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Metabolomic approach for the identification of therapeutic targets of erythropoietin against sepsis in rat models

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Abstract. – OBJECTIVE: Owing to a lack of effective treatment approaches, sepsis is considered a life-threatening clinical syndrome worldwide. Many therapeutic interventions combating sepsis have been evaluated in animal models and clinical cases over the past few decades. Due to the pleiotropic characteristics of EPO, many studies have shown that erythropoietin (EPO) would be used to alleviate sepsis-induced tissue injury beyond the hemoglobin elevation effect. Nevertheless, the organ protective activity of EPO could not be supported by all of the results. In order to address the unanswered questions, a new methodical approach is necessary to be considered. The latest progress in metabolomics could be helpful to interpret the underlying mechanisms of EPO on sepsis, via metabolite profiling, to bring in some potent predictable fact for clinical application.

MATERIALS AND METHODS: Twenty-one male Sprague-Dawley rats were divided into 3 groups of 7 rats each. Sepsis was induced by cecal ligation and puncture (CLP). Rats in the sepsis group were injected with equal volume of saline post-CLP. Rats from the EPO group were treated twice with EPO (immediately and 24 hours after CLP, 3750 IU/kg). The rats in the sham group were subjected to a sham surgery and injected with saline at the same time as the sepsis group. Serum samples were collected for biochemical and metabolomic analysis 72 hours post-CLP.

RESULTS: Biochemistry analysis revealed that erythropoietin improved the condition of multiple organs damaged by sepsis. Fifty-eight serum metabolites, including amino acids and fatty acids, displayed significant differences between the sepsis and sham groups. EPO treatment was found to attenuate the metabolic imbalances induced by CLP.

CONCLUSIONS: This study indicated that the metabolomic approach provided a comprehensive insight towards the metabolic targets of EPO treatment of sepsis.

Key Words:
Sepsis, Erythropoietin, Metabolomics.

Introduction

Sepsis, with its high morbidity and mortality rate, has been a major public health concern throughout the world over the past few years[1]. Sepsis is a systemic inflammatory response syndrome with a high probability of aggravating to multi-organ dysfunction syndrome, eventually leading to death at a fast pace[2,3]. Erythropoietin (EPO) is a blood cell regulator; erythropoietin receptors are widely distributed throughout the cardio-vascular system, renal tissue, central nervous system, and gastrointestinal tract. During recent years, exogenous EPO has been shown to relieve sepsis-induced renal, myocardial, lung, and small intestinal injury[4-7]. However, some results found that EPO does not modify the inflammatory response and lymphocyte apoptosis in endotoxemia model[8,9]. Comprehensive mechanisms of the pharmacological effects of EPO remain to be elucidated.

Metabolomics, a platform developed for the analysis of systems biology, is a comprehensive metabolic approach used to determine the integrated response of an organism to a specific stimulus. It can be applied to the diagnosis and

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treatment of diseases based on an analysis of the biological fluids, such as serum and plasma[10-12], as well as to assess the effects of drugs, in the levels of endogenous metabolites[13,14]. Thus, the emerging technical approach was used in the research of some major diseases[15], and sepsis made no exception[16-18]. Furthermore, the metabolomics based strategy has also focused on defining the treatment response in septic shock patients[19]. Therefore, aim to adding the metabolic mechanisms through which EPO exerts its therapeutic effect in a rodent model of sepsis, present study with serum metabolomics was conducted.

Materials and Methods

Chemicals and reagents

Chromatography-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Milli-Q water purification system (Millipore Corp., Billerica, MA, USA). Formic acid was obtained from Fluka (Buchs, Switzerland). All other chemicals used in this study were of analytical grade.

Animal Experiment

Male specific-pathogen-free Sprague-Dawley (SD) rats (6-8 weeks old, 220-230 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. The rats were randomly divided into model group (n=7, treated with 0.2 ml solution immediately and 24 hours after CLP), treatment group (n=7, treated with 3750 IU/kg erythropoietin (Sansheng Biopharm Co., Ltd. Shenyang, China) immediately and 24 hours after CLP), and sham group (n=7, treated with shame operation and saline). Sepsis was induced by cecal ligation and puncture (CLP) as per the method detailed by[20]. At the end of the study, 0.2 ml arterial and 1.5 ml venous blood samples were collected from the abdominal aorta. The arterial blood was used for PO_2 content measurement immediately while venous samples were clotted at 4°C for 2 h; centrifuged at 3500 g (RCF) for 5 min, and the sera harvested for biochemical analysis. All samples were stored at -80°C prior to analysis. All animal studies were performed at the Facility for Laboratory Animals of the Second Military Medical University. All animal handling and experimentation protocols were in compliance with the relevant national legislations and local guidelines. All experiments were approved by the Bioethics Committee of the Second Military Medical University (Shanghai, China), and were carried out according to the Use of Laboratory Animals and National Institute of Health Guide for the Care.

Sample Preparation and Test

The blood samples were defrosted at 4°C. Each 100 μL of the blood sample was mixed with 300 μL acetonitrile. This mixture was vortexed in order to extract the metabolites, and precipitate all component proteins. The clear supernatant was transferred to the sampling vial for ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis, after centrifugation at 10,000 g (RCF) for 15 min at 4°C. A quality control (QC) sample was used to monitor the data acquisition performance during analysis. The QC sample was prepared by pooling aliquots of whole blood samples obtained during analysis; these were inserted randomly among the sample sequences in order to ensure the stability of the system. The blood gas content was analyzed and the liver and kidney function assayed using the BC-2800Vet animal auto biochemistry analyzer (Shihai, Guangdong, China).

UHPLC-Q-TOF/MS profiling analysis was performed using an Agilent 1290 Infinity LC system equipped with an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separations were performed at 40° C using an Acquity UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm; Waters, Milford, MA, USA). The mobile phase was made up of 0.1% formic acid (A) and acetonitrile (can) modified with 0.1% formic acid (B). The optimized UHPLC elution conditions were set at: 0-2 min, 5%B; 2-10 min, 5%-15%B; 10-14 min, 15%-30%B; 14-17 min, 30%-95%B; and 17-19 min, 95%B. Post-elution, the mobile phase was run for 6 min to equilibrate the system. The samples were injected at a volume of 2 μL, the flow rate was set to 0.4 ml/min, and the auto-sampler maintained at 4°C. An electrospray ionization source (ESI) was used in the positive and negative modes of operation. The optimized conditions used were as follows: drying gas flow, 11 L/min; gas temperature, 350°C; capillary voltage, 4 kV for the positive mode and 3.5 kV for negative mode; fragment or voltage, 120 V;

Table I. Effect of EPO on arterial blood PaO_2 content, hepatic and renal function after CLP.

ALT (U/L)	Hct (%)	Group	PaO_2 (mmHg)	Scr (μmol/L)	BUN (mmol/L)	AST (U/L)
Sham	95.2 ± 4.1	12. 5 ± 4.3	6.87 ± 1.1	60.8 ± 18.2	25.8 ± 8.4	34.1 ± 2.4
Model (CLP)	75.2 ± 14.4*	67.4 ± 20.8*	29.4 ± 14.4*	459.1 ± 149.2*	124.3 ± 41.6*	30.3 ± 8.6
Treatment (EPO)	88.2 ± 17.1	33.6 ±12.4#	18.5 ± 10.8#	324.5 ± 150.9#	69.5 ± 27.4#	31.5 ± 6.3

Data are means±SD. *p <0.05 compare with sham group, #p <0.05 compare with model group.

nebulizer pressure, 45 psig; and skimmer voltage, 60 V. Data was collected in the profile mode from 50 to 1,100 m/z. The biomarkers were further analyzed by MS/MS, and the collision energy set from 10 to 40 eV.

Statistical Analysis

The UHPLC-MS raw data was converted to common data format files (mzdata) using the Agilent Mass Hunter Qualitative software. The threshold was set to 0.1%, all isotope interference was excluded. The XCMS program (http://metlin.scripps.edu/download/)[21] was applied for peak extraction, alignment, and integration, in order to generate a visual data matrix. The ions were filtered to 80% concentration[22]; however, in order to obtain the relative intensity of metabolites, all detected ions in each sample were normalized to the sum of the peak area. The data was then subjected to mean-centering and Pareto scaling. The three-dimensional data matrix, including the sample names, retention time, and m/z pairs, was imported. The ion intensities were normalized into the SIMCA-P program (version 11.0; Umetrics, Umea, Sweden) for multivariate statistical analysis. The relevant parameters and permutation tests were analyzed to evaluate the quality of the models obtained. All biochemical data with normal distribution were presented as mean ± standard deviation (SD). The statistical significance of the mean values was tested by the one-way analysis of variance (ANOVA) using the SPSS software (version 17.0; IBM, Armonk, NY, USA). The differences were considered to be significant when p <0.05.

Biomarkers Identication and Pathway Analysis

In order to identify the discovered biomarkers, the exact masses of ion were input into databases such as Metlin (http://metlin.scripps.edu), PubChem (http://pubchem.ncbi.nlm.nih.gov), etc.

Results

Based on the biochemistry assay about liver, pulmonary and renal function were showed in Table I, the CLP induced sepsis related tissue injury was valid. Meanwhile, the EPO treatment was determined to slow the progress of sepsis related damage, similar with previous studies[1,7].

Total ion current (TIC) profiles of typical samples from each group showed in Figure 1 displays the general information of UHPLC-Q-TOF/MS detection. Then supervised partial least squares discriminate analysis (PLS-DA) plot was a key method to differentiate metabolic phenotypes and demonstrated with visualization. The positive and negative modes were both employed to analyze the metabolites profiling. Visualized dispersion of each group in PLS-DA score plots (Figure 2A and 2B) revealed apparent differences in the three groups, as well as the absence of an extreme value. In addition, was constructed to determine potential biomarkers from the obtained data. From the score plots, the three groups showed obvious differences. And the cumulative R2Y and Q2 values were 0.992 and 0.833 in the positive mode, 0.997 and 0.811 in the negative mode of analysis, separately, which confirmed that the PLS-DA based math-model was valid. From the PLS-DA scatter plots and variable importance plots (VIPs), different metabolites among the three groups were defined, which were presented with points far away from the origin point in figure 2C and 2D. The metabolites with VIPs > 1.0 were designated as important differentiate biomarkers.

Based on the data from VIPs sequence, forty-seven metabolites were identified to reveal the EPO treatment effect, in the positive and negative mode by ANOVA and the Tukey's post hoc test with a significance level of 0.05. Among the 47 metabolites, pantothenic acid, SM (d18:1/16:0) and LysoPC (16:1) were observed in both modes. All identified different metabolites listed in Table

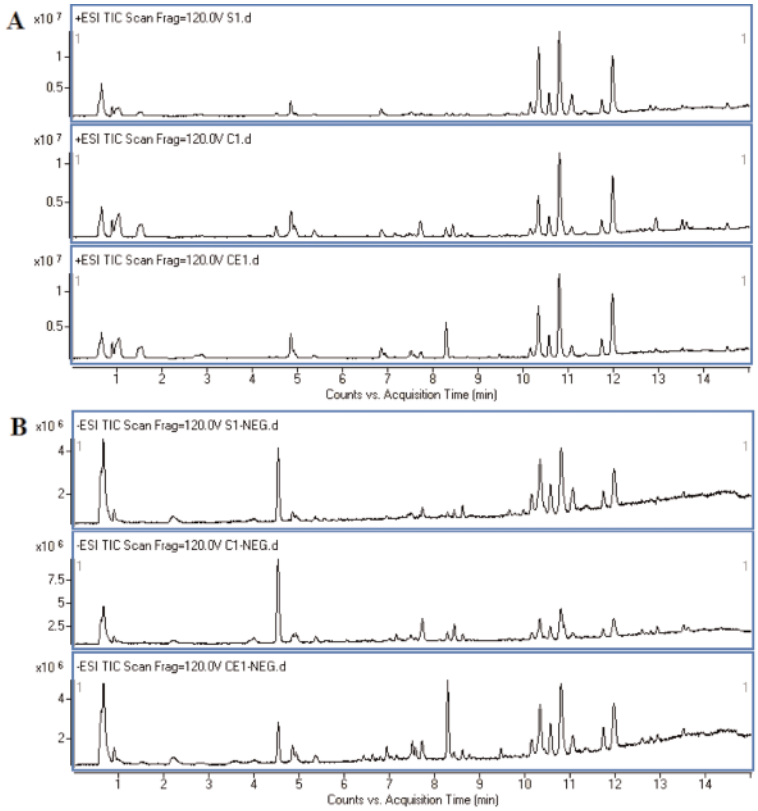

Figure 1. Representative total ion current (TIC) chromatograms of serum obtained from different groups of rats in the ***(A)*** positive mode and ***(B)*** negative mode. Upper panel: model group; Middle panel: sham group; Lower panel: treatment group.

II, with MASS related property and metabolic pathway characteristics. In order to make a comprehensive insight of metabolic change in sepsis under EPO treatment, an integrated metabolic network was constructed in Figure 3, using the Kyoto encyclopedia of genes and genomes (KEGG) pathway database (http://www.genome.jp/kegg/). The inherent pathways of the identified metabolites mainly focused on amino acid metabolism, fatty acid metabolism, cholic acid metabolism, and sphingosine metabolism.

Discussion

Based on the results obtained for the different serum metabolites, a few potential pathways were identified to interpret EPO interventional targets of rodent sepsis.

Energy Metabolism

Pyruvic acid plays an important role in the metabolism; it is the final product of the glycolysis pathway and the starting substrate of the tricarboxylicacid (TCA) cycle. Pyruvic acid acts as an endogenous antioxidant in cells[23,24]; it can also generate acetyl coenzyme A, which enters the TCA cycle to supply energy. Under the hyper-metabolic condition of sepsis, the body consumes large quantities of adenosine triphosphate (ATP) for energy; under these conditions, phosphocreatine forges a high-energy phosphate bond with adenosinediphosphate (ADP) in the presence of enzymes in order to produce ATP to fulfill all energy needs. Previous studies have shown that pyruvate affects rats via the TCA cycle. Phosphocreatine is rapidly concentrated in the cerebellum, hippocampus, cerebral cortex, and striatum; this is because sepsis may deplete the total ATP content in the body, leading the initiate body measures to protect the brain. Consistent to this observation, there was a significant concentration of pyruvic acid and creatine in the septic rats compared to the sham-operated rats in our experiment. This indicated that the bodies of septic rats were operating un-

der a high metabolic state, indicating the effectiveness of our sepsis model. The rats injected with EPO showed an apparent decrease in pyruvic acid and creatine expression, suggesting the therapeutic effect of EPO on sepsis; this effect may be exercised by manipulating the energy molecules in the metabolic pathways.

Amino acid Metabolism

Significant changes in the expression of some amino acids such as phenylalanine, tyrosine, tryptophan, and leucine were observed. Phenylalanine, tyrosine, and catecholamines regulate the upstream and downstream relationships in amino acid metabolism. Tyrosine was generated from phenylalanine through the action of phenylalanine hydroxylase. Meanwhile tyrosine could generate dopamine, epinephrine, norepinephrine, and other vascular-bioactive substances[26]. A recent study revealed that catecholamine, a metabolite of tyrosine, ameliorates hypoperfusion and metabolic failure during early sepsis. In addition, catecholamines are known to improve heart function, facilitating the release of sepsis. Recent studies conducted on inflammation and cancer models and in cancer patients revealed a phenylalanine polymer-induced increase in the serum content. Phenylalanine concentrations were also observed to increase in patient serum as a result of liver cell dysfunction[27]. We found similar amino acid expression rates in the CLP-induced sepsis rats. There was a significant decrease in phenylalanine concentration upon EPO administration, which indicated that phenylalanine and tyrosine act as biomarkers of sepsis, and that EPO may release sepsis through this metabolic pathway. As one of essential branch amino acids, isoleucine was recognized as a regulator of

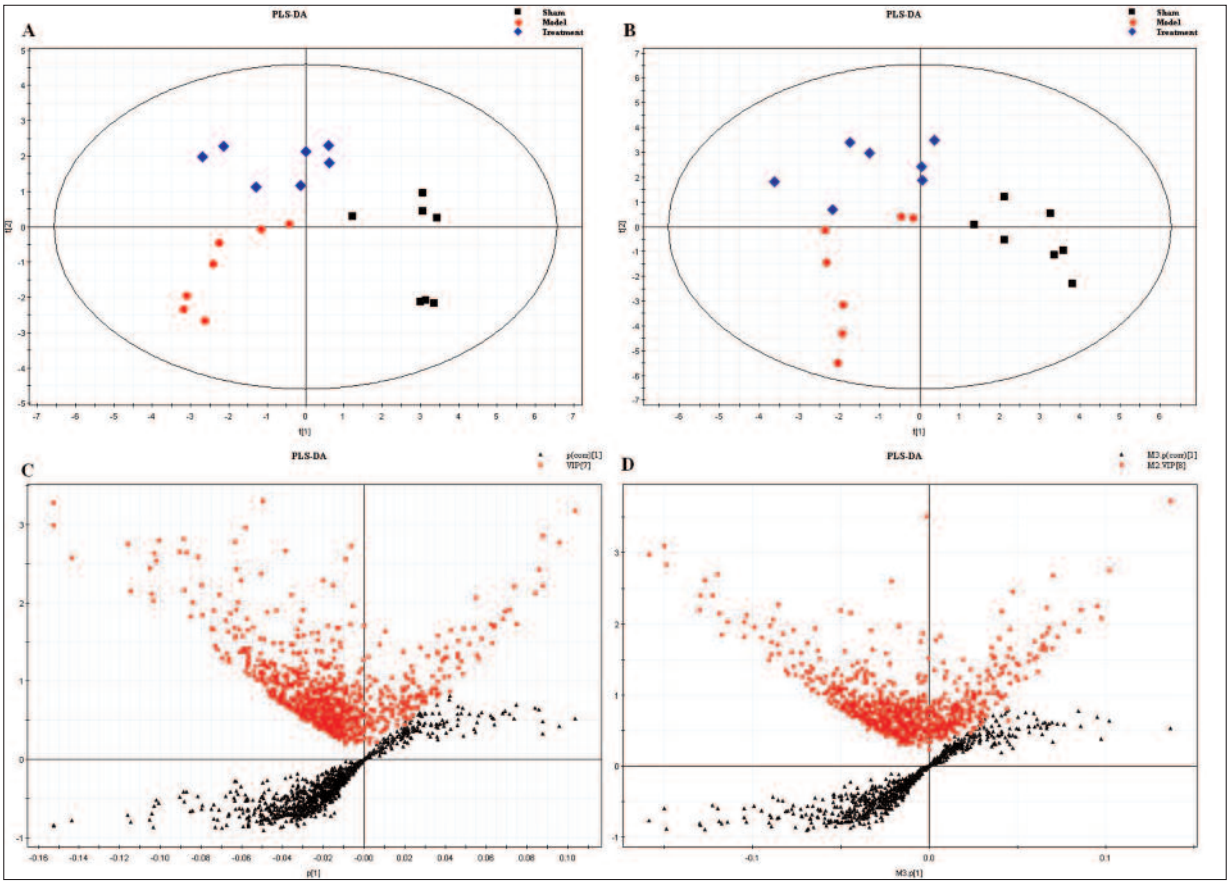

Figure 2. Score plots of the principal component analysis performed on the HPLC/MS profile of rat serum obtained from the CLP group or model (red rounds), sham group (black squares), and EPO or treatment group (blue rhombus) in the ***(A)*** positive mode and ***(B)*** negative mode. Samples obtained from the different groups showed obvious differences. The combination of S- and VIP-score plots in the ***(C)*** positive and ***(D)*** negative modes showed that a greater number of values obtained from serum analysis were further away from the origin.

Table II. Potential biomarkers facilitating EPO treatment in rat serum subjected to CLP, and their metabolic pathways.

No	Rt/ min	M/Z	Ion	Formula	Metabolites	Fold change CLP/Sham	Fold change EPO/CLP	Related pathway
1	4.95	283.083	$[M-H]^-$	$C_{13}H_{16}O_7$	p-Cresol glucuronide	7.45#	0.69	Tyrosine metabolism
2	2.88	205.097	$[M+H]^+$	$C_{11}H_{12}N_2O_2$	L-Tryptophan	1.16*	0.88	Tryptophan metabolism
3	3.99	212.003	$[M-H]^-$	$C_8H_7NO_4S$	Indoxylsulfuric acid	2.40#	0.46#	Tryptophan metabolism
4	6.53	188.072	$[M-H]^-$	$C_{11}H_{11}NO_2$	3-Indolepropionic acid	0.33#	0.67	Tryptophan metabolism
5	1.53	166.086	$[M+H]^+$	$C_9H_{11}NO_2$	L-Phenylalanine	1.26#	0.82	Phenylalanine metabolism
6	4.54	192.067	$[M-H]^-$	$C_{10}H_{11}NO_3$	2-Methyl hippuric acid	5.9#	0.33*	Phenylalanine metabolism
7	5.04	165.056	$[M-H]^-$	$C_9H_{10}O_3$	4-Methoxyphenylacetic acid	1.3#	0.74	Phenylalanine metabolism
8	1.06	130.088	$[M-H]^-$	$C_6H_{13}NO_2$	L-Isoleucine	0.63#	1.30	Valine, leucine and isoleucine metabolism
9	4.04	129.056	$[M-H]^-$	$C_6H_{10}O_3$	3-Methyl-2-oxovaleric acid	1.55*	0.49	Isoleucine metabolism
10	0.99	117.019	$[M-H]^-$	$C_4H_6O_4$	Methylmalonic acid	7.91#	0.44	Valine, leucine and isoleucine degradation
11	0.68	154.058	$[M+Na]^+$	$C_4H_9N_3O_2$	Creatine	1.77*	0.64	Glycine, serine and threonine metabolism
12	1.85	220.118	$[M+H]^+$	$C_9H_{17}NO_5$	Pantothenic acid	3.24#	0.62	Pantothenate and CoA biosynthesis
	1.85	218.104	$[M+H]^+$	$C_9H_{17}NO_5$	Pantothenic acid	2.86#	0.58	
13	0.71	133.015	$[M+FA-H]^-$	$C_3H_4O_3$	Pyruvate	1.82*	0.55	Citrate cycle (TCA cycle)
14	0.71	175.025	$[M+FA-H]^-$	$C_5H_6O_4$	Mesaconic acid	5.06#	0.70	C5-Branched dibasic acid metabolism
15	0.67	151.062	$[M+H]^+$	$C_6H_6N_4O$	1-Methyl hypoxanthine	5.11#	0.23*	Adenosine metabolism
16	0.71	173.01	$[M-H]^-$	$C_6H_6O_6$	Dehydroascorbic acid	1.90#	1.03	Vitamin C metabolism
17	4.88	519.255	$[M+Na]^+$	$C_{25}H_{40}N_2O_6S$	Leukotriene D4	1.97*	0.61*	Arachidonic acid metabolism
18	10.88	319.229	$[M-H]^-$	$C_{20}H_{32}O_3$	20-hydroxy-eicosatetraenoic acid	0.28#	0.29*	Arachidonic acid metabolism
19	9.99	398.326	$[M+H]^+$	$C_{23}H_{43}NO_4$	trans-hexadec-2-enoyl carnitine	2.34*	0.66	Fatty acid metabolism
20	8.45	357.278	$[M+H]^+$	$C_{24}H_{36}O_2$	tetracosahexaenoic acid	3.97#	0.18#	Fatty acid metabolism
21	11.57	331.263	$[M+H]^+$	$C_{22}H_{34}O_2$	Docosapentaenoic acid	0.14#	0.39#	Biosynthesis of unsaturated fatty acids
22	10.88	303.232	$[M+H]^+$	$C_{20}H_{30}O_2$	Eicosapentaenoic acid	0.31#	0.33	Biosynthesis of unsaturated fatty acids
23	2.13	232.154	$[M+H]^+$	$C_{11}H_{21}NO_4$	Butyryl-L-carnitine	2.21#	0.52	Fatty acid metabolism
24	8.30	407.281	$[M-H]^-$	$C_{24}H_{40}O_5$	Cholic acid	4.75*	0.84	Primary bile acid biosynthesis
25	8.44	437.291	$[M+FA-H]^-$	$C_{24}H_{40}O_4$	Chenodeoxycholic acid	3.3#	0.20#	Primary bile acid biosynthesis
26	7.79	498.29	$[M-H]^-$	$C_{26}H_{45}NO_6S$	Taurochenodeoxycholic acid	0.18#	2.16	Primary bile acid biosynthesis
27	9.63	300.289	$[M+H]^+$	$C_{18}H_{37}NO_2$	Sphingosine	2.72#	0.72#	Sphingolipid metabolism
28	9.87	302.305	$[M+H]^+$	$C_{18}H_{39}NO_2$	Sphinganine	2.39#	0.65#	Sphingolipid metabolism
29	14.62	703.574	$[M+H]^+$	$C_{39}H_{79}N_2O_6P$	SM(d18:1/16:0)	2.05#	0.63#	Sphingolipid metabolism
	14.62	725.556	$[M+Na]^+$	$C_{39}H_{79}N_2O_6P$	SM(d18:1/16:0)	2.05#	0.63#	
30	14.03	806.569	$[M+H]^+$	$C_{46}H_{80}NO_8P$	PC(20:2/18:4)	2.26*	0.68*	Phospholipid metabolism
31	10.75	546.355	$[M+H]^+$	$C_{28}H_{52}NO_7P$ Lyso	PC(20:3)	0.60*	1.42*	Phospholipid metabolism
32	10.23	482.324	$[M+H]^+$	$C_{23}H_{48}NO_7P$ Lyso	PC(15:0)	0.61*	1.27*	Phospholipid metabolism
33	9.99	494.324	$[M+H]^+$	$C_{24}H_{48}NO_7P$ Lyso	PC(16:1)	0.61*	1.18*	Phospholipid metabolism
	9.99	538.316	$[M+FA-H]^-$	$C_{24}H_{48}NO_7P$ Lyso	PC(16:1)	0.60#	1.03	
34	4.83	474.256	$[M+Na]^+$	$C_{21}H_{42}NO_7P$ Lyso	PE(0:0/16:1)	2.26*	0.69*	Phospholipid metabolism
35	10.44	544.269	$[M+FA-H]^-$	$C_{25}H_{42}NO_7P$	LysoPE(0:0/20:5)	0.48#	0.87	Phospholipid metabolism
36	11.06	566.347	$[M+FA-H]^-$	$C_{26}H_{52}NO_7P$	LysoPC(18:1)	0.71*	0.96	Phospholipid metabolism
37	10.75	452.279	$[M-H]^-$	$C_{21}H_{44}NO_7P$	LysoPE(16:0/0:0)	1.66#	0.78*	Phospholipid metabolism
38	11.35	592.362	$[M+FA-H]^-$	$C_{28}H_{55}NO_7P$	LysoPC(20:2)	0.40#	1.08	Phospholipid metabolism
39	10.23	526.315	$[M+FA-H]^-$	$C_{23}H_{48}NO_7P$	LysoPE(18:0/0:0)	0.57*	1.12*	Phospholipid metabolism
40	10.43	520.268	$[M+FA-H]^-$	$C_{23}H_{42}NO_7P$	LysoPE(0:0/18:3)	0.24*	0.57	Phospholipid metabolism
41	9.67	512.3	$[M+FA-H]^-$	$C_{22}H_{46}NO_7P$	LysoPC(14:0)	0.17#	0.44*	Phospholipid metabolism
42	10.25	588.331	$[M+FA-H]^-$	$C_{28}H_{50}NO_7P$	LysoPC(20:4)	1.22*	1.29#	Phospholipid metabolism
43	9.86	562.315	$[M+FA-H]^-$	$C_{26}H_{48}NO_7P$	LysoPC(18:3)	0.56#	1.14*	Phospholipid metabolism
44	10.74	590.347	$[M+FA-H]^-$	$C_{28}H_{52}NO_7P$	LysoPC(20:3)	0.59#	1.17*	Phospholipid metabolism
45	11.07	436.284	$[M-H]^-$	$C_{21}H_{44}NO_6P$	LysoPC(P-16:0)	0.70#	0.88	Phospholipid metabolism
46	10.30	500.279	$[M-H]^-$	$C_{25}H_{44}NO_7P$	LysoPE(20:4/0:0)	1.41*	0.77	Phospholipid metabolism
47	10.30	476.279	$[M-H]^-$	$C_{23}H_{44}NO_7P$	LysoPE(0:0/18:2)	0.79*	0.84*	Phospholipid metabolism

*$p < 0.05$; #$p < 0.01$; SM stands for Sphingomyelin; PC stands for phosphatidylcholine; LysoPE stands for lysophosphatidyl ethanolamine; LysoPC stands for lysophosphatidylcholine

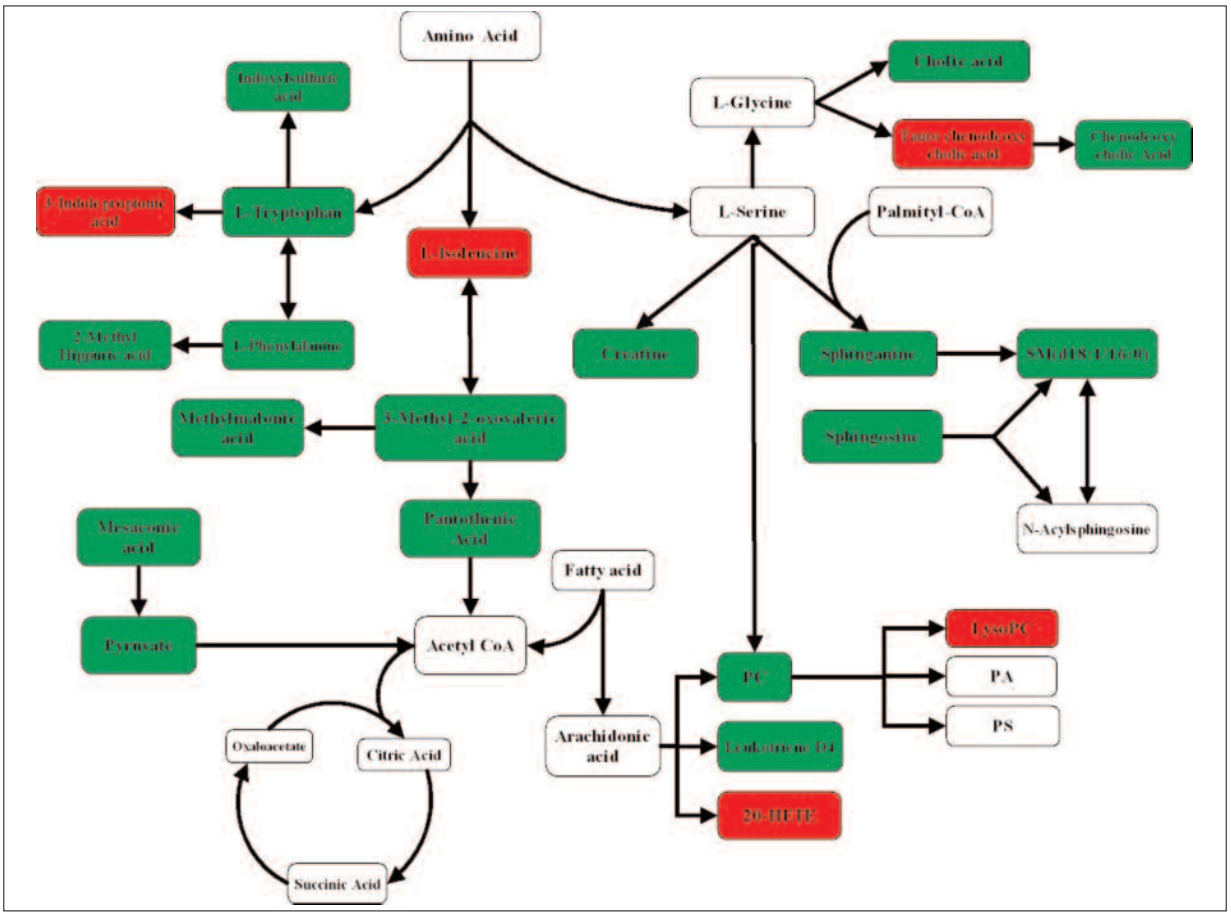

Figure 3. The metabolic pathway network was integrated from different metabolites between sepsis and EPO treatment rats. The components in green indicated the decreased relative intensity after EPO treatment, while that in red with increased after EPO-treatment.

protein regeneration, as well as a major substrate in protein synthesis. In the present study, decreased isoleucine expression in CLP-induced sepsis rats, suggesting that sepsis may cause irreversible damage. Previous literature[28,29] indicated that the human body does not generate sufficient glutathione during conditions of stress, cancer, sepsis and burns. Glutamate could be converted to gamma-aminobutyric acid (GABA), glutaminate, 2-oxoglutaric acid, glucose, and glutathione in mammalian cells[30]. These results implied that EPO might impact sepsis via the leucine and isoleucine metabolic pathways.

Fatty acid Metabolism

Hypermetabolism could be induced under the condition of sepsis through blood flow redistribution and changes in substrate utilization; this could result in tissue hypoperfusion and inadequate oxygenation. Carnitine regulates the acylcarnitine and free carnitine ratio (AC:FC) in order to prevent the accumulation of acylcarnitine, and enhance energy formation[3]. Lack of carnitine induces the contribution of long-chain fatty acids, the increase in LCAC/FC ratio, and the decrease in palmitoyl carnitine transferase activity. As the lipid metabolism mechanisms are affected under conditions of sepsis, carnitine could be used to enhance lipid metabolism and energy formation, as well as treatment option[19]. We observed an increase in the contribution of combined carnitine, which decreased with EPO administration. This indicated that EPO may be a therapeutical option for sepsis through the carnitine pathway.

Purine Metabolism

Increased levels of hypoxanthine were found in blood from septic patients[32]. The increase in hypoxanthine concentration was also detected in the

blood of rabbits and pulmonary artery blood of pigs suffering from endotoxin shock[33]. Hypoxanthine has been reported to be the major extracellular purine metabolite responding to endotoxemia. In consistent with these findings, significant increase of hypoxanthine concentrations was also found in sepsis rats in present work, which was subsequently reduced after EPO treatment. Thus we believed that EPO might alleviate the septic injury via the purine metabolic pathway.

Sphingolipid Metabolism

Sphingosine-1-phosphate (S1P) is the signaling molecule employed by endothelial cells involved in the amplification of cytokine during the influenza virus infection[34]. Increased vascular permeability, a complication of sepsis, was observed with the regulation of S1P in blood[35]. Patients with sepsis displayed a decrease in expression of apolipoprotein M (ApoM), which binds the S1P in blood. Under such conditions, the decrease in S1P content in the blood, and the consequent increase in vascular permeability, leads to an increase in the severity of the disease. It has been established that there is a decrease in lymphocyte content in patients with sepsis; the abnormality of mediated lymphatic outflow is one of the major reasons for this decrease[36]. Simultaneously, S1P would result in a cascade that could cause a persistent inflammatory response.

Conclusions

In this study, a sensitive metabolomics method based on liquid chromatography and mass spectrometry was used to analyze the complex disease patterns of sepsis and the therapeutic pathways of EPO. This could see widespread, worldwide application as the standard method for analysis of this serious disease. Using this method, we identified 47 biomaterials as the differential biomarker metabolites. These biomarkers regulated the energy metabolism, amino acid metabolism, fatty acid metabolism, purine metabolism, and sphingosine metabolism, in addition to playing an important role in other metabolic pathways. Based on the results of this study, it can be suggested that sepsis is treated by EPO via these pathways. This must be confirmed by future pharmacological studies; this would lead to a further understanding of the metabolomics regulating disease and treatment. This could also aid into the elucidation of the mechanism of sepsis and the therapeutic mechanism of EPO.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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