Xuebijing Injection Affects the Dynamic Change of Metabolism in CLP-Induced Septic Rats

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Abstract

Xuebijing injection has been widely applied to treat sepsis. However, its roles in the dynamic change of metabolism in sepsis are still unknown. In our study, Gas chromatography-mass spectrometer (GC-MS) combined with multivariate statistical techniques was used to detect the metabolic change in septic rats with or without XBJ injection treatment. The KEGG pathway analysis was used to further analyze the related metabolic pathways in which the identified metabolites were involved. Based on the fold change, variable important in projection, and $P$ value, we found 11, 33 and 26 differential metabolites in the sepsis group at 2, 6 and 12 hours post CLP, compared with the control group. Besides, we also found 32, 23 and 28 differential metabolites in the XBJ group at 2, 6 and 12 hours post CLP. The related pathways of differential metabolites were glycometabolism at 2h, glycometabolism and amino acid metabolism at 6h and amino acid metabolism at 12h post CLP in the sepsis group compared with the control group. Besides, glycometabolism, amino acid metabolism and lipid metabolism changed markedly after XBJ injection for 2 hours; while only amino acid metabolism changed significantly with the treatment of XBJ injection for 6 and 12 hours, compared with the sepsis group. Further analysis showed 3, 6 and 6 differential metabolites were overlapped in the sepsis group and XBJ group at 2, 6 and 12 hours post CLP. These identified differential metabolites were majorly involved in arginine and proline metabolism, suggesting that XBJ injection is capable of improving metabolic disorders in CLP-induced septic rat to a certain extent.

Introduction

Sepsis is a life-threatening organ dysfunction that is caused by dysregulated host responses to infection\([\text{1}]\). Sepsis is one of the most common causes of mortality in critically ill patients worldwide, and approximately 3 million cases and over 1 million deaths occur annually due to sepsis in China. It has been reported that the survival rate of septic patients decrease by 7.6% for each hour delay in treatment\([\text{2}]\). Therefore, the earlier the disease is identified and diagnosed, the better the expected outcomes\([\text{3}]\). The mechanism of sepsis has not been clarified completely, it has been reported that metabolic disturbance is one of the important features during the pathophysiology of sepsis\([\text{4}]\).

Metabolomics is a systematic method for the qualitative and quantitative analysis of all metabolites in a certain biological or cell-specific physiological period\([\text{5}]\). The alternation in the metabolic pathways, which results in impaired function of cells, perhaps plays an essential role in the pathogenesis of sepsis\([\text{6, 7}]\). Using a metabolomics approach to analyze relative concentrations of multiple metabolites\([\text{8}]\) is helpful for us to discover some effective prevention and treatment strategies by identifying the differential metabolites\([\text{9, 10}]\). GC-MS (gas chromatography - mass spectrometer), which combines gas chromatography with mass spectrometry, allows to detect the dynamic changes of metabolites and perform stoichiometric analysis, thus elucidating the molecular mechanisms of diseases\([\text{11, 12}]\).
Xuebijing (XBJ) injection, a traditional Chinese medicine, has been approved by the State Food and Drug Administration (SFDA) of China and showed a promising clinical therapeutic effect\cite{13}. Several studies reported that XBJ injection functioned to anti-endotoxin, inhibit inflammation, restore blood coagulation, and adjust immunity\cite{14,15}. XBJ injection is widely used in the treatment of sepsis and its beneficial effects have been observed. However, its effects in the dynamic metabolism changes during the early stage of sepsis are still unknown. In this work, a metabolomic approach based on GC-MS was applied to analyze the dynamic alternation of metabolite in cecal ligation and puncture (CLP)-induced septic rats at different time points. Besides, the effects of XBJ injection in the dynamic changes of metabolism were investigated.

**Materials And Methods**

**Animals**

Male Sprague Dawley (SD) rats (8 weeks old) were purchased from the Saike Jingda experimental animal Co. Ltd (Changsha, Hunan, China). All rats were housed in a specific pathogen-free environment at 25°C with a 12:12 h day/night cycle and raised in separate cages. The animals applied and the protocols operated in our study were approved by the Medical Ethics Committee of Hunan Provincial People's Hospital. The rats were divided into three groups randomly: (1) control group: rats were only freed the cecum but without ligation and perforation; (2) sepsis group: rats underwent CLP; (3) XBJ group: rats received XBJ injection treatment post CLP.

**CLP-induced sepsis and Xuebijing injection treatment**

All rats were fasting for 8 hours and depriving of water for 4 hours before CLP. Firstly, the animals were anesthetized by 2% isoflurane and kept them under anesthesia until the end of the operation. Then an incision was made along the abdominal to expose the cecum under aseptic conditions. The area 1 cm away from the cecum blind end was tightly ligated with 4-0 silk and then punctured twice with a 22-gauge needle. The cecum was returned to the peritoneal cavity and the abdomen was closed in two layers. Rats in the control group were operated similarly except for the ligation and perforation of the cecum. After the operation finished, the rats were injected intraperitoneally with 4 mL/kg Xuebijing or sterile normal saline.

**Serum collection**

The blood was obtained on the 2, 6, and 12 hours after CLP and collected in coagulation-promoting vacuum tubes. Then the obtained blood was incubated at room temperature for 30min and centrifuged at 3000rpm/min for 20min. Lastly, the acquired serum was stored at -80°C for further analysis.

**Sample preparation**

50μL of serum was used for the GC-MS analysis. Meanwhile, 10μL of serum was selected from each sample, which was mixed as quality control (QC) samples to verify the stability of the GC-MS system.
Firstly, 10\( \mu L \) L-2-chlorophenyl alanine (internal standard, 0.3mg/mL) was added to the sample. After vortex mixing for 15s, 150\( \mu L \) methanol and acetonitrile mixtures (2:1, v/v) were added to precipitate the proteins. After sonication on ice-bath, the samples were centrifuged for 15min (15000rpm, 4\(^\circ\)C). The supernatant was transferred to the glass-derived bottle and dried quickly by the rapid centrifuge concentrator. Then, 80\( \mu L \) methoxylamine pyridine hydrochloride solution was added and oximated at 37\(^\circ\)C in an oscillating incubator for 90min. After finished, 80\( \mu L \) BSTFA derived reagent and 20\( \mu L \) n-hexane were added. Then the samples were swirled for 2min and incubated at 70\(^\circ\)C for 1h. Lastly, the samples were removed and placed at room temperature (30\(^\circ\)C) for further GC-MS analysis.

**GC-MS analysis**

A DB-5 MS capillary column (30m×0.25mm×0.25um) was used for the non-targeted metabolites’ study. Helium(99.99%) was applied as the carrier gas with a flow rate of 1.0mL/min. The temperature program was set as follows: 15\(^\circ\)C/min, 50-125\(^\circ\)C; 5\(^\circ\)C/min, 125-210\(^\circ\)C; 10\(^\circ\)C/min, 210-270\(^\circ\)C; 20\(^\circ\)C/min, 270-305\(^\circ\)C and held at 305\(^\circ\)C for 5min. The temperature of injector and EI source was 260\(^\circ\)C and 230\(^\circ\)C, respectively. The mass spectrometer was operated at 70eV.

**Statistical processing and analysis**

The raw data acquired by GC-MS was processed by Chroma TOF and exported as CSV format, which contained the information of the sample, metabolite, retention time, molecular mass to electron charge (m/z) ratio, and mass spectrometry response intensity.

MetaboAnalyst 5.0 ([https://www.metaboanalyst.ca/](https://www.metaboanalyst.ca/)) was used to analyze the data obtained by GC-MS. Partial least squares discriminate analysis (PLS-DA) was used to detect the dynamic alternation of metabolites. Differential metabolites were screened based on the fold change (FC>1.5 or <0.67), variable important in projection (VIP>1.5), and \( P \) value (\( P<0.05 \)). KEGG (Kyoto Gene and Genomic Encyclopedia) pathway analysis was applied to analyze the signaling pathway in which the differential metabolites were involved.

**Results**

**Metabolic profiles among the control group, sepsis group and XBJ group**

A series of metabolites were obtained at 2h, 6h and 12h post CLP in rats with or without XBJ injection treatment via GC-MS analysis. All of these differential metabolites detected were showed in supplementary table 1, 2 and 3, respectively.

**Screening of differential metabolites in serum samples**

PLS-DA, a multivariate statistical analysis, acted to find differential metabolites and discriminate different groups. Our results showed that the PLS-DA model demonstrated a distinct separation in the metabolic profiles among the control group, sepsis group, and XBJ group (Figure 1).
To explore the effect of XBJ injection in the dynamic changes of CLP-induced septic rat. Statistical tests, including fold change (FC), P-values and VIP scores, were applied to detect the significant metabolites in the sepsis group and XBJ injection group. Before analysis, the raw data acquired by GC-MS were normalized by median (supplementary figure 1). Based on VIP scores (VIP >1.5), fold change (FC >1.5 or <0.67), P value (P <0.05), 11, 33 and 26 differential metabolites were identified at 2h, 6h, and 12h post CLP between the sepsis group and the control group (Table 1). Besides, 32, 23, and 28 differential metabolites were observed at 2h, 6h and 12h post CLP between the XBJ group and the sepsis group (Table 2). We also found 3, 6 and 6 differential metabolites overlapped between the XBJ group and the sepsis group at different time points post CLP (Figure 2 and Table 3). In addition, the heat map and VIP scores of differential expressed metabolites at different time points in the control group, sepsis group, and XBJ group were presented in Figure 3 and Figure 4.

**Related metabolic pathway analysis for the identified differential metabolites**

To explore the potential metabolic pathways in which the identified differential metabolites were involved, all of the identified metabolites with significant differences in each group were imported into MetaboAnalyst 5.0 to detect the metabolic pathways via KEGG. The pathway analysis results are shown in Figure 5. Cut-off value >0.1 was utilized to identify the significant metabolic pathways and filter the less important pathways. In our work, 1, 4 and 5 important metabolic pathways were investigated at 2h, 6h and 12h post CLP in the sepsis group compared with that of the control group. The related pathways of differential metabolites were galactose metabolism in 2h post CLP; aminoacyl-tRNA biosynthesis, arginine, and proline metabolism, selenocompound metabolism, starch and sucrose metabolism in 6h post CLP; aminoacyl-tRNA biosynthesis, arginine biosynthesis, arginine and proline metabolism, glyoxylate and dicarboxylate metabolism, glycine, serine, and threonine metabolism in 12h post CLP. Besides, 5, 1 and 1 significantly metabolic pathways were observed at 2, 6 and 12h post CLP in the rats with XBJ injection treatment compared with that of the rats without XBJ injection treatment. The related pathways of differential metabolites were tyrosine metabolism and galactose metabolism in 2h post CLP; aminoacyl-tRNA biosynthesis, arginine and proline metabolism, selenocompound metabolism, starch, and sucrose metabolism in 6h post CLP; aminoacyl-tRNA biosynthesis, arginine biosynthesis, arginine and proline metabolism, glyoxylate and dicarboxylate metabolism, glycine, serine and threonine metabolism in 12h post CLP compared with the control group (Figure 5). Besides, compared with the sepsis group, glutathione metabolism, arginine biosynthesis, arginine, and proline metabolism, sphingolipid metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis changed markedly at 2h; arginine and proline metabolism changed significantly at 6 and 12h post CLP in the XBJ group compared with that of the sepsis group (Figure 5). According to the related metabolic pathway we identified, we found that XBJ injection can affect arginine and proline metabolism in CLP-induced septic rat (Table 3).

**Discussion**
Sepsis is a multiorgan disease, accompanied by metabolic alterations\textsuperscript{[16]}. Metabolic disorders are an important characteristic in the development of sepsis, which makes the body fall into a state of negative nitrogen balance, resulting in a destroyed immune response, impaired tissue function and increased mortality\textsuperscript{[17]}. It has been reported that metabolic reprogramming is involved in the pathogenesis of sepsis\textsuperscript{[18, 19]}. In our study, we analyzed the dynamic metabolic changes and detected the metabolic alternation of septic rats responding to XBJ injection during the early stage. We found 11, 33 and 26 differential metabolites at 2, 6 and 12h post CLP in the sepsis group compared with that of the control group. Besides, we also found 1, 4 and 5 important metabolic pathways at different time points after CLP. These data suggested that metabolic disorder occurred in septic rats, which is consistent with previous reports\textsuperscript{[20]}.

The alternation of amino acid metabolism and energy metabolism were important metabolic characteristics of sepsis\textsuperscript{[21, 22]}. Our data demonstrated that most of the increased metabolites were amino acids during the early phase of sepsis, suggesting that the body was in a state of hypermetabolism and peripheral protein catabolism was activated. Further analysis showed the related metabolic pathway at 2h post CLP was glycometabolism, including galactose metabolism. As the disease progressed, not only the carbohydrate metabolism but also the amino acids metabolism changed strikingly at 6h post CLP, including aminoacyl-tRNA biosynthesis, arginine and proline metabolism, selenocompound metabolism, starch and sucrose metabolism. However, dysregulated amino acid metabolism is the prominently metabolic disorder after 12h post CLP, containing aminoacyl-tRNA biosynthesis, arginine biosynthesis, arginine and proline metabolism, glycine, serine and threonine metabolism, glyoxylate and dicarboxylate metabolism.

XBJ injection is a traditional Chinese medicine, which is consisted of Chuanxiong, Honghua, Chishao, Danshen and Danggui\textsuperscript{[23]}. Clinical studies showed that the addition of XBJ injection was capable of reducing the 28-day mortality and improving the prognosis of sepsis\textsuperscript{[24, 25]}. It has been reported that XBJ injection in combination with Biapenem can affect several key endogenous metabolites in septic patients\textsuperscript{[20]}. In our study, we found 32, 23 and 28 differential metabolites at 2h, 6h and 12h post CLP in the XBJ group compared with that of the sepsis group. Our further analysis showed the related metabolic pathways in septic rats with XBJ injection treatment for 2 hours were carbohydrate metabolism, amino acid metabolism and lipid metabolism, including glutathione metabolism, arginine biosynthesis, arginine and proline metabolism, sphingolipid metabolism, phenylalanine, tyrosine and tryptophan biosynthesis. However, as the treatment continued, only the amino acid metabolism altered significantly, including arginine and proline metabolism. Besides, 3, 6, 6 differential metabolites were overlapped in the sepsis group and XBJ group post CLP at different time points. These identified differential metabolites were majorly involved in arginine and proline metabolism, suggesting that XBJ injection is capable of affecting amino acid metabolism in CLP-induced septic rat.

Amino acid plays an essential role in the metabolism of sepsis and its level can reflect the energy metabolism of the body during sepsis\textsuperscript{[26]}. Arginine and proline metabolism was disturbed in acute liver
Several catabolic diseases such as injury and cancer will increase the utilization of arginine, thus resulting in arginine consumption. In septic rats, the levels of metabolites of arginine metabolism such as putrescine were decreased, indicating that sepsis leads to low consumption of arginine. With the treatment of XBJ injection, the level of putrescine was increased strikingly, indicating the disturbance of arginine and proline metabolism was alleviated to a certain extent.

In summary, numerical metabolites were detected in the sepsis group and the metabolites in serum were presented with dynamic alternation, indicating that the metabolic disorders may be involved in the pathophysiological process of sepsis. While with the treatment of XBJ injection, some metabolic pathways can be reversed, indicating that XBJ injection is capable of improving the metabolic disorder induced by CLP to a certain extent.

**Declarations**

**Author Contributions**

Yimin Zhu and Yu Jiang conceived and designed the project, Yanjuan Liu, acquired and analyzed the data, Qi Zeng, Wen Xiao, Fang Chen, Lianhong Zou and Xiehong Liu wrote the paper.

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**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Acknowledges**

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**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**
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Table 1 The differential metabolites identified in the sepsis group compared with the control group.
| Time Point | Metabolites                        | Fold Change | P Value   | VIP Score |
|-----------|-----------------------------------|-------------|-----------|-----------|
| 2h        | putrescine                        | 0.047982    | 6.40E-05  | 3.0587    |
|           | 5-Aminovaleric acid               | 5.2301      | 0.0009889 | 2.7585    |
|           | 5-Hydroxyindole-2-carboxylic acid | 69234       | 0.0049095 | 2.49      |
|           | 11-beta-prostaglandin-F-2-alpha   | 0.50839     | 0.0088991 | 2.3643    |
|           | ribitol                           | 938390      | 0.023304  | 2.1208    |
|           | glucose                           | 4760500     | 0.023796  | 2.1149    |
|           | galactose                         | 5.1009      | 0.025217  | 2.0982    |
|           | Phosphoglycolic acid              | 0.61445     | 0.030631  | 2.0406    |
|           | sucrose                           | 15992       | 0.034654  | 2.0025    |
|           | Digalacturonic acid               | 0.50353     | 0.036203  | 1.9887    |
|           | noradrenaline                     | 0.27026     | 0.047518  | 1.8993    |
| 6h        | 5-Aminovaleric acid               | 10.565      | 2.04E-08  | 2.1646    |
|           | proline                           | 2.7303      | 1.22E-06  | 2.0884    |
|           | alanine                           | 2.7177      | 2.92E-06  | 2.0639    |
|           | arachidonic acid                  | 0.030661    | 1.89E-05  | 1.9968    |
|           | putrescine                        | 0.10933     | 2.86E-05  | 1.9785    |
|           | Saccharic acid                    | 2.84E-07    | 7.06E-05  | 1.9336    |
|           | glucose                           | 25448000    | 7.31E-05  | 1.9317    |
|           | trans-4-hydroxy-L-proline         | 1.9037      | 7.55E-05  | 1.93      |
|           | serine                            | 1.7656      | 0.00024964| 1.8567    |
|           | methionine                        | 1.8695      | 0.00025598| 1.855     |
|           | asparagine                        | 1.7785      | 0.00047357| 1.81      |
|           | 3-Aminoisobutyric acid            | 2.499       | 0.00078522| 1.7688    |
|           | threonine                         | 1.7025      | 0.001084  | 1.7403    |
|           | 3-Hydroxypyridine                 | 9.54E-07    | 0.0020464 | 1.6784    |
|           | 2-Deoxy-D-galactose               | 2.87E-05    | 0.0021018 | 1.6756    |
|           | N-formyl-L-methionine             | 1.8498      | 0.0022643 | 1.6677    |
|           | ornithine                         | 1.6699      | 0.0023862 | 1.6621    |
| Metabolites                          | Fold Change | P value   | VIP Score |
|-------------------------------------|-------------|-----------|-----------|
| Glucose-1-phosphate                 | 1.7594      | 0.0024043 | 1.6613    |
| N-Carbamylglutamate                | 0.26442     | 0.0026002 | 1.6528    |
| Guanidinosuccinic acid             | 0.44776     | 0.0037652 | 1.6107    |
| D-erythro-sphingosine              | 0.5491      | 0.004276  | 1.5954    |
| Levoglucoosan                      | 0.19643     | 0.0046354 | 1.5855    |
| N-Oleoyldopamine                   | 8.1829      | 0.0047156 | 1.5834    |
| 3-phosphoglycerate                 | 0.14617     | 0.0049471 | 1.5774    |
| maltose                            | 1.5725      | 0.00513   | 1.5729    |
| 6-Methylmercaptopyrimine           | 0.26725     | 0.0053175 | 1.5683    |
| Halostachine                       | 1.5367      | 0.0054688 | 1.5647    |
| Lyxose                             | 2.5951      | 0.0059477 | 1.5538    |
| Allantoic acid                     | 1.5426      | 0.0063214 | 1.5458    |
| N-Methyl-DL-alanine                | 1.6245      | 0.0064244 | 1.5437    |
| pentadecanoic acid                 | 1.7549      | 0.0065637 | 1.5408    |
| Gluconic lactone                   | 1.6577      | 0.0068859 | 1.5344    |
| Sophorose                          | 1.6206      | 0.0081784 | 1.5107    |
| 12h galactose                      | 0.066139    | 7.12E-05  | 2.5154    |
|                                    | 12h 6-hydroxy caproic acid dimer | 0.0092841 | 0.0023862 | 2.1628    |
|                                    | ribitol     | 394910    | 0.0035719 | 2.1039    |
|                                    | Monoolein   | 2.887     | 0.008096  | 1.9676    |
|                                    | trans-4-hydroxy-L-proline | 2.2138    | 0.0091246 | 1.9455    |
|                                    | threonine   | 1.9936    | 0.010368  | 1.9213    |
|                                    | citric acid | 2.3689    | 0.012797  | 1.8797    |
|                                    | citrulline  | 1.969     | 0.013563  | 1.8678    |
|                                    | 3-Hydroxypyridine | 1.22E-06 | 0.013988  | 1.8615    |
|                                    | Allantoic acid | 1.8188 | 0.014857  | 1.8489    |
|                                    | N-Oleoyldopamine | 2.8411 | 0.015034  | 1.8465    |
|                                    | proline     | 2.1939    | 0.020058  | 1.7837    |
| Metabolite                        | Value 1  | Value 2   | Value 3  |
|----------------------------------|----------|-----------|----------|
| lysine                           | 1.8847   | 0.020698  | 1.7766   |
| ornithine                        | 1.846    | 0.022406  | 1.7584   |
| 3-Aminoisobutyric acid           | 2.3909   | 0.022938  | 1.753    |
| putrescine                       | 0.15919  | 0.025647  | 1.7266   |
| serine                           | 1.7544   | 0.029398  | 1.6934   |
| gluconic acid                    | 2.5066   | 0.032761  | 1.6662   |
| 3-Hydroxypropionic acid          | 1.8666   | 0.033349  | 1.6616   |
| L-Allothreonine                  | 33.75    | 0.03459   | 1.6522   |
| N-Methyl-L-glutamic acid         | 1.8074   | 0.036889  | 1.6355   |
| glutamine                        | 1.6696   | 0.043841  | 1.589    |
| methionine                       | 1.709    | 0.047496  | 1.5668   |
| Sedoheptulose                    | 0.19301  | 0.048091  | 1.5632   |
| maltose                          | 1.9615   | 0.048173  | 1.5628   |
| N-Carbamylglutamate              | 6.98E-06 | 0.049876  | 1.5529   |

Table 2 The differential metabolites identified in the XBJ group compared with the control group.
| Time Points | Metabolites                     | Fold Change | P Value | VIP Score |
|------------|--------------------------------|-------------|---------|-----------|
| 2h         | sucrose                         | 1.32E-06    | 2.67E-14| 2.086     |
|            | 2-Deoxy-D-galactose             | 2.10E-05    | 1.12E-13| 2.0826    |
|            | 1-Kestose                       | 5.20E-06    | 1.73E-13| 2.0814    |
|            | putrescine                      | 44.103      | 4.85E-07| 1.9315    |
|            | Dihydroxyacetone                | 5.242       | 5.20E-07| 1.9297    |
|            | phytosphingosine                | 0.002758    | 7.39E-07| 1.9206    |
|            | 0-Phosphorylethanolamine        | 3.265       | 5.50E-06| 1.8578    |
|            | glucose                         | 8.91E-08    | 5.86E-06| 1.8554    |
|            | alpha-D-glucosamine-phosphate   | 3.71E-06    | 7.36E-06| 1.8469    |
|            | glycine                         | 2.3258      | 8.14E-06| 1.843     |
|            | DL-dihydrosphingosine           | 0.021186    | 1.02E-05| 1.8342    |
|            | alpha-Ecdysone                  | 46512       | 1.06E-05| 1.8325    |
|            | Benzoin                         | 0.015864    | 1.09E-05| 1.8316    |
|            | glycocyamine                    | 2.334       | 1.67E-05| 1.8136    |
|            | Linoleic acid methyl ester      | 5.0072      | 2.17E-05| 1.802     |
|            | N-Oleoyldopamine                | 7.4008      | 3.34E-05| 1.7816    |
|            | resveratrol                     | 0.077484    | 9.34E-05| 1.7272    |
|            | Ethanolamine                    | 2.3731      | 0.00018792| 1.6845  |
|            | D-erythro-sphingosine           | 0.13773     | 0.00019388| 1.6825  |
|            | lactulose                       | 0.023982    | 0.00033392| 1.6454  |
|            | phthlastic acid                 | 9.35E-06    | 0.00037628| 1.6368  |
|            | ornithine                       | 2.2368      | 0.00040077| 1.6322  |
|            | citrulline                      | 1.9423      | 0.00056866| 1.6055  |
|            | methionine                      | 1.8042      | 0.00073976| 1.5844  |
|            | Leucose                         | 1.39E-05    | 0.00077086| 1.581   |
|            | 22-Ketocholesterol              | 2.1395      | 0.00090541| 1.5675  |
|            | 6-deoxy-D-glucose               | 1.7426      | 0.00094639| 1.5637  |
|            | phenylalanine                   | 1.8657      | 0.0009895 | 1.5598   |
| Metabolites                              | Fold Change | Pvalue   | VIP Score |
|------------------------------------------|-------------|----------|-----------|
| glutamic acid                            | 2.1101      | 0.001047 | 1.5549    |
| arachidonic acid                         | 0.039041    | 0.001093 | 1.5511    |
| asparaginene                             | 1.7854      | 0.001322 | 1.534     |
| Levogluconosan                           | 0.12008     | 0.001613 | 1.5155    |
| 5-Aminovaleric acid                      | 0.13888     | 4.15E-08 | 2.6564    |
| 2-deoxy-D-glucose                        | 0.31917     | 4.55E-06 | 2.498     |
| Dihydroxyacetone                         | 4.1652      | 5.28E-06 | 2.4908    |
| glucose                                  | 5.17E-08    | 5.80E-06 | 2.4863    |
| alpha-Ecdysone                           | 57855       | 6.76E-06 | 2.4786    |
| putrescine                               | 8.2252      | 2.15E-05 | 2.4145    |
| 3-Aminoisobutyric acid                   | 0.32711     | 6.23E-05 | 2.3442    |
| glycocyamine                             | 1.8539      | 9.35E-05 | 2.3139    |
| Dodecanol                                | 0.61305     | 0.000181 | 2.2599    |
| fructose                                 | 0.47178     | 0.000219 | 2.2431    |
| O-Phosphorylethanolamine                 | 1.8758      | 0.000536 | 2.1572    |
| phytosphingosine                         | 0.10136     | 0.001753 | 2.0197    |
| 6-hydroxy caproic acid                   | 0.5336      | 0.003377 | 1.9297    |

| Time Point | Metabolites                              | Fold Change | Pvalue   | VIP Score |
|------------|------------------------------------------|-------------|----------|-----------|
| 6h         | phthalic acid                            | 1.96E-05    | 0.003585 | 1.9209    |
|            | N-formyl-L-methionine                     | 0.65828     | 0.004735 | 1.8787    |
|            | D-erythro-sphingosine                     | 87319       | 0.005587 | 1.8524    |
|            | Thiocurcumin                              | 9.8328      | 0.006279 | 1.8333    |
|            | Biphenyl                                 | 0.1358      | 0.007853 | 1.7955    |
|            | Aminomalonic acid                        | 1.9731      | 0.01238  | 1.7128    |
|            | D-erythronolactone                       | 1.5356      | 0.0196   | 1.6208    |
|            | Indolelactate                            | 0.65568     | 0.020398 | 1.6123    |
|            | Allantoic acid                           | 0.64731     | 0.023452 | 1.5822    |
|            | Myristic Acid                            | 0.65838     | 0.023903 | 1.578     |
| 12h        | 11-beta-prostaglandin-F-2-alpha          | 368110      | 0.003807 | 1.8867    |
| Metabolite                                      | Value     | p-value   | Fold change |
|------------------------------------------------|-----------|-----------|-------------|
| D-erythronolactone                             | 190810    | 0.003769  | 1.8882      |
| N-Oleoyldopamine                               | 44.899    | 0.010026  | 1.7289      |
| putrescine                                     | 9.4346    | 0.0021924 | 1.9639      |
| galactose                                      | 8.5433    | 0.016034  | 1.6401      |
| Linoleic acid methyl ester                    | 7.4341    | 0.017629  | 1.6211      |
| Thiocitamide                                   | 6.1369    | 0.0092246 | 1.7437      |
| Galactonic acid                                | 2.9888    | 0.00030059| 2.185       |
| glycocyamine                                   | 2.1462    | 1.93E-05  | 2.3891      |
| Ethanolamine                                   | 1.9029    | 3.36E-06  | 2.4787      |
| Gallic acid                                    | 1.6451    | 5.36E-05  | 2.3238      |
| Allantoic acid                                 | 0.65538   | 7.92E-05  | 2.2957      |
| Bis(2-hydroxypropyl)amine                     | 0.63906   | 0.016088  | 1.6395      |
| Sitosterol                                     | 0.52268   | 0.018271  | 1.6138      |
| glucose                                        | 0.49626   | 0.011178  | 1.7091      |
| lactulose                                      | 0.4871    | 0.018207  | 1.6145      |
| N-Methyl-DL-alanine                            | 0.42983   | 0.00039017| 2.1603      |
| 3-Aminoisobutyric acid                        | 0.35089   | 0.00084995| 2.0793      |
| Nicotianamine                                  | 0.34493   | 0.01541   | 1.648       |
| phytosphingosine                               | 0.30819   | 0.010648  | 1.718       |
| fructose                                       | 0.30472   | 0.0071453 | 1.7877      |
| 2-deoxy-D-glucose                              | 0.062627  | 0.00088063| 2.0753      |
| Leucrose                                       | 1.47E-05  | 0.020308  | 1.5919      |
| Biphenyl                                       | 8.31E-06  | 0.00044535| 2.1473      |
| phthalic acid                                  | 5.57E-06  | 5.99E-06  | 2.4518      |
| 2-Deoxy-D-galactose                            | 4.44E-06  | 1.40E-21  | 2.7759      |
| alpha-D-glucosamine-phosphate                  | 4.42E-06  | 0.0041213 | 1.8749      |
| ribitol                                        | 1.81E-06  | 0.020185  | 1.5932      |

Table 3 The overlapped differential metabolites in sepsis group and XBJ group at different time points.
| Time Points | Metabolites                                      | The related metabolic pathway                        |
|------------|-------------------------------------------------|-----------------------------------------------------|
| 2h         | putrescine                                      | NA                                                  |
|            | glucose                                         |                                                     |
|            | sucrose                                         |                                                     |
| 6h         | 5-Aminovaleric acid                             |                                                     |
|            | glucose                                         |                                                     |
|            | putrescine                                      |                                                     |
|            | 3-Aminoisobutyric acid                          | Arginine and proline metabolism                     |
|            | N-formyl-L-methionine                            |                                                     |
|            | D-erythro-sphingosine                            |                                                     |
|            | Allantoic acid                                  |                                                     |
|            | 2-Deoxy-D-galactose                             |                                                     |
|            | 2-deoxy-D-glucose                               |                                                     |
| 12h        | N-Oleoyldopamine                                | Arginine and proline metabolism                     |
|            | putrescine                                      |                                                     |
|            | galactose                                       |                                                     |
|            | Allantoic acid                                  |                                                     |
|            | 3-Aminoisobutyric acid                          |                                                     |
|            | 2-deoxy-D-glucose                               |                                                     |
|            | ribitol                                         |                                                     |

**Figures**
Figure 1

Discriminant analysis of PLS-DA pattern recognition among the control group, sepsis group and XBJ group. (A-C): PLS-DA analysis score scatter plots illustrating that the metabolic profiles of the sepsis group and XBJ group are distinct from those of control group at 2, 6, 12h post CLP, respectively.
Figure 2

The dynamic changes of the overlapped differential metabolites in the rats with or without XBJ injection post CLP for different time points. (A-C): The overlapped differential metabolites in the rats with or without XBJ injection at 2, 6, 12 h post CLP, respectively.
Figure 3

VIP scores of differentially expressed metabolites at different time points. (A-C): VIP scores of differentially expressed metabolites in the sepsis group at 2, 6 and 12h post CLP compared with those of the control group. (D-F): VIP scores of differentially expressed metabolites in the XBJ group at 2, 6 and 12h post CLP compared with that of the sepsis group.
Figure 4

Heat map of differentially expressed metabolites at different time points. (A-C): Heat map of differentially expressed metabolites in the sepsis group at 2, 6 and 12h post CLP compared with that of the control group. (D-F): Heat map of differentially expressed metabolites in the XBJ group at 2, 6 and 12h post CLP compared with that of the sepsis group.
Figure 5

Metabolic pathways in which the differential metabolites are involved at different time points. (A-C): Metabolic pathways in which the differential metabolites involved in the sepsis group at 2, 6 and 12 post CLP compared with that of the control group. (D-F): Metabolic pathways in which the differential metabolites involved in the XBJ group at 2, 6 and 12 post CLP compared with that of the sepsis group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryfigure1.pdf
- supplementarytable1.csv
- supplementarytable2.csv
- supplementarytable3.csv