OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.XX.XX

Research Article
Mechanism of Saikosaponins from *Radix bupleuri* in the Treatment of Acetic Acid-Induced Gastric Ulcer in Rats

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Abstract

Background and Objective: *Radix bupleuri* ("Chaihu" in Chinese) is a classical Chinese herbal medicine that has a protective effect on the gastric lesion. The objective of this study was to explore the in-depth mechanisms that *Radix bupleuri* treating gastric ulcers. **Materials and Methods:** Gastric ulcer rat model was established via submucosal injection of acetic acid. Changes in food intake and weight of rats were observed as the physiological indexes and ulcer index as a pathological indicator to evaluate the efficacy of Saikosaponins (SSs) from "Chaihu". Metabonomics based on HPLC-QTOF-MS was used to explore the mechanism and Mass Profiler Professional (MPP) statistic software was used to find the diverse endogenous metabolites and related pathways and verified by the real-time quantitative PCR (RT-PCR) method. **Results:** The gastric tissue injury in SSs groups was alleviated compared with that of the model group. Eleven diverse endogenous metabolites were confirmed, including linoleic acid, taurocholic acid and Sphingosine-1-phosphate, etc. Pathway analysis indicated that the different metabolites were related to the bile acid secretion and sphingolipid metabolism. Compared with the model group, the mRNA expression levels of NF-κB and TNF-α were significantly down-regulated in SSs treatment groups ($p<0.01$). **Conclusion:** The mechanism of SSs in treating gastric ulcers is through regulating sphingolipid metabolism and bile acid secretion, which laid a foundation for the clinical rational application of SSs in the treatment of gastric ulcers.

Key words: *Radix bupleuri*, gastric ulcer, metabolomics, sphingosine-1-phosphate, cholic acid

Citation: Yang, X.X., S. Wang, T.J. Li, Y.K. Zhang, H.B. Wang, Y.R. Bao and X.S. Meng, 2022. Mechanism of saikosaponins from *Radix bupleuri* in the treatment of acetic acid-induced gastric ulcer in rats. Int. J. Pharmacol., 18: XX-XX.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gastric ulcer is an illness that affects a considerable number of people worldwide. There is about 10% of the world's population suffering from gastric ulcer[1]. A variety of endogenous and exogenous aggressive factors can lead to ulcers, such as gastric acid, pepsin, bile salts, nutritional deficiency, smoking, alcohol, stress, usage of nonsteroidal anti-inflammatory drugs (NSAIDs) and infection with Helicobacter pylori[2,3]. At present, the major strategies proposed to prevent peptic ulcer disease include reducing gastric acid production and increasing gastric mucosal protection[4]. Conventional regimens, like antacids, H_2-receptor antagonists and proton pump inhibitors, have been currently available[5]. However, their serious side effects (such as allergic reactions, tachycardia, alimentary tract haemorrhage, liver damage, leukopenia and severe diarrhoea) are often inevitable regardless of their potent therapeutic effect, thus possibly limiting their clinical applications[6,7]. Many experimental and clinical studies have demonstrated fewer side effects of herbal medicines with therapeutic benefits for gastric ulcer[1].

Qizhi weitong prescription is mainly composed of "Sini Powder" which comes from the classic medical book "Treatise on Febrile Diseases," written by Zhong-Jing Zhang of the Eastern Han Dynasty. It demonstrated significant efficacy in the treatment of gastric ulcer and epigastric pain and has been applied to treat these kinds of diseases in clinical for more than 30 years. *Radix Bupleuri* ("Chaihu" in Chinese, the dry root of *Bupleurum chinense* DC.) is the monarch drug in this prescription. It is originally recorded in the "Shen Nong's Herbal Classic" (Shennong Bencao Jing) as a medium-grade medicine and also listed in the 2015 edition of Chinese Pharmacopoeia. *Radix bupleuri* exhibits significant therapeutic effects in the clinic, so it has been extensively applied in the treatment of gastric ulcers for centuries in China[8,9]. Saikosaponins (SSs), which have a typical oleanane-type skeleton, has been known as the major bioactive compounds isolated from *Radix bupleuri*. SSs possess a wide spectrum of pharmacological activities and have been widely utilized to treat fever, influenza, malaria, nephritis, hepatitis, jaundice, dizziness, menstrual disorder, bitter taste in the mouth and hypochondriac pain in China, Japan and other Asian countries[10]. According to previous studies, SSs possess preventive and therapeutic effects on gastric mucosal damage in rats[11]. However, the therapeutic mechanism has not yet been well elucidated. In this study, a metabonomics approach based on High Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (HPLC-QTOF-MS) was used to find the drug-influenced pathways from the perspective of small molecular metabolites and then RT-PCR technology was applied to verify the related targets on multiple pathways. This study aimed to identify the biochemical pathways related to the SSs efficacy and to improve the understanding of the therapeutic mechanism of SSs, which can provide better insights on SSs for clinical application and lay a foundation for the rational clinical application of Qizhi weitong prescription.

MATERIALS AND METHODS

Study area: This study was carried out from September, 2019-November, 2020.

Reagents and materials: Acetonitrile of LC-MS grade was purchased from Merck (Darmstadt, Germany). Formic acid was obtained from Merck Fluka (Sigma, USA). Water was purified using the Milli-Q Ultrapure water system (Millipore, France). Other reagents and chemicals of analytical grade were provided by Tianjin Yongda Chemical Reagent Co., Ltd., (Tianjin, China). *Radix bupleuri* was collected from Benxi Sanyao, Co., Ltd., (Liaoning, China) and identified by Prof. Liang Xu (College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Liaoning, China) as the dry root of *Bupleurum chinense* DC. Voucher specimens (No. 201703 210005) were deposited in the Analysis and Testing Center of Liaoning University of Traditional Chinese Medicine, China. Its origin and quality were identified in line with the method stated in the Chinese Pharmacopoeia, 2020. The total contents of SSA and SSD conformed to the requirements of the Chinese Pharmacopoeia, 2020.

Animals: The 7 weeks old male Sprague-Dawley (SD) rats weighing 220-250 g were provided by Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China, License Key: SCXK (Liao) 2015-0001). All animals were housed in an environment under the following conditions, temperature, 23±2°C, humidity, 55±5% and light/dark cycle, 12/12-hrs. The rats had free access to a standard diet and water. All studies conformed to the approved experimental animal protocols and guidelines formulated by the Medicine Ethics Review Committee of Liaoning University of Traditional Chinese Medicine.

SS preparation: A water extract of *Radix Bupleuri* was prepared by extracting twice for 2 hrs each time. Then, the resultant extract was evaporated under reduced pressure. A final ratio of powder to the raw herb of 23.2% was obtained.

SS was prepared by its laboratory and detected through UV spectrophotometry at the wavelength of 541 nm. The purity of total SS was up to 90%[12].

Induction of gastric ulcer and experimental groups: Before the experiment, all rats were deprived of food but not water for 12 hrs. Gastric ulcer was induced in rats according to the previously reported method[13,14]. Thereafter, these rats were randomly divided into 7 groups, including normal control, model, SS high dose, SS medium dose and SS low dose, omeprazole and ranitidine groups, with 10 rats in each group. At 3 days after the induction of gastric ulcer, rats in normal control and model groups were given oral administration with saline. While rats in the remaining 5 groups were given oral administration with high dose SS (121.5 mg kg^{-1}), medium-dose SS (40.5 mg kg^{-1}), low dose SS (13.5 mg kg^{-1}), omeprazole (12 mg kg^{-1}) and ranitidine (90 mg kg^{-1}), accordingly. Then, the activities and changes in food intake and weight of rats were observed at the same time every day. At 7 days after the induction of gastric ulcer, plasma samples were harvested and stored at -20°C for analysis. Besides, the stomach was exposed to observe and calculate the Ulcer Index (UI) by referring to the Guth standard[15]. The other gastric ulcer tissues were frozen in liquid nitrogen and stored until the extraction of total tissue RNA.

Analysis of plasma samples: The previously prepared samples were precipitated with methanol. After brief vortex mixing of samples, the supernatants were collected after centrifugation at 15,000×g for 15 min and transferred into the vials for metabolomic analysis. Besides, chromatography was performed on the Agilent 1260 series HPLC system (Agilent Technologies, USA). The separation was carried out on the 4.6 mm i.d.,×100 mm ZORBAX SB-C18 column (Agilent Technologies, USA) at the column temperature of 45°C. The gradient mobile phase consisted of solvent A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) at the flow rate of 1 mL min^{-1}. The gradient conditions of the mobile phase were as follows: 0-7.0 min, B 30.0-67.0%, 7.0-12.0 min, B 67.0-12.0%.

Mass Spectrometry (MS) was conducted on the Agilent 6530 Accurate-Mass Q-TOF equipped with an ESI source (Agilent Technologies, USA). The optimal conditions were shown below: Drying gas temperature of 350°C, drying gas flow rate of 9 L min^{-1} and fragment or voltage of 365 V, with nitrogen as the dry gas and nebulizer. The full-scan MS data were collected from m/z 50-1050 in the centroid mode. For further identification, the ESI ion source was set in the negative ion polarity mode to acquire all MS data. All data collected in the centroid mode were acquired using the Mass Hunter software (Agilent Technologies, USA).

RT-PCR analysis on the mRNA expression of NF-κB and TNF-α: Total RNA was extracted, cDNA was synthesized by reverse transcription under suitable conditions and PCR was then performed. For NF-κB, the PCR amplification conditions were as follows, 30 cycles of 3 min at 94°C for initial denaturation, 30 sec at 94°C for denaturation, 30 sec at 58°C for annealing and 45 sec at 72°C for the extension. For TNF-α and β-actin, the PCR amplification conditions were shown below, 30 cycles of 3 min at 94°C for initial denaturation, 30 sec at 94°C for denaturation, 30 sec at 60°C for annealing and 45 sec at 72°C for the extension. The following primers were used in this study: For NF-κB: 5′-GAGAGCCCTTG CATCCTTTA-3′ (forward) and 5′-CTTCCCTTTGGTCTTTCTGT-3′ (reverse), for TNF-α: 5′-CCTTCCTGGGCATGGAGTCCTG-3′ (forward) and 5′-GGAGCAATGATCTTGATCTTC-3′ (reverse), for β-actin, 5′-CCTTCCTGG-GCATGGAGTCCTG-3′ (forward) and 5′-GGAGCAATGATCTTGATCTTC-3′ (reverse). The gel imaging analysis system (Bioshine, USA) was utilized to quantitatively assess the RT-PCR products.

Metabolomic data processing: The mass data were processed with profinder (version B.06.00, Agilent Technologies, USA) and imported to the mass hunter qualitative analysis software to generate an extracted compound chromatogram. Then, the compound mass spectrum and the resultant data matrices were introduced into MPP software for hierarchical clustering analysis and principal component analysis. The methods referred to the Integrated Biology with Agilent Mass Profiler Professional Workflow Guide. Potential markers were extracted after analysis with the ID Browser features of MPP when exploring the deep differences between the 7 groups.

Statistical analysis: The SPSS 19.0 software, along with one-way analysis of variance (ANOVA) and independent sample t-test, was employed for all statistical analysis. Data were expressed as Mean±SEM. A difference of $p<0.05$ indicated statistical significance and that of $p<0.01$ suggested highly statistical significance.

RESULTS

Effect of SSs on the healing of gastric ulcers: Rats in the normal control group had a great food intake (31.4±2.4 g), obviously increased weight and flexible response. By contrast, rats in the model group had low food intake (20.6±2.3 g), slightly increased weight, marasmus, slow response and

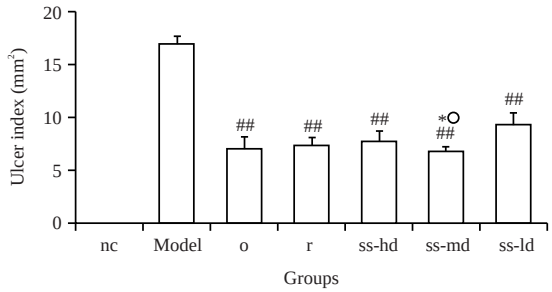

Fig. 1: Ulcer index indicating the Mean±SEM of 10 rats

##p<0.01 vs. model group, *p>0.05 vs. omeprazole group, ○p>0.05 vs. ranitidine group, horizontal axis denotes normal control group, model group, omeprazole group, ranitidine group, SS high dose group, SS medium dose group and SS low dose group, respectively, from left to right

Table 1: Comparison of food intake and weight of rats in each group

Groups	Food intake (g)	Weight (g)
Normal control	31.4±2.4#	288.1±8.9#
Model	20.6±2.3	227.1±7.4
SS high dose	26.4±2.9#	249.6±8.6#
SS medium dose	29.2±2.8#□◇	273.0±7.4#□◇
SS low dose	24.1±2.6#	244.1±6.5#
Omeprazole	25.6±2.7#	247.1±8.4#
Ranitidine	24.9±3.2#	245.9±7.7#

Each value indicates the Mean±SEM of 10 rats. ◇#p<0.05 vs. model group, □p<0.05 vs. omeprazole group and ◇p<0.05 vs. ranitidine group

weakness. Besides, rats treated with SSs had superior performances of the above indexes to those in the model group (p<0.05), whereas rats in the SS medium-dose group had the best performances (29.2±2.8 g) and close to those in the normal control group (31.4±2.4 g). In comparison, rats in the omeprazole group had low food intake (25.6±2.7 g), slightly increased weight and flexible response, while rats in the ranitidine group had similar performances (24.9±3.2 g) to those in the omeprazole group, except for the slow response in Table 1.

Well-defined gastric ulcers were observed 3 days after the induction of gastric ulcers in all rats and the ulcer area was 16.9±0.8 mm^2 in the model group. Oral administration for seven consecutive days significantly decreased the ulcer area and there was a significant difference compared with the model group (p<0.01). Among the various doses of SSs, the medium-dose group achieved the best effect (6.8±0.8 mm^2), which was slightly better than those of the omeprazole group (7.0±1.2 mm^2) and ranitidine group (7.3±0.8 mm^2) (p>0.05) in Fig. 1.

Analysis of metabolic profiles: Using the HPLC-QTOF-MS condition described above, the Typical Total Ion Current (TIC) of different group samples were observed as (Fig. 2a-g). The abscissa is the acquisition time and the ordinate is counts, representing the corresponding strength. With the molecular feature extraction function of Mass Hunter Qualitative analysis software, the number of compounds extracted from each group was as follows: Model group (Fig. 2a) 3542, normal control group (Fig. 2b) 3516, SS high dose group (Fig. 2c) 3567, SS medium-dose group (Fig. 2d) 3556, SS low dose group (Fig. 2e) 3549, omeprazole group (Fig. 2f) 3524 and ranitidine group (Fig. 2g) 3533. Further statistical analysis was performed to find differences between them. Hierarchical clustering analysis was performed in the form of a heat map to obtain clearer results. As observed from Fig. 3, the 3 columns denoting normal control group, omeprazole group and SS medium-dose group were clustered together and there was a clear separation between these 3 groups and model group. To identify more subtle changes, PCA was carried out. The results showed that the cumulative contribution rate was 79.35%, including 39.84% for the X-axis first principal component, 22.06% for the Y-axis component and 17.45% for the Z-axis component. Figure 4 suggested that the model group was far away from the remaining 4 groups, indicating that there were significant differences in the metabolites between the model group and other groups. The SS high dose group was close to the SS low dose group, while the SS medium-dose group was far away from these 2 groups, indicating a difference among them.

Biomarker identification: In the present study, some potential biomarkers were identified by analysis using the significance testing and fold change of MPP software. To verify the accuracy of the above results, data were further analyzed by the target MS/MS using the HPLC-Q-TOF-MS and METLINE metabolites database/library (Agilent Technologies, USA). There were 11 known biomarkers, such as linoleic acid, taurocholic acid and Sphingosine-1-Phosphate (S1P). Table 2 and Fig. 2a-g shows the contents with significant changes in treatment groups compared with the model group. Histidine, dihydroxyacetone, L-Aspartyl-4-phosphate, chenodeoxycholic acid, taurocholic acid, leucine, cholic acid and taurochenodeoxycholic acid (TUDCA) were up-regulated, whereas Arachidonic acid (AA), linoleic acid, S1P and stearic acid were down-regulated. 7 pathways were found to be related to the above biomarkers based on the HMDB, KEGG and PubChem databases. The relationship between these biomarkers and pathways was shown in Fig. 5.

The identification of S1P and cholic acid was elaborated as an example. For S1P Fig. 6a, its precise molecular weight was 379.2535 and the molecular formula was speculated as

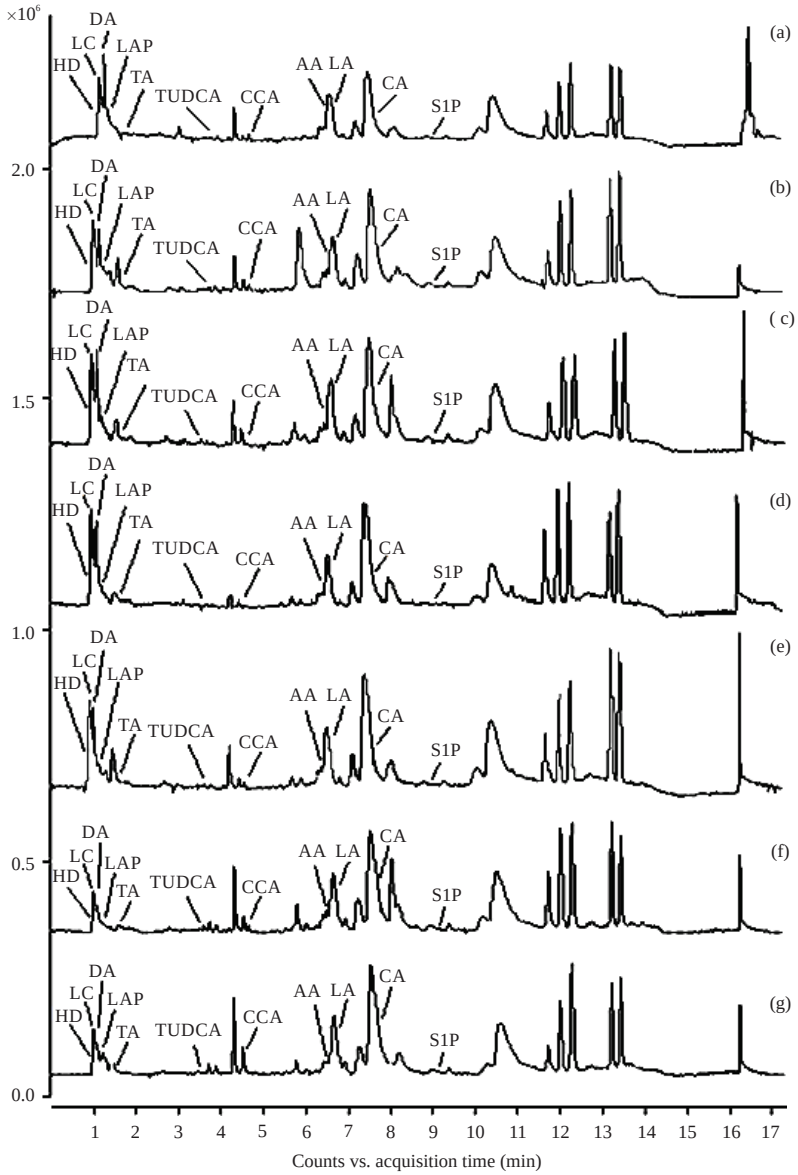

Fig. 2(a-g): Representative total ion chromatograms of different groups based on HPLC-QTOF-MS, (a) Model group, (b) Normal control group, (c) SS high dose group, (d) SS medium-dose group, (e) SS low dose group, (f) Omeprazole group and (g) Ranitidine group

HD: Histidine, LC: Leucine, DA: Dihydroxyacetone, LAP: L-Aspartyl-4-phosphate, TA: Taurocholic acid, TUDCA: Taurochenodeoxycholic acid, CCA: Chenodeoxycholic acid, AA: Arachidonic acid, LA: Linoleic acid, CA: Cholic acid and S1P: Sphingosine-1-phosphate

Table 2: Analysis of potential biomarkers and metabolic pathways from MPP

RT (min)	Ion mode	m/z	Molecular formula	Identification	Regulation	Pathways
0.947	$[M-H]^-$	155.0694	$C_6H_9N_3O_2$	Histidine	Up	Histidine metabolism
1.056	$[M-H]^-$	131.0934	$C_6H_{13}NO_2$	Leucine	Up	Aminoacyl-tRNA biosynthesis
1.124	$[M-H]^-$	90.0318	$C_3H_6O_3$	Dihydroxyacetone	Up	Glycerolipid metabolism
1.398	$[M-H]^-$	213.0098	$C_4H_8NO_7P$	L-Aspartyl-4-phosphate	Up	Amino metabolism
1.762	$[M-H]^-$	515.2925	$C_{26}H_{45}NO_7S$	Taurocholic acid	Up	Bile acid secretion
3.481	$[M-H]^-$	499.2956	$C_{26}H_{45}NO_6S$	Taurochenodeoxycholic acid	Up	Bile acid secretion
4.518	$[M-H]^-$	392.2924	$C_{24}H_{40}O_4$	Chenodeoxycholic acid	Up	Bile acid secretion
6.572	$[M-H]^-$	304.2403	$C_{20}H_{32}O_2$	Arachidonic acid	Down	Linoleic acid metabolism
6.618	$[M-H]^-$	280.2403	$C_{18}H_{32}O_2$	Linoleic acid	Down	Linoleic acid metabolism
7.663	$[M-H]^-$	408.2875	$C_{24}H_{40}O_5$	Cholic acid	Up	Bile acid secretion
9.115	$[M-H]^-$	379.2535	$C_{18}H_{38}NO_5P$	Sphingosine-1-phosphate	Down	Sphingolipid metabolism

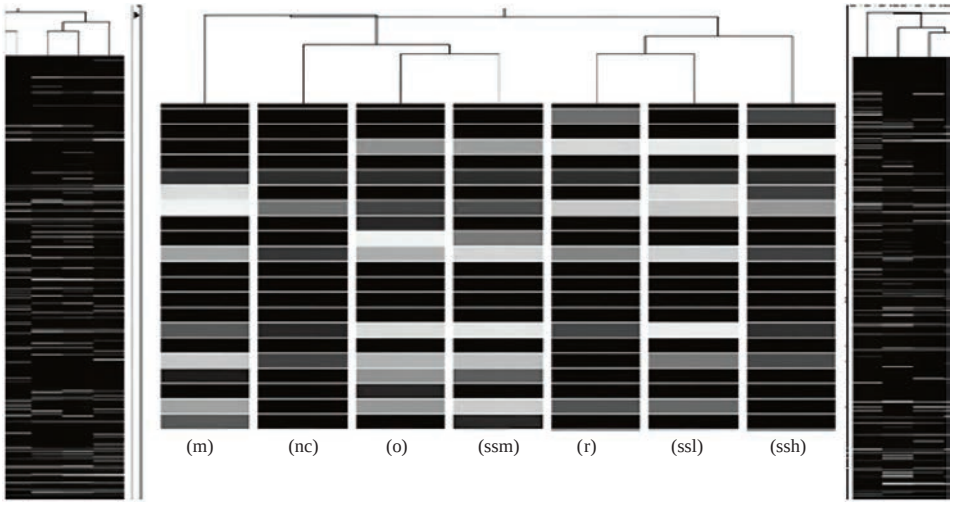

Fig. 3: Unsupervised clustering analysis of the biomarkers in gastric ulcer

A rampage delegates a kind of material, different colours indicate the different amounts. Model group, normal control group, omeprazole group, SS medium-dose group, ranitidine group, SS low dose group and SS high dose group are presented from left to right, respectively

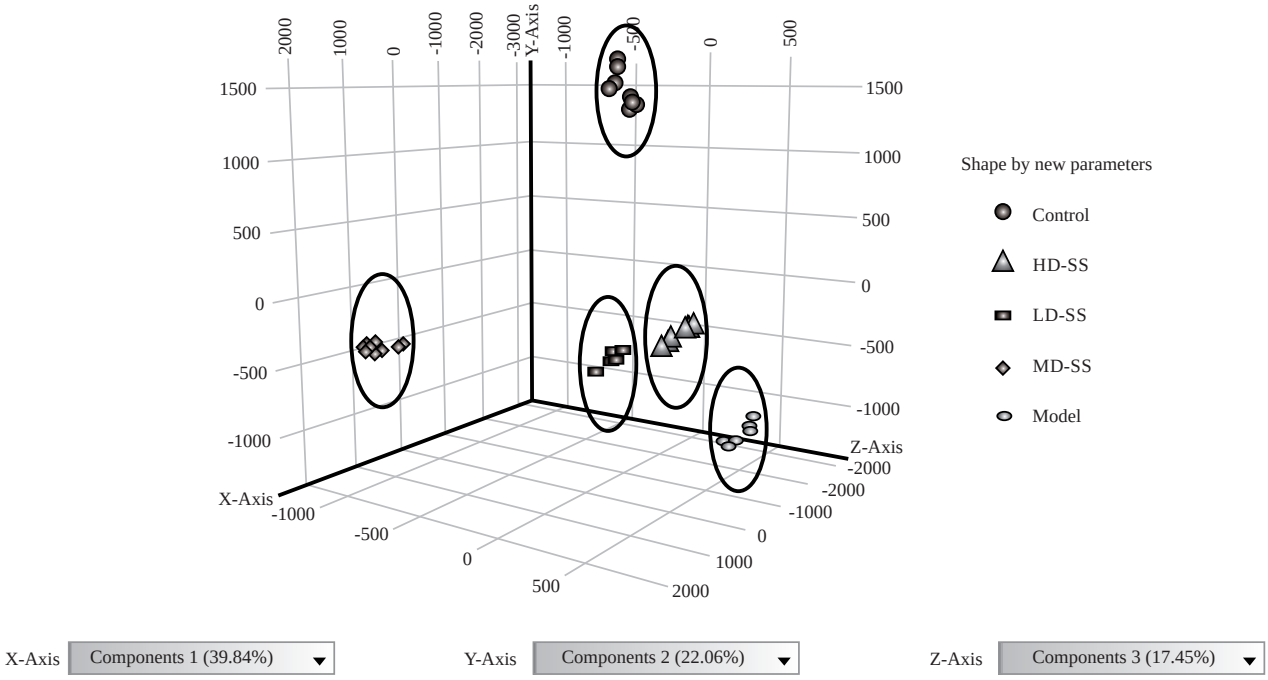

Fig. 4: PCA score plots representing the separate clustering of MS, each coloured point represents a group of samples

First, 2nd and 3rd principal components are displayed on the X, Y and Z-axis, respectively. These three components account for the largest proportion of the overall variability

$C_{18}H_{38}NO_5P$. As shown by tandem mass spectrometry analysis, the main fragment ions were m/z 378.2415 ($C_{18}H_{37}NO_5P$) and 78.9594 ($[M-2H]^-$, H_2PO_3) according to the polarity and references. Finally, S1P was identified as the biomarker. Similarly, the precise molecular weight of cholic acid was 408.2875 and its molecular formula was speculated as $C_{24}H_{40}O_5$. Based on tandem mass spectrometry analysis, the main fragment ions were m/z 407.2802 ($C_{24}H_{39}O_5$), 343.2639 ($C_{23}H_{35}O_2$), 289.2175 ($C_{19}H_{29}O_2$), 251.2011 ($C_{16}H_{27}O_2$), 205.1596 ($C_{14}H_{21}O$) and 69.0350 (C_4H_5O) according to the polarity and references. At last, cholic acid was identified as the biomarker in Fig. 6b.

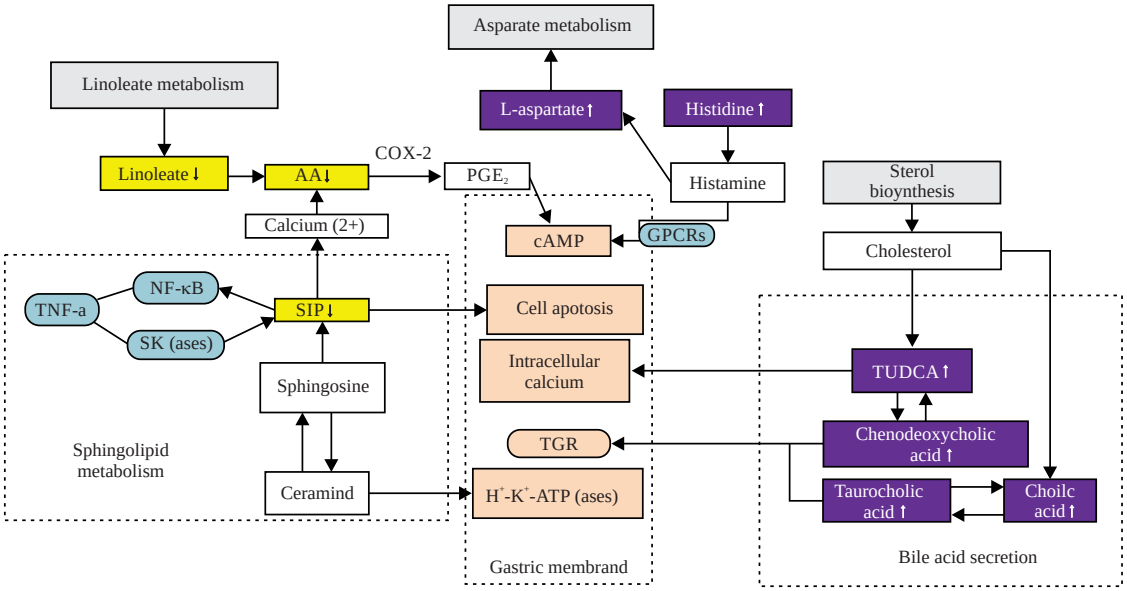

Fig. 5: Metabolite pathways involved in the SS intervention on gastric ulcer

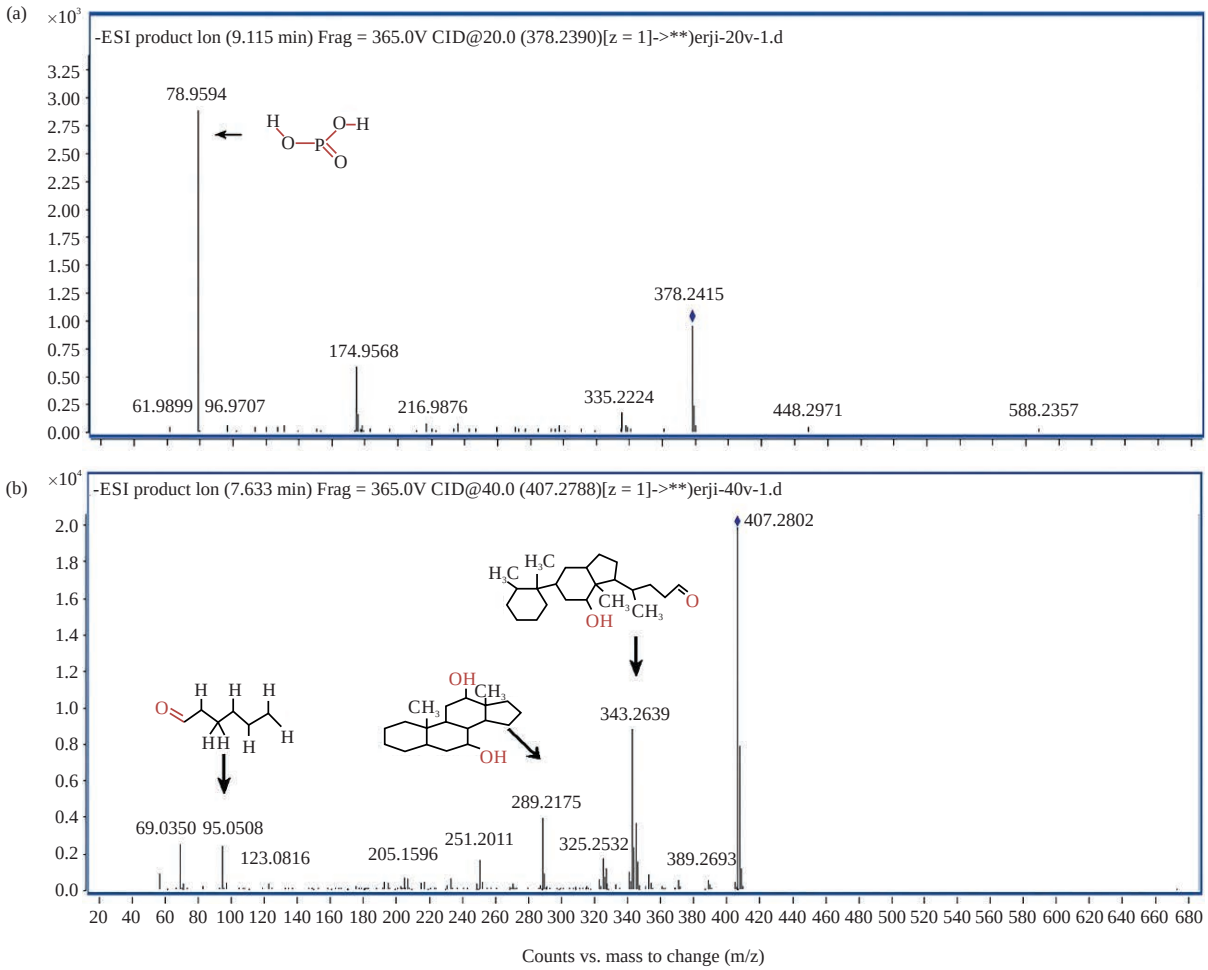

Fig. 6(a-b): Analysis results of potential biomarkers by HPLC-Q-TOF-MS, the Mass Hunter Software and the METLINE database/library, (a) Data of the chemical structures and mass fragments of S1P and (b) Cholic acid

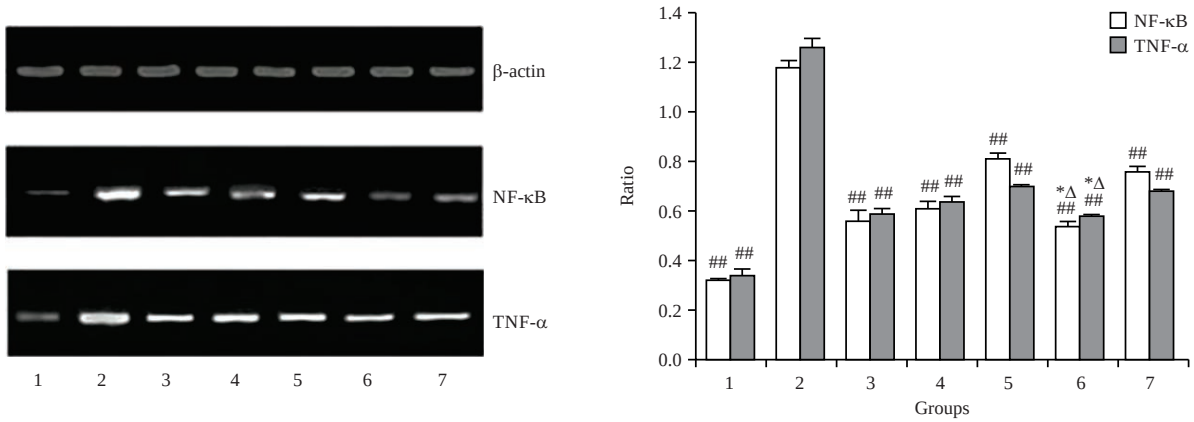

Fig. 7: Comparison on the mRNA expression levels of NF-κB and TNF-α in each rat gastric tissue

n = 3, $\bar{x}\pm S$. ##p<0.01 vs. model group, *p>0.05 vs. omeprazole group, $^{\Delta}$p>0.05 vs. ranitidine group, 1: Normal control group, 2: Model group, 3: Omeprazole group, 4: Ranitidine group, 5: SS low dose group, 6: SS medium-dose group and 7: SS high dose group

Effect of SS on the mRNA expression of NF-κB and TNF-α: The results are displayed in Fig. 7. Compared with the model group (1.18±0.03), the mRNA expression levels of NF-κB were significantly reduced in medicine treatment groups (p<0.01), while those in the SS medium-dose group (0.54±0.02) showed greater declines compared with those in low (0.81±0.02) and high (0.79±0.03) dose groups. Besides, the reduced NF-κB mRNA expression level was close to those in the ranitidine (0.62±0.03) and omeprazole group (0.55±0.04) (p>0.05). While the mRNA expression levels of TNF-α were significantly reduced in medicine treatment groups (p<0.01) compared with the model group (1.27±0.03), in which SS medium-dose group (0.58±0.02) showed greater declines compared with low (0.71±0.01) and high (0.70±0.02) dose groups. Whereas the down-regulated TNF-α mRNA expression level was close to that in the omeprazole group (0.59±0.01) (p>0.05).

DISCUSSION

Gastric ulcer is a kind of frequently occurring disease. Its clinical symptoms are mainly characterized by abdominal pain that affects the normal life of patients as a result, it is extremely essential to find the curative treatment for gastric ulcers. The existing drugs such as omeprazole and ranitidine only alleviate the symptoms of ulcers but do not radically cure them[16]. At present, the Traditional Chinese Medicine (TCM) treatment of gastric ulcers has aroused increasing attention all over the world.

SSs are the important constituents of Bupleurum, which are commonly used in the clinic. More than 75 monomer SSs have been isolated from Bupleurum, such as SSA, SSB, SSC, SSD, SSM, SSN, SSP and SST[17,18], among which, SSA, SSB2, SSC and SSD are the main active ingredients. In recent years, some studies find that inflammatory response is the main cause of peptic ulcer recurrence and difficulty in healing[19], while SSA and SSD possess distinct anti-inflammatory activities[20] and can regulate the immune response. It is reported that the cellular immune function in patients with a gastric ulcers is significantly weakened[21]. In this study, it was confirmed that SS, omeprazole and ranitidine showed therapeutic effects on gastric ulcers in rats but the latter 2 affected food intake, weight and activity of rats to a certain extent. However, SS avoided the above problems on the premise of achieving good therapeutic effects and the mechanism might be related to anti-inflammatory and immune regulation. SS has been utilized to treat gastric ulcers in the clinic for years, yet its therapeutic mechanism remains unclear so far. Therefore, further efforts are needed to study the therapeutic target and illustrate the therapeutic mechanism.

Metabolomics provides a powerful tool to discover and identify novel biomarkers to improve the drug efficacy in biological systems[22,23]. Metabolites are the end products of gene expression, so the information provided by metabolomics analysis helps to reveal the relationships between genes, monitor and infer gene functions[24]. This study explored the mechanism of SS in the treatment of gastric ulcers in rats from the perspectives of metabolomics and associated genetic analysis. Eleven significant biomarkers were found and identified, including S1P, AA and chenodeoxycholic acid. In addition, 7 pathways related to the above biomarkers were also identified. The metabolite pathways involved in the SS intervention on gastric ulcers were shown in Fig. 5, among which, sphingolipid metabolism

was the main pathway associated with inflammation. It is reported that S1P is a bioactive lipid with important functions in regulating the inflammatory response closely related to gastric ulcer[25,26] and its level increases in numerous inflammatory conditions. Many potential therapeutic targets may be identified in the regulation of the SK/S1P pathway and the sphingolipid metabolism may serve as a viable target for the treatment of gastric ulcer[27]. More importantly, S1P can activate the NF-κB pathway, which is essential for regulating the inflammatory response[28]. The TNF-α expression level significantly increases after the activation of NF-κB and the accumulation of these 2 factors will reduce the gastric mucosal blood flow, eventually leading to the formation of gastric ulcer[29,30]. Meanwhile, TNF-α can activate SK, an important rate-limiting enzyme that catalyzes the generation of S1P by SP[31], thereby further controlling the generation of S1P. In this study, the S1P level, together with the NF-κB and TNF-α mRNA expression, was significantly up-regulated during the induction of gastric ulcer in rats. After SS treatment, the NF-κB mRNA expression level was significantly reduced, which in turn suppressed the TNF-α mRNA expression, inhibited the SK activation and significantly decreased the S1P content. Thus, it was concluded that SS treated gastric ulcers by controlling the expression of S1P, together with the activation of NF-κB and TNF-α.

When patients with gastric ulcers are treated with H_2 receptor antagonists, they will increase bile acid hydrolysis binding due to bacterial overgrowth, which may be related to the transition of gastric juice pH to neutral[32]. TGR5 is a cell membrane bile acid receptor and G protein-coupled receptor, which plays an important role in lipid metabolism regulated by bile acid[33-35]. TGR5 receptor present in the metaplastic and neoplastic gastric epithelial cells mediates the promoted cell proliferation induced by the bile acid TUDCA. Taurocholate is known to interact with the cell membranes as a lipophilic compound, it may diffuse into the cell membranes, thus increasing the permeability to extracellular agents. In this study, 3 significantly up-regulated bile acids were found, including chenodeoxycholic acid, taurocholic acid and TUDCA, which activated cell proliferation and acted on the damaged gastric mucosa.

In addition, 6 other potential biomarkers were also identified, including histidine, linoleic acid and L-Aspartyl-4-phosphate and their contents changed obviously during the occurrence and treatment of gastric ulcer. Histamine exerts pleiotropic effects in humans, which involves the immune and neuroendocrine systems, neurotransmission and gastric secretion. In the gastrointestinal system, histamine acts on the parietal cells to activate H^+-K^+-ATPases, thus resulting in gastric acid secretion[36]. Linoleic acid also affects gastric acid secretion and its down-regulated expression can reduce acid secretion and prevent mucosal lesion, possibly due to the augmented synthesis of endogenous prostaglandins in the gastric mucosa. SSs treat gastric ulcers by regulating multiple targets, which represents one of their greatest advantages.

CONCLUSION

Through metabonomics combined with RT-PCR technology, this study illustrates the mechanism of SSs in treating gastric ulcers by regulating sphingolipid metabolism and bile acid secretion, which laid a foundation for the clinical rational application of SSs in the treatment of gastric ulcers.

SIGNIFICANCE STATEMENT

Based on the clearly defined components and efficacy of SSs, this study further explores the mechanism of SSs in the treatment of gastric ulcers. It can be illustrated that SSs treating gastric ulcers were through regulating multiple biomarkers as sphingosine-1-phosphate, cholic acid, etc. and the expression of related genes. This study is the first to explain the mechanism of SSs in treating gastric ulcers by regulating sphingolipid metabolism and bile acid secretion, which can provide better insights on SSs for clinical application.

ACKNOWLEDGMENT

This study was supported by the National Key Research and Development Project (2018YFC1704805).

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