comparing with NIST 2005 database and authentic standards.

[b] Metabolites identified by comparing with literature and NIST 2005 database.

the previous report that tryptophan deficiency may induce depressive disorders.[27] The results provide evidence for the association between the 5-HT pathway and the onset of depression. Tyrosine was significantly decreased in the CUMS group compared with the control group. Tyrosine was produced by phenylalanine hydroxylation from phenylalanine, and its decreased level suggested the lower phenylalanine hydroxylase activity, which was in agreement with a previous report that the plasma phenylalanine-tyrosine ratio was increased in depressed patients.[28]

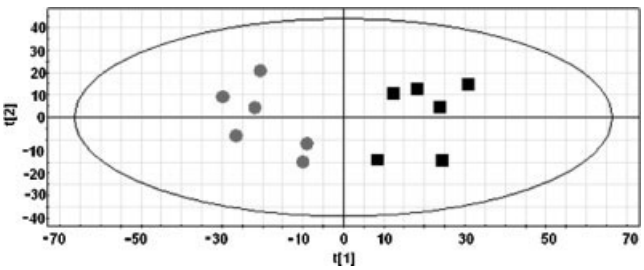

Figure 5. PCA scores (■ – CUMS treated rats, ● – control rats) plot based on the data derived from the plasma samples of rats.

Energy metabolism disturbance

Fatty acids can be decomposed by β-oxidation to acetyl coenzyme A to participate in the energy supply for the body. In this study, hexadecanoic acid, linoleate and octadecanoic acid, associated with metabolic pathways related to the tricarboxylic acid (TCA) cycle, were lowered considerably in the CUMS group compared with the controls, suggesting the dysfunction of the TCA cycle. The decrease of fatty acid levels detected in this study may be caused by fatigue due to resisting the physical stressors. Energy deficiency or fatigue is one of the most frequently represented depressive symptoms in major depressive disorder.[29]

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Glycometabolism

The concentrations of frucose and glucose in the CUMS group were increased significantly. It has been reported that depression is associated with glucose metabolism in the biological mechanisms and glucose metabolism may be affected by the abnormal secretion of depression-related hormone.[30] Most scholars believed that the increased content of sugar was related with the lack of norepinephrine (NE) and 5-HT (5-HT) in the brain.[31,32] The previous study found that the concentrations of adrenocortical hormone or its metabolites were increased in patients with depression. In addition, the concentration of blood sugar can be further increased by the increased secretion of adrenocortical hormone.[33,34]

CONCLUSIONS

In this study, we applied GC/MS to the metabolomic analysis of plasma obtained from CUMS rats, aiming to investigate the biomarker of depression. Thirty-seven metabolites were identified among the detected compounds from TIC chromatograms using the authentic standards and the NIST 2005 mass spectral database. The concentrations of 12 metabolites were observed to be significantly changed in the CUMS group when compared with the controls. The elevated or decreased endogenous metabolites in the plasma of CUMS rats suggested a different metabolic pattern between the CUMS and control groups. Using the KEGG pathway database, it was found that amino acid metabolism, energy metabolism and glycometabolism were affected after the CUMS treatment. This is the first report of a metabolomics study on the CUMS depression model based on the GC/MS metabolic profiles of rats plasma, and 12 endogenous metabolites could be considered as potential biomarkers of depression. When compared with the previously reported urinary biomarkers,[20,21] seven of them, including linoleate, octadecanoic acid, glycine, butanedioic acid, 2,3-dihydroxypropanoic acid, fructose, and glucose, were newly discovered. In addition, tyrosine, linoleate, octadecanoic acid, butanedioic acid, 2,3-dihydroxypropanoic acid, fructose, and glucose were also different from those biomarkers in brain regions.[19] It is noteworthy that glycometabolism was found to be related to the depression from a plasmatic metabolomics study of the CUMS depression model. The above findings suggested that the biomarkers were different in various biological samples, and they were complementary to each other for fully understanding the progress of disease. It is interesting to note that the lowered level of octadecanoic acid in the plasma of CUMS rats was in agreement with the previous study on the plasma of elderly depression, suggestive of alterations in fatty acid metabolism in depressed patients.[22] The determination of potential biomarkers of depression may be useful for the early clinical diagnosis of depression, evaluating the treatment strategy, and measuring the outcomes.

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