Effect of Shenling Baizhu San on Lipid Metabolism and Intestinal Barrier of UC with Spleen Deficiency

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Research

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Abstract

**Background:** Ulcerative colitis (UC) is a chronic inflammatory bowel disease and spleen-deficiency (SD) is considered as the common Chinese medicine syndrome of UC. However, the mechanism of Shenling Baizhu San (SLS) on UC with spleen-deficiency has not been clarified. The aim of this study was to explore the influence of SD on lipid metabolism and intestinal barrier in UC mice and reveal the underlying mechanism of SLS on UC mice with spleen-deficiency.

**Methods:** The male C57BL/6 mice were randomly divided into control group, Dextran Sulfate Sodium (DSS) group, DSS+SD group and SLS group. The data of disease activity index, body weight and length of colon were recorded to evaluate the colitis, the spleen and thymus weight were recorded to evaluate the spleen-deficiency, the serum metabolites were detected by untargeted metabolomics, the tight junction and intestinal mucus barrier were evaluated by Alcian Blue-Periodic acid Schiff staining and immunochemical, the activity of ATPase were detected by spectrophotometer, the proinflammatory factors and the copy number of mitochondrial DNA were detected by RT-PCR, the relative expression of Sirtuin 1 (SIRT1), Peroxisome proliferator-activated receptor γcoactivators-1α (PGC-1α) and Mitochondrial transcription factor A (TFMA) were detected by RT-PCR and western blot.

**Results:** The mice treated by compound method showed decreased spleen and thymus weight as well as more serious intestinal inflammation than DSS group. Compared with DSS+SD group, SLS improved intestinal inflammation and spleen deficiency, ameliorated lipid metabolism and intestinal barrier as well as promoted expression of SIRT1, PGC-1α and TFMA in UC mice with spleen-deficiency.

**Conclusion:** SLS could be considered as an effective treatment for spleen-deficiency in UC and its underlying mechanism might be related to improve lipid metabolism and intestinal barrier through mediating SIRT1/PGC-1α pathway.

**Background**

Ulcerative colitis (UC), a chronic inflammatory bowel disease, is featured as abdominal pain, diarrhea and bloody stools on clinical manifestations and continuous and diffuse inflammation of the colorectal mucosa on pathological manifestations[1]. The pathogenesis of UC has not been clarified and it is thought that genetic susceptibility individuals were influenced by environmental, microbial and immune-mediated factors and then lead to intestinal inflammation[2].

Lipid has an important role in constituting biological membranes and maintaining the integrity of the intestinal barrier[3]. The dysfunction of lipid metabolism was founded in UC[4] and our previous clinical research showed there was abnormal alteration of lipid metabolism in UC patients with spleen-deficiency (SD). SD is a common syndrome of UC[5], however, the influence of SD on lipid metabolism and intestinal barrier in UC has not been clarified.
There has been amounts of studies reported that improving mitochondrial function is helpful to maintain lipid metabolism balance[6, 7]. Mitochondrial biogenesis refers to adaptive increase of the number and quality of mitochondria to produce ATP when human body was in energy-deficiency[8] and Peng et al found sulforaphane can improve lipid utilization and regulate lipid metabolism by promoting mitochondrial biogenesis[9]. SIRT1/PGC-1α pathway has the effect of promoting mitochondrial biogenesis. Peroxisome proliferator-activated receptor γ coactivators-1α (PGC-1α) is involved in regulating the expression of mitochondrial antioxidant genes and preventing oxidative damage and mitochondrial dysfunction[10]. Sirtuin 1 (SIRT1) can active PGC-1α by deacetylation and enhance mitochondrial biogenesis[11, 12]. Mitochondrial transcription factor A (TFAM) has influence in regulating the transcription of mitochondrial nuclear genes and improving the efficiency of mitochondrial biogenesis[13].

Shenling Baizhu San (SLS) is a well-known prescription used to treat diarrhea with SD and was reported as effective treatment on UC in previous researches[14, 15]. However, the mechanism of SLS on UC with SD had not been researched. This study aimed to explore the influence of SD on lipid metabolism and intestinal barrier in UC mice and reveal the underlying mechanism of SLS intervene on UC mice with spleen-deficiency.

**Methods**

**Preparation of SLS**

The composition of SLS: Ren Shen (root of Ginseng Radix et Rhizoma), Bai Zhu (root of Atractylodis Macrocephalae Rhizoma), Fu Ling (sclerotium of Poria cocos.), Shan Yao (Rhizoma of Dioscorea opposite), Bai Bian Dou (seed of Dolichos lablab), Lian Zi (seed of Nelumbinis semen), Yi Yi Ren (kernel of Coicis semen), Sha Ren (fruit of Amomi fructus), Jie Geng (root of Platycodon grandiflorum), Gan Cao (root of Glycyrrhiza uralensis).

All the crude medicinal materials were purchased from Jiangsu Province Hospital of Chinese Medicine. The composition ratios of SLS were shown as follow: Ren Shen 20g, Bai Zhu 20g, Fu Ling 20g, Shan Yao 20g, Lian Zi 10g, Bai Bian Dou 15g, Yi Yi Ren 10g, Sha Ren 10g, Jie Geng 10g, Gan Cao 20g according to “Chinese Pharmacopoeia”[16]. All herbs were soaked in 10-fold volumes pure water for 1 hour and decocted at 100℃ for 40min. The liquid medicine was collected and the process was repeated. Then the twice liquid was put together and centrifuged 4000g for 10min. After that, the centrifuged liquid medicine was rotary evaporate (60℃ 70rpm) in a rotary evaporator to 64.5ml with a concentration of 2.4g/ml. Previous study found the dosage of 24g/kg/day achieved the best therapeutic effect, so this study applied the dosage.

**Animals and grouping**

A total of 24 male C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. All mice were housed in a condition with temperature (22-26℃) and relative humidity (50-60%)
on a reverse 12 h light/dark circle. After acclimating for 1 week, the mice were randomly divided into four groups: control group (Ctrl group) were treated by free drinking and eating on day1-day21; UC mice group (DSS group) were treated by free drinking and eating on day1-day14 and treated by 3% Dextran Sulfate Sodium (DSS) on day15-day21; UC mice with spleen deficiency group (DSS+SD group) were induced to spleen deficiency on day1-day14 and were treated by 3% DSS on day15-day21; Shenling Baizhu San group (SLS group) were induced as spleen deficiency on day1-day14 and treated by 3% DSS and SLS on day1-day21.

**Reagents**

DSS powder (cat. no. 160110, molecular weight: 36,000–50,000) was purchased from MP Biomedicals; methanol and acetonitrile (chromatographically pure) were purchased from Tedia in USA; formic acid (chromatographically pure) was purchased from Adamas in China; NH3Ac (chromatographically pure, 99%) was purchased from Damas Reagent Co.Ltd in China; RT SuperMix and ChamQ SYBP qPCR Master Mix were purchased from Vazyme; ATPase kits were purchased from Nanjing Jiancheng Bioengineering Institute; SIRT1, PGC1, TFAM antibody were purchased from Proteintech Group, Inc.

**Modeling of UC in mice**

3g DSS powder was dissolved into 100ml autoclaved tap water to make 3% DSS solution. The 3% DSS solution were free given to mice 5ml per mouse per day to induce UC[17]. The bottles were emptied and filled with fresh DSS solution every other day.

**Modeling of spleen-deficiency in mice**

Spleen-deficiency was induced by limited feeding and overstrain according to published studies[18]. Food was provided on odd-numbered days and fast on even-numbered days to limit feeding and mice were forced to swim until they lose the ability to rise to the water surface within 10 seconds every day to induce overstrain.

**Macroscopic and histological analysis**

Body weight, length of colon and disease activity index (DAI) of mice were determined and hematoxylin-eosin staining (H&E) were used to evaluate the histological alteration.

**Table 1 Disease activity index (DAI)**
| Score | Reduction of body weight (%) | Stool traits | Blood in the stool |
|-------|------------------------------|--------------|-------------------|
| 0     | None                         | Normal       | None              |
| 1     | 1-5                          |              |                   |
| 2     | 6-10                         | Loose stool  | Occult blood in stool |
| 3     | 11-15                        |              |                   |
| 4     | >15                          | Diarrhea     | Bloody stool      |

Spleen and thymus weight in different groups of mice

The spleen and thymus were washed using pre-cooled 0.9% saline, dried on filter paper and weighed respectively. The spleen index and thymus index were calculated according to the following formula: spleen index=spleen weight/mouse weight, thymus index=thymus weight/mouse weight.

Untargeted metabolomics

The LC/MS analysis was performed as previous described. The serum was extracted with 550μl methanol and the liquid was vibrated for 20 min. After centrifugation at 15000rpm for 10min, 500μl supernatant was transferred to 1.5ml Eppendorf tube and evaporated under vacuum to completely dryness. The extract was dissolved with 200 μl methanol and vibrated for 20 min. After centrifugation at 18000rpm for 10min for twice, 60μl supernatant was transferred to injection tube to perform metabolomics analysis using UPLC/MS SYNAPT G2-Si equipped with Waters ACQUITY UPLCH-Class (Waters Technologies, USA).

The T3 chromatographic column (100 mm × 2.1 mm ×1.8μm, Waters, USA) was used for compound separation at 55 °C. The mobile phases were 0.1% formic acid in 60% acetonitrile (solvent A) and 0.1% formic acid in 90% isopropanol (solvent B). The flow rate of the mobile phase was set at 0.4 mL/min. The gradient conditions were used as follows: 0-2min gradient 60% A, 2-2.1min gradient 57% A, 2.1-12min gradient 50%A, 12-12.1min gradient 60%A, 12.1-18min gradient 60%A, 18-18.1min gradient 60%A, 18.1-20min gradient 60%A. Data were acquired in negative mode. The data was imported into Progenesis QI and calibrated with internal standards, then a multivariate data matrix containing precursor ions (m/z), retention time, and peak area was imported into EZinfo data. MetaboAnalyst was used to perform the orthogonal projections to latent structure discriminant analysis (OPLS-DA), heatmap analysis and VIP score analysis.

The metabolites were identified by comparing the m/z and MS/MS fragmentation patterns from the METLIN (http://metlin.scripps.edu) and Kyoto Encyclopedia of Genes and Genomes (https://www.kegg.jp/).

Alcian Blue-Periodic acid Schiff staining (AB-PAS) and immunohistochemical analyses
Colon tissue was rinsed with ice-cold 0.9% normal saline, fixed in 10% buffered formalin and prepared into sections by dehydrating and paraffin embedding. Then the sections (5 μm thick) were stained with AB-PAS, zonula occludens 1 (ZO-1) and Mucin-2 (Muc-2).

**Real-time PCR**

Total RNA was extracted from colon tissue using Trizol reagent (Invitrogen) and the RNA concentration was determined by Nanodrop 2000. cDNA was synthesized using HiScript Ш RT SuperMix (Vazyme). qPCR assays were performed using ChamQ SYBP qPCR Master Mix (Vazyme) with LightCycler96 (Roche). The primers were provided by Invitrogen. The relative mRNA expression was calculated by normalizing the expression of each target gene to that of β-actin using the $2^{-\Delta \Delta Ct}$ method.

**Table 2 The primers used for RT-PCR**

| Gene    | Primers Sequence                  |
|---------|----------------------------------|
| GAPDH   | (F) TGTCATCAACGACCCCTTCA         |
|         | (R) GGTCTCGCTCCTGGAAATCG         |
| TNF-α   | (F) CCACCACGCTCTTCTGTCTA         |
|         | (R) AGGTTCTGGGCCCATAGAACT        |
| IL-1β   | (F) GAAATGCCACCTTTTGACAGTG       |
|         | (R) TGGATGCTCTCATCAGGACAG        |
| IL-6    | (F) TAGTCCCTTCTACCCCAATTTC       |
|         | (R) TTGGTCCCTTAGCCACTCCTTC       |
| PGC-1β  | (F) GCAGCCAAGACTCTGTATGG         |
|         | (R) TTCCGATTGGTGCTACACC          |
| SIRT1   | (F) TGACAGAACGTCACACGCC          |
|         | (R) AACAATCTGCCACACGGTCA         |
| TFAM    | (F) GAGCGTGCTAAAAGCACTGG         |
|         | (R) CCACAGGGCTGCAATTTC          |
| gDNA    | (F) AGCAGGAGGCCTAAATTTGAGTC      |
|         | (R) GAAGTAGCCAGGGTGGG           |
| mtDNA   | (F) TGAACGGCTAAACGAGGTC          |
|         | (R) AGCTCCATAGGGTCTTCTCGT        |
Western blotting analysis

Total protein of colon tissue was extracted using lysis buffer and the protein concentration was measured by BCA kit (Beyotime, Shanghai). The proteins were separated by SDS-PAGE and transferred to PVDF membrane (Millipore). The membranes were blocked in the blocking buffer for 1 hour at 37°C, and incubated with the primary antibodies at 4°C overnight. After that, the membranes were incubated with the second antibodies for 90 min at room temperature. Finally, the proteins were imaged using ECL reagent kit (Millipore) with Chemiluminescence Imaging System (Bio-Rad) and the images were measured by Image Lab Software.

Statistics analysis

The statistics analysis was conducted by SPSS 16.0 software package. The data was analyzed using one-way ANOVA or non-parametric test according to data distribution. The pairwise comparison was conducted using LSD method. \( P < 0.05 \) was be considered that the difference was statistically significant. The differential metabolites with \( P < 0.05 \) and normalised abundance > 1000 were screened by using Kruskal-Wallis test.

Results

SLS reduced loss of weight and DAI of model mice

The time-related change of body weight of mice was shown in Fig1A. There was gradual downregulation of body weight in DSS group compared with Ctrl group, which was consisted with previous studies. DSS+SD group exhibited greater loss of body weight than DSS group. However, SLS reduced the loss of weight UC mice with spleen-deficiency. The time-related change of DAI was showed in Fig 1B. In comparison with Ctrl group, DAI in DSS and DSS+SD group both increased, while DAI in SLS group decreased significantly in comparison with the DSS+SD group. It was suggested that spleen-deficiency might exacerbate the macroscopic manifestations in mice intervened by DSS, and SLS could relieve the manifestations in mice of UC with spleen-deficiency.

Length of colon in different groups of mice

The comparation of colon length was shown in Fig1C and D. Compared with Ctrl group, the colon length in DSS and DSS+SD group both reduced significantly and it was even shorter in DSS+SD group, while the mice in SLS group exhibited longer colon than that in DSS+SD group. It was suggested that DSS shorten the colons of mice successfully and spleen-deficiency exacerbated this alteration, however, SLS treatment ameliorated the shortening of colon in mice of UC with spleen-deficiency.

Spleen and thymus weight in different groups of mice

As shown in Fig 2, the spleen weight and spleen index increased in the DSS group and decreased in the DSS+SD group compared with the Ctrl group. After intervention with SLS, both the spleen weight and
spleen index increased significantly compared with DSS+SD group. Compared with the Ctrl group, both thymus weight and thymus index decreased significantly in DSS and DSS+SD group, however those in SLS group exhibited significant elevation compared with DSS+SD group. It was suggested the intestinal inflammatory induced by DSS upregulated the spleen weight and downregulate the thymus weight in mice, while spleen-deficiency both downregulated the spleen and thymus weight.

Histopathological observation of colon tissue in different groups of mice

The histopathological alteration in different groups was observed by H&E staining. As Fig 3 showed, there was complete colonic epithelial structure and normal crypt morphology in colon tissue of Ctrl group. However, colon tissue of DSS and DSS+SD group were damaged. The histopathological alteration of DSS group were characterized as destroyed colonic epithelial structure, incomplete crypt morphology and inflammatory infiltration in mucosal layer, and that of DSS+SD group were characterized as completely destroyed colonic epithelial structure, disappeared crypt morphology and a large amount of inflammatory infiltration in the mucosal layer and submucosa layer. After intervention with SLS, the colon tissue was shown as relatively complete colonic epithelial structure and crypt morphology and inflammatory infiltration in the mucosal layer. It was indicated that spleen-deficiency aggravated histopathological damage of colon tissue induced by DSS and SLS ameliorated the damage at a microscopic level.

The level of inflammatory cytokines in different groups

In comparison with the Ctrl group, the relative mRNA expression of IL-1β, TNF-α and IL-6 in DSS and DSS+SD group increased significantly and those in DSS+SD group was higher. After SLS treatment, lower mRNA expression of inflammatory cytokines was shown than DSS+SD group (Fig 4), which signified spleen-deficiency promoted the intestinal inflammation in UC mice and SLS reduced the intestinal inflammation.

The relative intensity of serum metabolites in different groups of mice

The result of sPLS-DA analysis showed there were differences in metabolites among the four groups (Fig 5A), and the heatmap verified the difference (Fig 5B). In comparison with Ctrl group, DSS+SD group exhibited decreased relative intensity in PC(0-7:0/O-7:0), Phosphocholine, PC(0-18:1(1E)/0:0), PC (14:0/0:0), LysoSM(d18:0), LysoPC(20:0), Docosatrienoic Acid and increased relative intensity in LysoPC(22:6) and Glycerophospho-N-Palmitoyl Ethanolamine. After intervention with SLS, the mice showed consistent tendency with Ctrl group (Fig 5D-L) and it was indicated that UC mice with spleen-deficiency suffered from imbalance of metabolites, while SLS had an effect on regulating the metabolites balance.

The intestinal barrier in different groups of mice

As shown in the AB-PAS staining results, colonic goblet cells and mucus decreased in DSS and DSS+SD group, while SLS treatment relieved the reduction of goblet cells and restored the mucus. As shown in the IHC results, the expression of ZO-1 and Muc-2 decreased compared with Ctrl group, while SLS treatment
upregulated the expression of ZO-1 and Muc-2 compared with DSS+SD group (Fig 6). It was indicated that spleen-deficiency exacerbated the damage of tight junction and mucosal barrier in colon and SLS ameliorated the damage effectively.

**The expression of SIRT1-PGC1-TFAM mRNA in different groups of mice**

The relative mRNA expression of SIRT1, PGC1 and TFAM were detected by RT-PCR as shown in Fig 7. Compared with Ctrl group, both the DSS and DSS+SD group exhibited significant reduction in the relative mRNA expression and DSS+SD group exhibited further reduction. Compared with DSS+SD group, the relative mRNA expression of SIRT1, PGC1 and TFAM increased significantly in SLS group. The results signified UC mice existed mitochondrial biogenesis dysfunction and spleen deficiency might aggravate this dysfunction. Besides, SLS could promote mitochondrial biogenesis in UC mice with spleen-deficiency through SIRT1/PGC-1α pathway.

**The protein expression of SIRT1-PGC1-TFAM in different groups of mice**

The relative protein expression of SIRT1, PGC1 and TFAM were detected by western blot as shown in Fig 7. The relative expression of above proteins performed the same trend as mRNA expression. Compared with Ctrl group, DSS and DSS+SD group exhibited significant reduction in the relative protein expression and DSS+SD group exhibited further reduction. After SLS intervention, the relative protein expression of SIRT1-PGC1-TFAM in mice upregulated significantly in comparison with DSS+SD group.

As shown in above results, spleen-deficiency was considered as an unfavorable factor to mitochondrial biogenesis in UC mice and SLS could be acted as an effective intervention to promote mitochondrial biogenesis through SIRT1/PGC-1α pathway.

**The activity of Na⁺-K⁺-ATPase and Ca⁺-Mg⁺-ATPase in different groups of mice**

Compared with Ctrl group, Na⁺-K⁺-ATPase and Ca⁺-Mg⁺-ATPase activity in DSS and DSS+SD group reduced significantly ($P < 0.05$, $P < 0.01$) and those in DSS+SD group showed further reduction, while the ATPase activity in SLS group performed upregulation compared with DSS+SD group. (Fig 8A and B) The results suggested there was cell transport dysfunction in UC mice and spleen-deficiency aggravated the dysfunction and SLS was suggested could improve cell transport and mitochondrial function in UC mice with spleen-deficiency.

**The copy number of mitochondrial DNA in different groups of mice**

Compared with the Ctrl group, the copy number of mitochondrial DNA (mtDNA) in DSS and DSS+SD group were significantly reduced ($P < 0.05$, $P < 0.01$) and the DSS+SD group showed further reduction. However, the copy number of mitochondrial DNA in SLS group exhibited significant elevation compared with DSS+SD groups after SLS treatment. (Fig 8C) The results suggested there was mitochondrial transcription dysfunction in UC mice and spleen-deficiency exacerbated the dysfunction and SLS was
considered as an effective measure to improve mitochondrial DNA transcription in UC mice with spleen-deficiency.

**Discussion**

The animal model of Chinese medicine syndrome is a kind of biological characterization model that uses some biological characteristics of animals to simulate the characteristics of human syndrome[19]. There had been no unified method to preparing animal models of UC with spleen-deficiency syndrome so far and it was reported that the common methods included bitter cold purging, limited feeding, overstrain, reserpine and so on. These methods can be used alone or in combination. The evaluation indicators of spleen-deficiency syndrome included macroscopic characterization and objective indicators. The macroscopic characterization included decreased food intake, loss of weight, loose stools, dry hair, arched back, unresponsiveness and so on[20]. The objective indicators included decreased D-xylose absorption rate, decreased spleen weight, decreased thymus weight and so on[21]. The compound method consists of DSS, limited feeding and overstrain was applied to prepare model of UC mice with spleen-deficiency in this study was referred to a published study[18] and it was adjusted according to the feature of UC. The results of this study showed UC mice with spleen-deficiency exhibited macroscopic manifestation such as decreased food intake, decreased mobility, dry and yellow hair and abnormal objective indicators such as decreased spleen and thymus weight. Furthermore, the model mice showed bloody stools and increased expression of pro-inflammatory factors, which suggested the compound method is an effective and reliable method to prepare animal model of UC with spleen deficiency.

In the theory of Chinese medicine, the main physiological function of the spleen (Pi) includes govern transportation and transformation and control blood circulating in the vessels. Spleen-deficiency lead to dysfunction of transporting and transforming nutrient substances as well as blood circulating, which finally resulted in diarrhea and bloody stools. For the reason that spleen-deficiency is thought as an unfavorable factor to patients with UC. The spleen weight of mice in DSS group significantly increased compared with control group, suggesting that UC mice exhibited splenomegaly and hyperfunction of immunity. However, the spleen weight in DSS + SD group significantly decreased compared with DSS group, suggesting that the UC mice with spleen deficiency exhibited hypofunction of immunity. The reduction of thymus weight in DSS group suggested UC mice exhibited thymus atrophy and the thymus weight of mice in DSS + SD group further decreased, which indicated spleen deficiency aggravate the thymus atrophy of UC mice. The thymus index in DSS + SD group were higher than that in DSS group, it might be related to the body weight in DSS + SD group were lower than DSS group.

SLS was recorded in "Taiping Huimin Heji Jufang" in Song Dynasty. Lin et al reported SLS relieved colitis associated colorectal cancer through inhibiting epithelial mesenchymal transition induced by transforming growth factor β1[22]. Chao et al reported SLS improved DSS-induced UC in mice via MAPK/NF-κB and pyroptosis signaling pathway[23]. In this study, mice treated by SLS exhibited increased spleen and thymus weight and decreased expression of pro-inflammatory cytokines, which
confirmed the effect of SLS on UC with spleen-deficiency and disproved the reliability of compound method to prepare animal model.

The lipid metabolism imbalance was observed in UC with spleen-deficiency. There were decreased relative intensity of PC(O-7:0/O-7:0), Phosphocholine, PC(O-18:1(1E)/0:0), PC (14:0/0:0), LysoSM(d18:0), LysoPC(20:0), Docosatrienoic Acid and increased peak area of LysoPC(22:6) and Glycerophospho-N-Palmitoyl Ethanolamine in DSS + SD group. As a component of lipid, PC was reported accounts for 35%-72% of phospholipid in animal gastrointestinal mucus and plays a key role in enhancing the resistance of mucus barrier to cavity attack[3]. PC depletion may lead to decrease of surface hydrophobicity and invasion of harmful substances, especially bacteria, in the cavity. It was also reported that oral PC can improve the intestinal barrier function in rats[24]. Additionally, the anti-inflammatory effect by inhibiting TNF-α and NFκB has been detected in PC[25, 26], and exogenous PC makes a positive impact on ulcerative colitis and intestinal barrier defense against clostridium difficile toxin[27]. The results indicated that the upregulation of PC may be the reason of SLS improve intestinal barrier of DSS + SD mice.

Mitochondria is the "energy factories" and where β-oxidation occurs. When mitochondrial biogenesis was inhibited, the increased NAD+/NADH ratio activated SIRT1 and PGC-1α. Activated PGC-1α is translocated to the nucleus and combined with the key downstream factor nuclear respiratory factor-1/2, and then activated TFAM[10]. TFAM could protect mtDNA from oxidative damage and further improve the efficiency of mitochondrial biogenesis[28]. Na+-K+-ATPase and Ca+-Mg+-ATPase located at the mitochondrial membrane are the core enzymes for energy conversion in organisms and copy number of mtDNA represents mitochondrial transcription function. Previous study showed resveratrol can upregulate the expression of SIRT1 and PGC-1α in rats with type 2 diabetes to regulate mitochondrial biogenesis[6]. Our study showed reductive expression of SIRT1, PGC-1α, TFAM and declining mitochondrial function in DSS + SD group, suggesting there was mitochondrial biogenesis dysfunction in the colon tissue of UC mice with spleen-deficiency. However, SLS upregulated SIRT1, PGC-1α, TFAM expression and promote mitochondrial function, implying that SLS regulated lipid metabolism and intestinal barrier function by mediating SIRT1/PGC-1α pathway.

Conclusions

In conclusion, spleen-deficiency was an unfavorable factor for the intestinal inflammation, lipid metabolism and intestinal barrier of UC mice with spleen deficiency and SLS could be considered as an effective intervention to regulate the abnormal alteration. The underlying mechanism of SLS treatment might be related to activation of SIRT1/PGC-1α pathway.

Abbreviations

UC: Ulcerative colitis; SD: spleen-deficiency; SLS: Shenling Baizhu San; DSS: Dextran Sulfate Sodium; PGC-1α: Peroxisome proliferator-activated receptor γcoactivators-1α; SIRT1: Sirtuin 1; TFAM:
Mitochondrial transcription factor A; AB-PAS: Alcian Blue-Periodic acid Schiff staining; mtDNA: mitochondrial DNA; ZO-1: zonula occludens 1; Muc-2: Mucin-2.

Declarations

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Not applicable.

Authors’ contributions

ZL, YL and JH designed the research; ZL, LZ and HS wrote the main manuscript text; ZL, CC, and JH performed the animal experiments; ZL and YL analysed the data and prepared the figures. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experiment was approved by the ethical committee of Affiliated Hospital of Nanjing University of Chinese Medicine (2019NL-187-03).

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

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Figures
Effect of SLS on macroscopic manifestations. a The time-related change of body weight. b The time-
related change of DAI. c, d Comparison of length of colon in different groups. The data were shown as
the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.
**Figure 2**

Effect of SLS on spleen and thymus weight.  

a Comparison of spleen weight in different groups.  
b Comparison of spleen index in different groups.  
c Comparison of thymus weight in different groups.  
d Comparison of thymus index in different groups. 

The data were shown as the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.

**Figure 3**

H&E staining of colon tissue. Scale bar=40μm

A  

![Image of TNF-α expression](image1)

B  

![Image of IL-1β expression](image2)

C  

![Image of IL-6 expression](image3)
**Figure 4**

Effect of SLS on inflammatory cytokines. a Comparison of TNF-α in different groups. b Comparison of IL-1β in different groups. c Comparison of IL-6 in different groups. The data were shown as the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.

**Figure 5**

The relative intensity of serum metabolites in different groups of mice. a sPLS-DA analysis. b Heatmap. c VIP score. d-l Comparisons of serum metabolites. The data were shown as the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.
Figure 6

The intestinal barrier in different groups of mice. a AB-PAS staining of colon tissue. b Immunohistochemistry for ZO-1 of colon tissue. c Immunohistochemistry for Mucin-2 of colon tissue. Scale bar=50μm

Figure 7

Effect of SLS on SIRT1/PGC-1α pathway. a, b, c Comparison of SIRT1, PGC1α, TFAM mRNA in different groups. d Comparison of SIRT1, PGC1α, TFAM, SOD1 protein in different groups. The data were shown as the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.
Figure 8

Effect of SLS on mitochondrial function. a Comparison of Na+K+-ATPase activity in different groups. b Comparison of Ca+-Mg+-ATPase activity in different groups. c Comparison of mitochondrial DNA in different groups. The data were shown as the mean ± S.E.M. #P < 0.05, ##P < 0.01 vs. the Ctrl group; *P < 0.05, **P < 0.01 vs. the DSS+SD group.