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Original Article

Lizhong Decoction Ameliorates Ulcerative Colitis in Mice via Regulation of Plasma and Urine Metabolic Profiling*

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ABSTRACT **Objective**: To elucidate the mechanism of Lizhong Decoction (LZD) in treating dextran sodium sulfate (DSS)-induced colitis in mice based on metabonomics. **Methods**: Thirty-six mice were randomly divided into 6 groups, including normal, model, low- (1.365 g/kg), medium- (4.095 g/kg) and high dose (12.285 g/kg) LZD and salazosulfadimidine (SASP) groups, 6 mice in each group. Colitis model mice were induced by DSS admistration for 7 days, and treated with low, medium and high dose LZD extract and positive drug SASP. Metabolic comparison of DSS-induced colitis and normal mice was investigated by using ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass (UPLC-Q-TOF/MS) combined with Metabolynx™ software. **Results**: The metabolic profiles of plasma and urine in colitis mice were distinctly ameliorated after LZD treatment (P<0.05). Potential biomarkers (9 in serum and 4 in urine) were screened and tentatively identified. The endogenous metabolites were mainly involved in primary bile acid, sphingolipid, linoleic acid, arachidonic acid, amino acids (alanine, aspartate, and glutamate), butanoate and glycerophospholipid metabolism in plasma, and terpenoid backbone biosynthesis, glycerophospholipid and tryptophan metabolism in urine. After LZD treatment, these markers notably restored to normal levels. **Conclusions**: The study revealed the underlying mechanism of LZD on amelioration of ulcerative colitis based on metabonomics, which laid a foundation for further exploring the pathological and physiological mechanism, early diagnosis, and corresponding drug development of colitis.

KEYWORDS Lizhong Decoction, ulcerative colitis, metabonomics, biomarkers, Chinese medicine

Ulcerative colitis (UC), a chronic inflammatory disease, starts from the rectum and usually extends downward through part or all of colon in a continuous manner. In recent years, UC is prevalent worldwide with the steadily increasing incidence.(1) UC is generally characterized by severe diarrhea, weight loss, bloody stools, etc., resulting in a decrease in quality of life. Furthermore, UC patients normally have a markedly increased risk of colon cancer and other related diseases.(2) Although the pathogenesis and pathological correlations of UC are still unclear, idiopathic, genetic, immunological and environmental factors, which can cause immune imbalance and inflammatory factor burst, are generally considered to play a significant role in the etiology of UC.(3,4) The effective therapies for UC mainly include 5-aminosalicylic acid, steroids, immunosuppressants and anti-tumor necrosis factor-alpha.(5,6) Unfortunately, due to high dosage and long-term use, the abovementioned treatments can cause serious side effects such as infection, acute pancreatitis, and osteoporosis.(7,8) Therefore, there is an urgent need to find new drugs with high efficiency and low toxicity for the treatment of UC. As an effective and classic way of treating chronic diseases, the application of Chinese medicines (CMs) to cure UC has aroused wide interests due to their good curative effects, lower toxicity, fewer side effects and lower cost.(6)

Lizhong Decoction (LZD, 理中汤), which includes 4 herbs *Zingiberis Rhizoma*, *Radix Ginseng*, *Rhizoma Atractylodis* Macrocephalae and *Radix Glycyrrhizae*,(9) has been commonly prescribed for the treatment of digestive diseases in East Asian countries for over 1,000 years and is mostly used for the relief of nausea

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or vomiting, stomachache, diarrhea, or watery stools, and rugitus.[10-13] However, the mechanism of LZD on the amelioration of intestinal inflammation is poorly understood.

Clinically, UC patients are usually diagnosed too late due to the interference of serious complications. The identification of more accurate diagnostic biomarkers therefore has a high impact on the improvement of UC outcome. Meanwhile, CMs usually achieve overall therapeutic effects and reduce drug-related side effects through targeting multiple pathways. A systematic in-depth research using some holistic techniques is needed to accurately diagnose UC and confirm the anti-inflammatory effects of LZD.

The degree of metabolic changes and types of metabolites served as a good indicator of cytokines-mediated inflammation in UC.[14] What's more, through the identification of biomarkers, analysis of metabolic pathways, discovery of drug-target interactions, etc., it is also possible to elucidate the pathogenesis of diseases and the mechanism of drugs.[14] Metabolomics is a system biology method that measures the dynamic multi-parametric metabolic responses of living systems to pathophysiologic stimuli or genetic modification by performing the simultaneous and non-targeted analysis of dynamic changes of endogenous metabolites in complex biological matrices.[14-16] Therefore, metabolomics has aroused extensive interest in studying UC and evaluating drug treatment outcomes.

In this study, mice with colitis induced by dextran sodium sulfate (DSS) were used to study the effect of intervention after oral administration of LZD. To further elucidate its mechanism, metabolic profiles in plasma and urine from DSS-induced colitis mice were explored by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass (UPLC-Q-TOF/MS) combined with Metabolynx™ software. The potential biomarkers and metabolic pathways were preliminarily identified.

METHODS

Chemicals, Reagents and Materials

Acetonitrile and formic acid were supplied by Merck (Darmstadt, Germany). *Radix Glycyrrhizae* (batch No.1910009), *Radix Ginseng* (batch No.190801), *Rhizoma Atractylodis* Macrocephalae (batch No. 190801) and *Zingiberis Rhizoma* (batch No.19040301) were purchased from Jiayou Chinese Medicine Decoction Pieces Co., Ltd. (Anhui, China). DSS (LLC, France) was purchased from MP Biomedicals Co., Ltd. (Shanghai, China)

LZD Extract Preparation

Radix Glycyrrhizae, *Radix Ginseng*, *Rhizoma Atractylodis* Macrocephalae and *Zingiberis Rhizoma* were mixed and soaked in water (1:6, w/v) for 20 min, and then decocted for 30 min. The filtrate was collected and the residue was then decocted in water (1:4, w/v) for 20 min. Then, 2 filtrates were combined and concentrated to 1.0 g/mL.

Animals

Thirty-six male specific pathogen-free C57BL/6 mice, weighing 18–22 g, were supplied by Vital River Laboratory Animal Technology Co., Ltd. [Beijing, China; experimental animal license No. SCXK (Su)-2016-0003]. The animals were kept in a clean room with controlled temperature and humidity (23 ± 2 ℃ and 55% ± 10%, respectively), with a 12-h light/dark cycle. Strictly implement animal welfare and experimental procedures were in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996), all the experimental procedures were approved by the institutional and local committee on the care and use of animals of Nantong University.

Induction of Colitis Mice Model and Treatment Protocol

Referring to the previously established method of the colitis model,[17] DSS was dissolved in distilled water to form 2% and 2.5% (W/W) DSS solution. 2% DSS solution was given for the first 3 days and 2.5% DSS solution was given to induce colitis for the following 4 days. After 7 days of adaptation, mice were randomly divided into 6 groups, including the normal, model, low-, medium-, and high-dose LZD groups (1.365, 4.095 and 12.285 g/kg, LZD-L, M and H groups, respectively) and positive drug group (sulfasalazine, SASP, 0.758 g/kg). Acute colitis were induced by drinking DSS-containing water for 7 days. All the treatments were given for 14 days by gastric gavage once daily.

Biological Effects of LZD on UC

According to previous studies, weight loss, colon length, diarrhea and blood stool were used to

evaluate the ulcerative colitis induced by DSS.[18,19] The Disease Activity Index (DAI) score was an indicator of the severity of colitis and was based on the results including weight loss, stool charter and fecal occult blood.[20] Histological characteristics of the colons were observed by HE staining.

Biological Sample Collection and Preparation

After the last treatment, all mice were placed in metabolic cages and urine was collected every 12 h. Each fresh urine sample was centrifuged at 2,000 *g* for 10 min at 4 ℃ and the supernatant was collected stored at –80 ℃ until use. Urine samples were prepared by mixing 100 μL urine with 300 μL organic solvent mixture (acetonitrile: MeOH, 3:1 v/v) and centrifuged at 13,000 *g* at 4 ℃ for 10 min for deproteinization and removal of particulates. The supernatant was evaporated under vacuum, and the residue was reconstituted in 100 μL of methanol.

Blood samples were collected into the tubes containing EDTA-2Na from eyeballs and immediately centrifuged at 12,000 *g* at room temperature for 10 min. A 300 μL volume of extraction mixture (MeOH: acetonitrile: acetone, 1:1:1 v/v/v) was added to 100 μL plasma aliquots for deproteinization, followed by centrifugation at 13,000 *g* for 15 min at 4 ℃. The supernatant was evaporated under vacuum and the dry residue was reconstituted in 100 μL of methanol. Totally 2 μL aliquot of each plasma or urine sample was injected for LC/MS analysis.

UPLC-Q-TOF/MS Analysis Conditions

The plasma or urine samples were divided into 2 groups by unsupervised PCA mode Ⅰ, including DSS-induced colitis model group and normal group.

UPLC-Q-TOF/MS analysis platform provides precise molecular weight and corresponding product fragments within retention time and measurement error (<5 ppm) for the identification of metabolites. Based on the precise molecular weight, the possible element composition was predicted, and the possible molecular formulas were retrieved from Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) and human metabolic database (http://www.hmdb.ca). Collectively, 13 potential biomarkers were identified by above methods.[14]

Metabolites separation was performed using a Waters Acquity™ UPLC system (Waters, USA). Totally 2 μL sample solution was injected into an ACQUITY™ UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm, Waters Corporation, Milford, USA) with the flow rate was 0.4 mL/min at 35 ℃. The optimal mobile phase consisted of water (A, 0.1% formic acid) and acetonitrile (B). The optimized UPLC elution conditions were: 0–8 min, 95%–70% A; 8–11 min, 70%–30% A; 11–13 min, 30%–5% A; 13–14 min, 5% A. The auto-sampler was maintained at 4 ℃.

MS spectral analysis was performed on Waters Synapt™ Q-TOF/MS (Waters Corp., Milford, MA, USA). The conditions for the use of the electrospray ion (ESI) source were as follows: capillary voltage 3.0 kV, sample cone voltage 30.0 V; extraction cone voltage 2.0 V, source temperature 120 ℃, desolvation temperature 350 ℃. Nitrogen was used as desolvation and cone gas with the flow rate of 600 and 50 L/h, respectively. Leucine-enkephalin was used as the lock mass generating an [M+H]+ion (m/z 556.2771) and [M-H]-ion (m/z 554.2615) at a concentration of 200 pg/mL and flow rate of 100 μL/min to ensure accuracy during the MS analysis by a syringe pump.[14]

Metabolomic Data Processing and Multivariate Data Analysis

UPLC/MS data were detected and further processed by Massylynxv4.1 software. The characteristics of potential biomarkers were confirmed by comparing the retention time of mass spectrometry and chromatography with available reference standards and the full spectrum library of MS/MS data obtained under positive and negative ion modes. According to the precise molecular weight, the possible element composition was predicted, and the possible molecular formula was searched in the human metabonomics database (http://www.hmdb.ca).[14] Metabolic pathway interpretation was performed by metabolic analyst 3.0 (http://www.metaboanalyst.ca).

Using OPLS-DA technology, the classification recognition rate and the determination of potential metabolites were maximized. A "left-one-out" algorithm was used to detect 100% sensitivity and 95% specificity of supervised OPLS-DA.

Statistical Analysis

All the experimental data were analyzed by

GraphPad Prism software (version 5). All the results were expressed by an mean ± standard deviation ($\bar{x} \pm s$). Under the appropriate circumstances, the significant difference was analyzed by one-way ANOVA. P<0.05 was considered to be statistically significant.

RESULTS

Improvement of Lizhong Decoction on DSS-Induced Colitis

As shown in Appendix 1A–D, compared with the normal group, the weight of mice in the model group decreased significantly and DAI score was significantly higher (P<0.05 or P<0.01). Compared with the model group, the weight of mice in the LZD-treated and SASP groups increased on the 10th day. The DAI scores of LZD-treated groups were improved and the colon length was significantly longer (P<0.05).

The normal mice maintained complete normal colonic structure and mucosal epithelium. There were no obvious signs of ulcer or inflammatory cell infiltration (Appendix 1E). However, DSS treatment caused the pathological deterioration such as destruction of epithelial cells and crypts. Compared with the normal group, crypt lack, mucosal inflammation and cell infiltration led to a significant increase in histological scores. There was a significant difference in histopathological score between LZD-treated and DSS groups (P<0.05).

LC-MS Analysis of Metabolic Profiles and Intervention Effects of LZD Extracts on Metabolic Profiles of Colitis Mice

The OPLS-DA scoring map showed that two groups were separated obviously (Appendix 2). The results showed the obvious difference between two groups (Appendixes 2A, C, E, G), indicating that the DSS-induced colitis model was successfully established. Based on the above results, potential metabolites associated with the progression of colitis were screened using OPLS-DA scoring map (Appendixes 2B, D, F, H). The details of endogenous metabolites detected are summarized in Table 1.

The metabolic status of plasma and urine of model rats treated by SASP and LZD at different doses returned to the levels of normal rats in various degrees. Additionally, in the direction of principal component, different administration and model groups were separated obviously, which was close to that of the normal group (Appendix 3).

Furthermore, metabolic pathways are successfully established by introducing potential metabolites into a network-based database MetPA. The influence value calculated from pathway to topology analysis with MetPA greater than 0.1 was selected as the potential target pathway. As shown in Figure 1A, among the

Table 1. Identified and Change Trend of Potential Biomarkers of DSS Mice Intervened by LZD

t_R/min	$[M-H]^-$/ $[M+H]^+$	Metabolites	Normal	Model	SASP	LZD-L	LZD-M	LZD-H	Pathway	Resource	Ion mode
14.69	465.6227	Glycocholic acid	–	↓**	↑△△	↑△△	↑△△	↑△	Primary bile acid biosynthesis	Plasma	ESI-
13.5	329.5179	Dihydroceramide	–	↑*	↓△	↓△△	↓△△	↓	Sphingolipid metabolism	Plasma	ESI-
4.24	184.1507	Phosphorylcholine	–	↓**	↑△△	↑△△	↑△	↑△	Glycerophospholipid metabolism	Urine	ESI-
4.92	204.2252	Tryptophan	–	↓**	↑△△	↑△	↑△	↑	Tryptophan metabolism	Urine	ESI-
12.82	280.4455	Linoleic acid	–	↓*	↑△	↑△△	↑△	↑	Linoleic acid metabolism	Plasma	ESI+
11.38	304.2402	Arachidonic acid	–	↓*	↑△	↑△	↑△	↑	Arachidonic acid metabolism	Plasma	ESI+
1.33	103.0946	Gamma-aminobutyric acid	–	↑*	↓△△	↓△△	↓△△	↓△	Butanoate metabolism	Plasma	ESI+
2.07	132.1179	L-asparagine	–	↓**	↑△△	↑△△	↑△△	↑△	Alanine, aspartate and glutamate metabolism	Plasma	ESI+
11.98	496.3199	LysoPC (16:0)	–	↑*	↓	↓△	↓△	↓	Glycerophospholipids	Plasma	ESI+
12.83	524.3340	LysoPC (18:0)	–	↑**	↓△	↓△△	↓△	↓	Glycerophospholipids	Plasma	ESI+
11.97	267.2413	Adenosine	–	↑**	↓△△	↓△△	↓△△	↓△	Purine metabolism	Plasma	ESI+
12.66	140.1399	Methyl-imidazoleacetic acid	–	↑**	↓△	↓△△	↓△	↓△	Terpenoid, backbone biosynthesis	Urine	ESI+
12.22	148.1800	2-Oxo-4-methylthio butanoic acid	–	↑**	↓△	↓△△	↓△△	↓△	Terpenoid, backbone biosynthesis	Urine	ESI+

Notes: ↑ indicates increase; ↓ indicates decrease. ESI: electrospray ion. LZD-L: low dose LZD group; LZD-M: medium dose LZD group; LZD-H: high dose LZD group. *P<0.05, **P<0.01, *vs* normal group; △P<0.05, △△P<0.01 *vs*. model group

10 pathways in plasma, (1) linoleic acid metabolism, (2) arachidonic acid metabolism, (3) sphingolipid metabolism and (4) alanine, aspartate and glutamate metabolism were filtered out as the most important metabolic pathways. In Figure 1B, 6 pathways in urine mainly involving (1) linoleic acid metabolism, (2) tryptophan metabolism and (3) cysteine and methionine metabolism were filtered out.

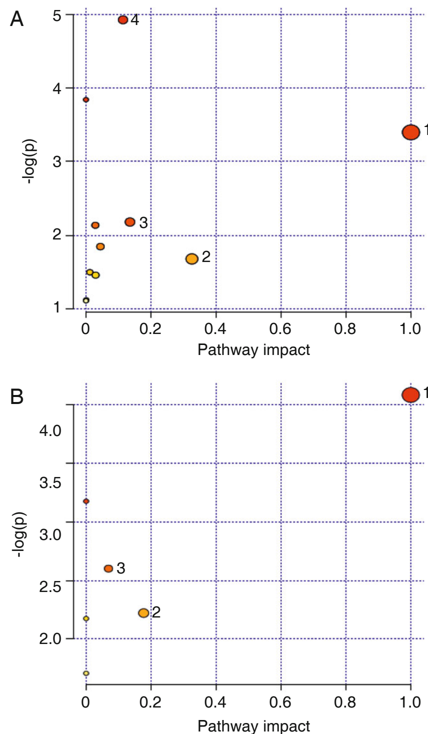

Figure 1. Metabolic Pathways Involved in Potential Biomarkers in Plasma and Urine

Notes: A: (1) linoleic acid metabolism; (2) arachidonic acid metabolism; (3) sphingolipid metabolism; (4) alanine, aspartate and glutamate metabolism; B: (1) linoleic acid metabolism; (2) tryptophan metabolism; (3) cysteine and methionine metabolism)

DISCUSSION

Our previous experiments thoroughly reported the physiological and pathological changes of mice with colitis after oral administration of LZD extracts which showed that LZD could significantly ameliorate the symptoms of DSS-induced colitis mice.[11] The above results indicated that the targeted pathways might promote to the development of colitis and were significantly interfered by LZD treatment.

The physiological intestinal barrier mainly consists of the epithelial cell layer, which protects the body from potential damage caused by bacteria, nutrients, and toxins in the lumen. Lysophosphatidylcholine (LysoPCs), due to its natural detergent properties, participates in the process of barrier destruction.[21] LysoPCs can alter intestinal permeability and destroy cell membranes, thus allowing larger molecules (such as potential antigens and toxic molecules) to cross the intestinal wall, thereby destroying the mucosal barrier. High LysoPCs protein concentration impairs intestinal barrier function and increases gastrointestinal permeability.[22] Additionally, LysoPCs participates in the pathogenesis of inflammation, acts as a chemokine of monocytes and T cells, and exhibits pro-inflammatory properties.[23] In this study, plasma levels of lysoPCs, including LysoPC (16:0) and LysoPC (18:0), were notably up-regulated in the DSS-treated rats. These results were consistent with the destructive and pro-inflammatory properties of LysoPCs on intestinal barrier, suggesting that LysoPCs played a role in mucosal inflammation in UC. LysoPC (16:0) is one of the most prominent LysoPC species produced by endothelial lipase on high density lipoprotein, which can promote the synthesis of endothelial interleukin (IL)-8.[21] IL-8 in the pathogenesis of UC is a key pro-inflammatory factor, promotes neutrophil into non-inflammatory mucosa. Therefore, elevated levels of LysoPCs indicated the possibility of intestinal barrier damage and inflammation. However, our findings indicated that LZD could inhibit inflammation by reducing levels of LysoPC, leading to the prevention and treatment of UC mice in different dose groups.

It is documented that bile acids (BAs), is one of the most important components in bile which is composed of bilirubin, cholesterol, phospholipids, proteins, water, and electrolytes.[24] Inflammatory bowel disease (IBD) has been reported to be closely related to intestinal flora and bile acid metabolic disorders.[25] Therefore, BAs might play a pivotal role in the pathological process of UC. BAs could act as signaling molecules to mediate hormone action by activating BA receptors (such as farnesoid X receptor (FXR/NR1H4) and membrane-bound BA receptor TGR5 (also known as GPBAR1 or M-BAR/BG37).[26] Ligand-activated nuclear receptors (NRs) such as FXR regulated a wide range of metabolic processes, including liver BA transport and metabolism, lipid and glucose metabolism, drug treatment, liver regeneration, inflammation, fibrosis, cell differentiation and tumorigenesis.[27] BA-dependent FXR activation also regulated bacterial overgrowth and maintained intestinal mucosal integrity by inducing transcription of

multiple genes involved in intestinal mucosal defense microorganisms under physiological conditions.[28] In this study, the level of glycocholic acid in plasma of mice treated with DSS was notably decreased, while the trend was markedly reversed by different dosages of LZD. Thus, it was rationally speculated that the amelioration of LZD on colitis might activate FXR by regulating glycocholic acid to further regulate inflammatory response.

Tryptophan played a key role in the immunosuppression, immune system regulation, protein synthesis and production of serotonin (5-HT) and melatonin in inflammatory diseases.[27,29] Tryptophan was also an effective endogenous free radical scavenger and antioxidant, which could protect oxidative damage and play an anti-inflammatory role in DSS-induced colitis.[30] 5-HT activated immune cells to produce inflammatory mediators, inhibited nerve transmission and regulated gastrointestinal secretion, movement and perception.[31] In this study, tryptophan contents in DSS-induced mice were significantly reduced, which indicated that tryptophan metabolism in UC might be enhanced. While the notable increase of tryptophan was detected in plasma from colitis mice after oral administration of low and medium dosages of LZD. So, it could be inferred that LZD might ameliorate the immune dysfunction of UC by the modulation of tryptophan metabolism.

Increasing evidences have shown that the disorder of energy metabolism is also closely related to UC.[32] This study found that the content of adenosine in DSS-induced mice was remarkably higher than that in normal mice. Adenosine is an important intermediate for the synthesis of adenosine triphosphate (ATP), adenine, adenylate and adenosine arabinose. It was speculated that the improvement of normal ATP supply might contribute to the restoration of UC mucosal epithelial injury.[33] Thus, it was necessary to modulate energy metabolism to maintain mucosal epithelial barrier.[34] The data suggested that increased adenosine might play an important role in promoting the development of intestinal barrier dysfunction induced by DSS.

Dihydroceramide, a sphingolipid substance, could be produced from acetylated sphinganine by ceramide synthases, while the latter was degraded from 3-keto-dihydrosphingine by 3-keto-dihydrosphingine reductase.[35] Dihydroceramide could be transformed to ceramide by desaturase and subsequently hydrolyzed to sphingosine.[36] And the latter was further phosphorylated to sphingosine-1-phosphate (S1P) by sphingosine kinase, which occurred in membrane and cytoplasm.[37] Accumulation of S1P could lead to the increased inflammation and affect mucosal homeostasis and inflammatory signals.[38] Compared to the DSS-induced mice, the levels of dihydroceramide decreased significantly after treatment of LZD, which indicated that LZD might improve ulcerative colitis by regulating sphingolipid metabolism to alleviate the inflammation.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mature central nervous system (CNS).[39] It is well known that GABA not only plays a role in the CNS, but also exists in various peripheral tissues, such as gastrointestinal tract and immune cells.[40] Intestinal barrier dysfunction makes an important impact in the occurrence and development of UC. However, GABA increased intestinal permeability and bacterial infiltration in the colon by activating GABAA receptors (GABAARs)[41] and accelerated the onset and progression of DSS-induced acute colitis.[42] In this study, it can be found that LZD might reduce bacterial infiltration and restore intestinal barrier by blocking the combination of GABA and GABAARs.

Recently, some researches indicated that nutrients such as amino acids and fatty acids were thought to be regulators for the repair of intestinal barriers.[43] Especially, L-glutamine was essential to maintain the integrity of intestinal mucosa.[41] L-asparagine (ASN), a non-essential amino acid with similar chemical structure (less than one methylene) to glutamine could be a substitute for glutamine.[43] ASN could alleviate intestinal barrier dysfunction, which was partly related to the regulation of CRF/CRFR1 signaling pathway and mast cell activation.[44] In this study, LZD could notably increase the contents of ASN in plasma from UC mice, which suggested that LZD might ameliorate UC by modulating amino acid metabolism.

Besides the above biomarkers and metabolic pathways, we all know that linoleic acid might inhibit adverse reactions by reducing the yield of inflammation factors such as tumor necrosis factor α and IL-1. Linoleic acid could also be metabolized into arachidonic acid (an unsaturated fatty acid) and the later was further degraded to prostaglandins,

leukotrienes, and other inflammatory substances. It was documented that many prostaglandins and leukotrienes produced by arachidonic acid metabolism were released in mice with ulcerative colitis.[44,45] In this study, linoleic acid and arachidonic acid were notably decreased in UC mice, while their plasma concentrations were markedly increased by low- and medium-dose of LZD. Furthermore, phosphatidylcholine, an important product of choline metabolism and a major cell component of the biofilm, was significantly decreased in DSS-induced colitis mice, which was similar to the previous report.[46] The absence of phosphatidylcholine might cause abnormal cell membrane biosynthesis. However, LZD remarkably restored the tendency.

Taken together, LZD significantly ameliorated the symptom of UC mice. Thirteen potential biomarkers, which were markedly regulated by LZD, were identified by UPLC-Q-TOF-MS. Their metabolic pathways mainly involved linoleic acid, arachidonic acid, sphingolipid, alanine, aspartate, glutamate, tryptophan, cysteine, and methionine metabolisms. This study explored the possible mechanism of LZD on UC treatment, which was beneficial for further clinical application.

Conflict of Interest

The authors have declared that there is no conflict of interest.

Author Contributions

Tao JH and Jiang S conceived and designed research. Tao JH, Shen YM, Wang L and Chen YF conducted experiments. Jiang S contributed new reagents or analytical tools. Tao JH, Shen YM and Jiang S analyzed data. Tao JH, Wang L, Chen YF and Shen YM wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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