UPLC-G2Si-HDMS untargeted metabolomics for identification of metabolic targets of Yin-Chen-Hao-Tang used as a therapeutic agent of dampness-heat jaundice syndrome

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ABSTRACT

Yin-Chen-Hao-Tang (YCHT), the classic formulae of traditional Chinese medicine (TCM), is widely used to treat dampness-heat jaundice syndrome (DHJS) and various liver diseases. However, the therapeutic mechanism of YCHT is yet to have an integrated biological interpretation. In this work, we used metabolomics technology to reveal the adjustment of small molecule metabolites in body during the treatment of YCHT. Aim to discover the serum biomarkers which are associated with the treatment of DHJS against YCHT. Pathological results and biochemical indicators showed that the hepatic injury and liver index abnormalities caused by DHJS was effectively improve after treatment with YCHT. On the basis of effective treatment, ultra-high performance liquid chromatography (UPLC-G2Si-HDMS) combined with the multivariate statistical analysis method was utilized to analyze the serum samples. Finally, 22 biomarkers were identified by using mass spectrometry and illuminated the correlative metabolic pathways which play a significant role and as therapeutic targets in the treatment of DHJS. This work demonstrated that mass spectrometry metabolomics provides a new insight to elucidate the action mechanism of formulae.

1. Introduction

Traditional Chinese medicines (TCMs) have a long history for the treatment or preventing disease. It has been extensively used in many Asian countries, and recognized by some Western countries over the past few decades. Symptoms and formulas are the core of TCM theory and they are directly related to the diagnosis of disease and clinical treatment [1]. However, the ambiguity of symptoms and complexity of formulas is two main problems that hinder the modernization of TCM. To find out a research method that accords with characteristics of TCM are the key point to determine diagnostic criteria of symptoms and explain the efficacy of relevant formulas.

Metabolomics is an emerging “omics” science of Post-Genomic generation. It analyzes the metabolic profiling changes in organisms and tissues and explores biology state of the whole organism by monitoring changes in micro-molecule metabolite level [2]. It accords with differentiation theory and treatment way of holism view in traditional Chinese medicine. Metabolomics can be used to describe biological changes in potential metabolism pathways caused by symptoms. Therefore, we can find out biomarkers related to symptoms, thus explaining scientific nature of symptoms and laying a foundation for accurate diagnosis of symptoms [3]. Besides, we can also observe its treatment effect by detecting changes of relevant biomarkers after formula treatment and establish the technical system to evaluate the efficiency of formulas. It indicates that metabolomics may refresh our understanding about TCM theory and provide a new way for the development and modernization of TCM [4]. Dampness-heat jaundice syndrome (DHJS), a kind of yanghuang syndrome (YHS), which caused by damp heat evil and mostly associated with acute jaundice hepatitis. The clinical manifestation of DHJS is the patients show yellow staining in the sclera and skin, and accompanied by a certain degree of liver injury. The patient will have physical fever, thirst, irritability and other symptoms [5]. Yin-Chen-Hao-Tang (YCHT) is a classical traditional Chinese medicine formula, which is an aqueous extract consist of three herbal drugs, Gardenia jasminoides Ellis, Artemisia capillaries Thunb and Rheum officinale Baill.
As a representative formula for the treatment of DHJS, YCHT has the exact therapeutic effect and be praised by Chinese medicine practitioners since ancient [6,7]. Advanced UPLC-Q/TOF-G2Si-HDMS system, strong data processing platform and comprehensive metabolic network analysis were made use of to determine pathogenesis of DHJS and metabolism target of YCHT treatment. The protective effect of YCHT on bile stasis liver injury was also explored from the perspective of metabolites.

2. Materials and methods

2.1. Drugs and chemical reagents

Alcohol was acquired from Beijing Reagent Company (Beijing, China). Olive oil was purchased from Kerry Oils & Grains Trade Co., Ltd. (Shenzhen, China). Rhihoma Zingiberis was supplied by Harbin Tong Ren Tang Drug Store (Harbin, China). Gardenia jasminoides Ellis and Rheum officinale Baill were purchased from Harbin Tong Ren Tang Drug Company (Harbin, China). The Artemisia capitata Thunb was purchased from Xinyeexin Pharmaceutical Inc. (China Branch Office, Japan). All the crude drugs were authenticated by Prof. Xi-jun Wang of the Pharmacognosy Department, Heilongjiang University of Chinese Medicine. Ultrapure water was bought from Watson’s Food & Beverage Co., Ltd. (Guangzhou, China). Acetonitrile (HPLC grade) was obtained from Fisher Scientific Corporation (Loughborough, UK); Methanol (HPLC grade) was supplied by Merck (Darmstadt, Germany); Leucine enkephalin was purchased from Sigma-Aldrich (St Louis, MO, USA); formic acid was of an analytical grade supplied by Beijing Reagent Company (Beijing, China); 3a-naphthylisothiocyanate (ANIT) was supplied by Sigma-Aldrich (St Louis, MO, USA). Assay kit for glutathion peroxidase (GSH-Px), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), malondialdehyde (MDA) and alanine aminotransferase (ALT) were purchased from Nanjing jiancheng Biotech Company (Nanjing, China). The assay kits for total bile acid (TBA), total superoxide dismutase (T-SOD), aspartate amino transferase (AST), direct bilirubin (D-BIL), total bilirubin (T-BIL), were obtained from BioSino Bio-Technology & Science Inc. (Beijing, China).

2.2. Animals and experimental design

Male Brl/c mice, 20 ± 2 g, were obtained from Shanghai Slac Laboratory Animal Co., Ltd (Shanghai, China). Mice had free access to food and water, with comfortable environmental conditions (temperature, 24 ± 1 °C; relative humidity, 60 ± 5%), and a 12-h light/dark cycle. All the mice were randomly divided into seven groups of eight mice each: two control groups (CON1, CON2), two DHJS groups (DHJS1, DHJS2) and three groups of different therapeutic doses of TCHT: YCHT-High group (YH), YCHT-Middle group (YM) and YCHT-Low group (YL). DHJS models mice were established as follows: the mice in DHJS groups and YCHT groups were orally administered Rhizoma Zingiberis extracting solution (0.013 g/mL) and alcohol (3.125% (v/v)) at a dose of 0.1 mL/10 g once daily from the first day to day 14. On days 15 and 16, mice were given different concentrations of ANIT mixture (1.5 mg/mL, 1 mg/mL, dissolved in olive oil) at a dose of 0.1 mL/10 g once daily, respectively. Meanwhile, the mice in the control groups were orally administered olive oil at a dose of 0.1 mL/10 g once daily from the first day to day 14. Three YCHT administration groups were treated as follows: from day 17 to day 22, the mice of YH group were treated with YCHT at 30 g/kg/day, the mice of YM group were treated with YCHT at 15 g/kg/day and the mice of YL group were treated with YCHT at 5 g/kg/day. Meanwhile, mice in the CON2 group were orally administered distilled water at a same solution volume as YCHT once daily from the first day to day 14. The weight and rectal temperature of mice was monitored and recorded regularly. Animals in CON1 and DHJS1 groups were sacrificed on the day16 to evaluate the model of DHJS, and the other groups were sacrificed on the day22.

After mice anesthetized with ether, blood was collected by removing the eyeball and liver tissues were sampled for histopathology study. Blood was immediately centrifuged at 3500 rpm for 10 min at 4 °C and collected the upper serum. A mixed sample was prepared as the quality control (QC) sample, which containing aliquots of all the collected serum samples. Methanol was added to the plasma sample and QC sample, vortex for 30 s, centrifuged (13,000 rpm, 4 °C) for 10 min and the supernatant was dried under nitrogen. Then the residue was added 80% methanol for reconstituted and centrifuged (13,000 rpm, 4 °C) 10 min, the supernatant was filtered through a 0.22 μm membrane and the filtrate into the UPLC-Q/TOF-G2Si-HDMS analysis. The above investigation was approved by the Ethical Committee of Heilongjiang University of Chinese Medicine and was conducted according to the principles expressed in the Declaration of Helsinki.

2.3. Pathological detection and clinical chemistery analysis

According to the instructions of the manufacturer, we quantified the levels of AST, ALT ALP, D-Bili, γ-GT, GSH-Px, KMAS, MDA, T-SOD activities using assay kit. The fresh liver samples were obtained and immediately delivered to the affiliated hospital of Heilongjiang University of Chinese Medicine for conducting the histopathology analysis.

2.4. Chromatography

We employed an ultra-high performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA/USA) for the global analysis of serum samples using MasslynxTM software (V4.1 SCN901). We employed a Waters ACQUITY UPLC system (Waters Corp., Milford, MA/USA) for the comprehensive analysis of mice serum samples using MassLynxTM software (V4.1 SCN901, Waters Corporation, Milford, USA). The separation was performed with an ACQUITY UPLC HSS T3 column (100 mm × 2.1, 1.8 μm; Waters Corporation, Milford, USA) at 40 °C and the flow rate was set at 0.4 mL/min. The optimal mobile phase consisted of a linear gradient system of (A) acetonitrile with 0.1% formic acid and (B) water with 0.1% formic acid as the mobile phase, 0–3 min, 1%–10% A; 3–5 min, 10–20% A; 5–8.5 min, 20%–40% A; 8.5–9.5 min, 40%–99% A; 9.5–11.5 min, maintain 99% A; 11.5–12 min, linear decrease from 99% to 1% A; held at 1% A for 3 min for equilibration of the column. In addition, the QC sample was utilized to optimize the conditions for UPLC, as it contained the most information about the serum samples. When the samples were analyzed, QC sample was injected twice at first and then inserted one QC sample after each five serum samples.

2.5. Mass spectrometry

Global detection of biological information was performed using the high throughput G2Si High-definition mass spectrometry (Waters Q-TOF SYNAPTTM, Waters Corp, Manchester, England). After the comprehensive exploration of conditions, the optimal detailed parameter as follows: The MS source temperature was set at 110 °C, and the desolvation temperature was set at 350 °C. Nitrogen was used as the dry gas, the desolvation gas flow rate was set at 800 L/h, and cone gas flow was maintained at 50 L/h. In both positive and negative modes, capillary voltage was set at 3000 V and cone voltage at 30 V. The mass spectra were recorded across the range of 50–1200 Da. To ensure stable and precise scanning, leucine enkephalin (positive ion mode ([M + H]+ = 556.2771) and negative ion mode ([M – H]– = 554.2615)) was used as the reference compound at a concentration of 0.2 mg/mL under a flow rate of 100 μL/min.
2.6. Multivariate statistical analysis

The positive and negative ion of serum samples were collected by using the established metabolomics analysis condition to obtain the BPI chromatograms of the corresponding metabolic profile, and observe the differences between the groups. The UPLC-MS data were imported into Progenesis QI software (Nonlinear Dynamics, 2014, version 1.0) for peak picking and normalization. There was a temporary file automatically generated after data preprocessing and the file contained all the biological information. Then we import all the metabolic data into Ezinfo 2.0 software for multivariate data analyses, including principal components analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA) [8].

2.7. Metabolites identification and pathway analysis

To confirm the biomarker alterations between control and model groups, we compared the peak height intensity of differential metabolites by t-test. And ions which conform to VIP > 1, P < 0.05 were selected. These ions were deemed to have a large contribution rate to the change of the metabolic profile. Then, qualifying ions were screened for endogenous biomarkers through the Human Metabolome Database (HMDB) (http://www.hmdb.ca/). Fragment ion information of the corresponding ions collected in the G2-Si-MS/MS mode is matched with the possible cleavage patterns of the obtained precursor ions and fragment ions according to databases such as HMDB and MassFragment manager (Waters Corp., Milford, USA) to finally determine the chemical structure. Pathway analysis and visualization were performed using Metabolomics Pathway Analysis (MetPA) database (http://www.metaboanalyst.ca) and KEGG pathway database (http://www.genome.jp/kegg/).

3. Results

3.1. Histopathology and biochemical analysis

The biochemistry parameters and histopathology analysis of the control groups, DHJS groups, YH group, YM group and YL group were summarized in the Fig. 1. On day 16, compared with control group, H&E staining of the DHJS group liver tissue showed the tissue was infiltration by inflammatory cell, while the arrangement of liver cells was loose and irregular. The regional laminar necrosis and edema were found to exist around the central vein. It is indicated that liver injury has occurred in the DHJS group (Fig. 1A). On day 22, compared with the DHJS group, there is no obvious tissue necrosis in YH, YM, YL group, the edema is reduced. The liver tissue structure (leaflets, liver plate, leaflets of the central vein, bile duct) is clear and no obvious abnormalities and the number of inflammatory cells are reduced in the liver tissue (Fig. 1B). During the 0–22 days, the weight of the CON2 group increased steadily and rectal temperature did not change significantly. Meanwhile, the weight of mice in the DHJS2 group was always lower than CON2 group and the rectal temperature was significantly higher than CON2 group (P < 0.05). During the 0–16 days of modeling, the trend of weight and rectal temperature indicators was basically same as the DHJS2 group. During the 17–22 days of treatment, the weight gain rate of the mice in YCHT groups was increased and the rectal temperature was significantly lower than that in DHJS2 group (P < 0.01), above indicators were trend to the blank group remarkable and YL group is more obvious (Fig. 1C).

As showed in the clinical biochemical analysis, the level of liver index was significantly different among the experimental groups (Supplementary Table 1). Compared with the control group, the serum concentrations of TBA, ALT, ALP, γ-GT in the DHJS group was markedly increased (P < 0.01) and the level of T-Bili, AST was increased (P < 0.05). Meanwhile, the levels of MDA in liver tissue were significantly increased (P < 0.01) and the contents of GSH-Px in liver tissue were decreased in DHJS group (P < 0.05). On day 22, after the oral administration of YCHT treatment, the level of liver index in the YH group, YM group and YL group has a tendency to call-back to the control group (Fig. 1D). The results show that YCHT can effectively relieve the abnormal liver index caused by DHJS, such as bilirubin indicators of elevated and other symptoms. And the DHJS mouse liver cell edema with lobular structural disorders, local focal necrosis, inflammatory cells infiltration and other phenomena has also been alleviated. This indicated that YCHT can curb the further development of DHJS and improved the body function. After a comprehensive comparison, we found the treatment effect of YL group was better than YM group and YH group.

3.2. Metabolite profiling analysis and biomarker identification

All the serum samples from YH, YM, YL group, control groups and DHJS groups were analyzed by UPLC-Q/TOF-G2Si-HDMS system. Using the established metabolomics analysis method to collect the data of the positive ion (ESI+) and negative ion (ESI−) pattern of blood samples, and obtain the BPI chromatogram of the corresponding metabolic profile (Fig. 2). The raw data was imported into the Progenesis QI software to perform Peak Picking and normalization, then further conduct multivariate statistical analyses. We exported the result to EzinfoTM software (Waters Corporation) for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). The PCA model was used to determine the difference of metabolite profiles between DHJS groups and the control groups. OPLS-DA and cloud plot was used to find the differential ions: In the OPLS-DA plot, the points far away from original were having the major contribution to the differences between different groups. The points in the cloud plot represent the ions have been detected and the ions which larger circles represent were having the larger VIP value (Fig. 3). These differential ions were selected to be potential biomarkers, and then the structures were identified based upon databases and cleavage patterns. Finally, 22 serum biomarkers were determined, included Glycerophosphocholine, l-Acetylcarnitine, Prostaglandin E2, Leukotriene A4 (Supplementary Table 2). Among these, 12/22 biomarkers had a tendency to call-back to the control level in the YH group, 13/22 biomarkers had a tendency to call-back in the YM group and 15/22 biomarkers had a tendency to call-back in the YL group (Fig. 4). The dynamic changes of 22 biomarkers in each group were analyzed by heat map. The hue of the chunks reflects the relative signal intensities and cluster identification. The result show that compared with the control group, the metabolic profile of DHJS mice was changed obviously. While the metabolic profile of three YCHT groups was closer to the control group and the profile of the YL group was the closest (Fig. 5).

3.3. Analysis of correlative metabolic pathways of serum biomarkers

MetPA is a network tool for metabolic data visualization analysis. MetPA combines a number of advanced pathway enrichment analysis procedures and pathway topology characterization to assist in determining the most relevant metabolic pathways associated with metabolic research. The information of the identified biomarker of DHJS was introduced into the metabolic pathway analysis website (http://www.metaboanalyst.ca) for analysis, and 8 metabolic pathways were disturbed: Glycerophospholipid metabolism, arachidonic acid metabolism, steroid hormone biosynthesis, sphingolipid metabolism, glyco-sphingolipidylinositol (GPI)-anchor biosynthesis, linoleic acid metabolism, ether lipid metabolism and alpha-linolenic acid metabolism (Fig. 6, Supplementary Table 3). The results indicate that these endogenous metabolites have a strong disturbance throughout the metabolic trajectory and these phenomena are closely related to the DHJS (Fig. 7). These metabolic disorders caused by modeling may be utilized to explain the mechanism of DHJS.
4. Discussion

The high-throughput metabolomics are powerful approaches for identification of metabolic targets of TCM [8–14], via investigating unique metabolites [15–17]. YCHT has a long story in treatment of DHJS and other liver disease in China, and it is widely used in the clinic of modern Chinese medicine. In recent years, the effectiveness of YCHT has been extensively studied, but there is not any in-depth research based on the classical disease model [18,19]. Based on TCM syndrome theory combined with blood metabolomics, we establish an effective method to explain the pathogenesis of DHJS and the therapeutic mechanism of YCHT in treating DHJS. In accordance with the theory of TCM, mice were orally administered Rhizoma Zingiberis and ethanol to establish the damp-heat background and α-naphthylisothiocyanate (ANIT) was given in the last two days of modeling to cause acute liver injury [20,21]. Histopathology analysis and biochemical index were used to evaluate the DHJS model. Metabolic profile characterization and pattern recognition were used to analyze serum metabolites of DHJS mice and control mice by means of metabolomics platform. On the end of model replication, the DHJS group and control group were clustered obviously. Through PCA and OPLS-DA analysis to find the differential metabolites, and use HMDB, MetPA, and KEGG databases to authenticate biomarkers. 22 potential biomarkers that have a significant effect on clustering were eventually locked. We focused on the changes in metabolic pathways associated with biomarkers and found that these metabolic pathways were associated with clinical symptoms of DHJS. The details about the related metabolic pathway network are illustrated in Fig. 8.

Glycerol phospholipids are the most abundant kind of phospholipids, which constitute the biofilm. They constitute the surface active
Fig. 2. Metabolic fingerprints. UPLC-MS BPI serum chromatograms of each experimental group in positive mode and negative mode.

Fig. 3. Multivariate statistical analysis of the UPLC-MS serum spectra data. PCA score plots based on serum metabolites discriminating (○) control group and (●) DHJS group, in positive mode and negative mode. OPLS-DA-S-VIP plot analysis for DHJS in both positive ion mode and negative ion mode. Cloud plot for DHJS in positive mode and negative mode.
substances of bile and membrane, and participate in cell membrane recognition and signal transduction of proteins [22,23]. The basic structure of glycerol phospholipids is phosphatidic acid and the substituents (X) linked to phosphoric acid. It can be divided into various classes depending on the substituents, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and so on. Studies have shown that phosphatidylcholine (PC) levels are lowered in liver lesions [24,25]. This may be related to a decrease in PC content leading to decreased cell membrane fluidity and decreased antioxidant capacity. Glycerol phospholipids degrade under phospholipase to produce lysophosphatidylcholine (LPCs). LPCs are mainly metabolized in the liver and can undergo significant changes in liver disease and hepatotoxicity. LPCs are cytotoxic and bind to the glucose transfer protein on the cell membrane to inhibit glucose transport. It can also activate JNK or PKC signaling pathway to improve the degree of activation of PLA2 to accelerate cell membrane hydrolysis. Research has shown that the content of LPCs has decreased in the cholestatic liver disease [26–28]. In this study, the levels of phosphatidylycerol and lysophosphatidylcholine in the serum of the mice were reduced and the metabolic disorder of glycerophospholipid resulted in impaired cholestatic liver.

Arachidonic acid is crucial in phospholipid-bound structural lipids in the blood, liver, muscle and other organ system [29]. It is a direct precursor of biologically active substances such as prostaglandin E2 (PG2), prostacyclin (PGI2), thromboxane A2 (TXA2) and leukotriene and C4 (LTC4). These arachidonic acid metabolites play an important role in regulating the physiological functions of the liver and the pathophysiology of the liver as a lipid medium [30,31]. Studies have shown that some of the arachidonic acid metabolites increase during liver injury and the level of change in arachidonic acid metabolites are directly related to the degree of liver damage [32]. PGE2 is a metabolite of arachidonic acid cyclooxygenase, which level has increased or is significantly elevated in the tissue homogenate of liver injury. The development of liver injury is strongly related to neutrophils and macrophages gathered in the liver. Under the action of pathogenic factors, macrophages accumulate to the liver and are activated, resulting in a large number of releases of arachidonic acid metabolites such as PGs, LTs, TXs, free radicals, TNF-α, IL-1 and other toxic substances. These toxic substances cause liver cell damage [33,34]. When liver injury occurs, intrahepatic infiltration of neutrophil 5-lipoxigenase activity was significantly enhanced leading to increased intrahepatic leukotrienes (LTs), promote liver injury [35]. In our study, levels of PGE2 and leukotriene A4 in the serum of the mice were significantly higher than those in the control groups. According to the observation of liver pathology, the liver tissue of the model group showed obvious damage, balloon-like changes and inflammatory cell infiltration. Thus we conclude that liver injury is accompanied by significant metabolic disorders of arachidonic acid.

Steroid hormones, including estrogen, androgens and adrenal hormones, play a major role in life support, body development and immune regulation. Androstenedione involved in this study is androgen, and its level decreased can cause apathetic, sparse hair, anorexia and other symptoms. We found the androstenedione levels in DHJS groups were lower than that in control groups. The mice in control groups were lively and body weight increased steady, while the mice in DHJS groups were lack of spirit and growth slow. This phenomenon may be related

Fig. 4. Relative signal intensities of metabolic biomarkers identified by UPLC-MS. Bar plots represent the relative peak area ratios of 22 biomarkers. Data is expressed as mean ± SD. *Significant difference from control at P < 0.05. **Significant difference from control at P < 0.01; †Significant difference from DHJS at P < 0.05. ‡Significant difference from DHJS at P < 0.01.
Sphingolipids include kinds of lipid molecules, they are not only an important part of the biofilm, but also own a variety of biological activity. Among the sphingolipids, ceramide is the basic skeleton of complex sphingolipid synthesis, which is situated in the center of sphingolipid metabolism. Under the action of sphingomyelinase, all sphingomyelins are eventually degraded to ceramide, and ceramide is deacetylated under the action of ceramidase (CEase) to form sphingosine [36]. Sphingosine is one factor that induces hepatocyte apoptosis. After the cells are stimulated by outside, the content of sphingomyelin increased, resulting in an increase in ceramide, and sphingosine produced by degradation of ceramide causes an increase in lysosome membrane permeability, cathepsin release and ultimately apoptosis [37–39]. In this study, it was found that the content of sphingomyelin in the blood of model mice was markedly increased. This indicates that changes in the sphingolipid metabolic pathway of DHJS mice cause the liver cells to be damaged.

α-Linolenic acid (ALA) is a polyunsaturated fatty acid with three double bonds and is an omega-3 essential fatty acid. It is the basic materials that constitute the cell membrane and the biological enzyme. Linoleic acid (in the form of glycerides) is present in animal fats with other fatty acids [40]. Phosphatidylcholine (PC) produces linoleic acid under the action of cytoplasmic phospholipase A2. Under the effect of cytoplasmic phospholipase A2 and KRAS-like inhibitory factor, PC will produce ALA. The content of PC in DHJS mice was significantly lower than that in normal mice result in the content of ALA in vivo was decreased, but the recovery of cells requires a lot of fatty acids like ALA.
When contents of the body fatty acids are not enough to maintain long-term use in cells, it will increase the burden of synthesis and transform fatty acid derivatives in the mitochondrial, eventually leading to mitochondrial damage and fatty acid-related metabolic abnormalities [41].

Glycerylphosphocholine (GPC) is a product of two fatty acyl groups that are completely hydrolyzed on phosphatidylcholine (PC) molecules. Studies have shown that choline substances are mainly oxidized into betaine in the liver and kidney. When the liver and kidney damage, choline metabolic pathway will be blocked, resulting in increased choline ingredients. So when the serum metabolites test showed the content of glycerol phosphocholine increased, this change will suggest that the liver has been injured in [42]. In the present study, the serum GPC content in the DHJS group was significantly higher than that in the control group. Combine with the results of liver histopathology, this may be related to the abnormality of the ether lipid metabolism pathway in the DHJS group, resulting in an increase in GPC content and eventually liver injury.

5. Conclusion

This study established DHJS mice model according to the pathogenesis of TCM theory and verified the rationality of this model by analyzing weight, anus temperature, histopathology and clinical biochemical criterion. On the basis of the successful model, it accurately identified a series of potential endogenous metabolite in the serum and observed relevant metabolism pathway changes via non-targeted and high-throughput strategy. The pathway analysis released a series of key enzyme and metabolite directly related to DHJS pathogenesis and locked core metabolic pathway: glycerophospholipid metabolism, rachidonic acid metabolism, glycosylphosphatidylinositol-anchor biosynthesis, steroid hormone biosynthesis, sphingolipid metabolism, linoleic acid metabolism, ether lipid metabolism, alpha- ‑linolenic acid metabolism. These pathways may be the potential biological chemistry mechanism of DHJS occurrence. In conclusion, we adopted advanced Metabonomics technology based on mass spectrum to discover abnormal metabolic pathways and analyze treatment targets of YCHT for treating DHJS. The above work proved that serum metabonomics has great potential in revealing the pathogenesis of TCM symptoms and effective target of formulas, so it provides a new effective way for accurate diagnosis of TCM symptoms and action mechanism of formulas.
Fig. 8. Related metabolic pathway maps of potential biomarkers related to the Yang-Huang syndrome mouse model based on KEGG network. (Red font represents the biomarker detected in this experiment.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)


