A Network Pharmacology-Based Study of the Mechanism of Renshen Baidu Powder in Treating Ulcerative Colitis

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Research Article

Keywords: Inflammatory Bowel Disease, Crossover Genes Enrichment Analysis, Protein-protein Interaction, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, Anti-inflammatory Activities

Posted Date: February 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-199959/v1>

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Abstract

Ulcerative colitis (UC), one forms of chronic inflammatory bowel disease (IBD), is characterized by recurrent inflammation, and Renshen Baidu Powder (RSBDP) is often used as a traditional Chinese medicine prescription for treating of UC, but the pharmacological mechanisms remains unclear. This research aims to explore the mechanisms of RSBDP in the treatment of UC based on network pharmacology. Through multiple databases collected the compounds and targets, and constructed the network. The crossover genes enrichment analysis were performed by protein-protein interaction (PPI) construction and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Finally, 181 compounds and 266 targets were obtained. Potential candidate ingredients mainly include phytosterols, flavonoids, and coumarins, all of which have anti-inflammatory activities. GO and KEGG enrichment analysis suggested that RSBDP have anti-inflammatory and immunomodulatory effects. The effect of RSBDP on UC might be achieved by regulating the balance of cytokines (i.e., IL-6, TNF, IL-1) and the downstream mediators (i.e., STAT1, STAT3) in the immune system. And inflammation-, immune- and hypoxic-related pathways, like TNF, Toll-like receptors and HIF-1 signaling pathway. These results provide a theoretical basis for studying the effective substances of RSBDP in the treatment of UC and their mechanism of action for further research.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease with pathological manifestations in the rectum and colon. The clinical manifestations are repeated abdominal pain, diarrhea, mucus pus and bloody stools. At the turn of the 21st century, ulcerative colitis has become a global disease. Ulcerative colitis is a leading cause of morbidity and prevalence trends in Asia, the Middle East and South America increased, but it has stabilized in Western Europe, North America and Australia. The global risk of UC indicates the importance of environmental factors, immune response, microbial flora and genetic susceptibility. The exact cause of UC is remain clarified. It is believed to be closely associated with epithelial cells (colonocytes), mucous barrier and epithelial barrier defects¹⁻⁵.

Besides, patients with long-standing IBD, especially UC, have an increased risk of developing colorectal cancer (CRC), which is one of the most critical complications⁶. According to reports, the incidence of CRC in IBD patients (i.e., colitis-associated cancer, CAC) was 60% higher than the general population⁷. Eaden's meta-analysis based on population cohort studies has shown that the overall prevalence of CRC in UC patients was 3.7%. The incidence rate is equivalent to a cumulative probability of 2% at 10 years, 8% at 20 years, and 18% at 30 years⁸. Bopanna et al. systematic review and meta-analysis have shown that the overall prevalence of CRC in Asia UC patients was 0.85%. The cumulative risks of 0.02%, 4.81%, and 13.91% at 10 years, 20 years, and 30 years⁹. These results showed that the connection between the occurrence and development of inflammation and cancer is inevitable and intertwined, and inflammatory lesions may increase the risk of cancer occurrence and development. Therefore, it is necessary to reduce the risk of these patients suffering from colorectal cancer.

The aim of UC management is to induce and maintain partial remission and improve the lives of patients. Current treatments for mild to moderate UC mainly contains 5-aminosalicylic acid (5-ASA) and corticosteroids (budesonide)^{2,10-11}. However, these therapies have low efficacy, and even with drug treatment, patients have a high short-term recurrence rate. Besides, there are many different management drugs for moderate to severe UC, including anti-TNF- α drugs (infliximab, adalimumab and golimumab), immunomodulators (thiopurines, methotrexate) and biological drugs^{2,12}. Moreover, these therapies are accompanied by toxic side effects and have individual differences, and high financial costs are a barrier for patients. Thus, the current main task is to seek a more majorization and comprehensive treatment method.

So far, several studies have shown that traditional Chinese medicine (TCM) can treat UC, with low toxic side effects and easy acceptance by patients¹³⁻¹⁵. Renshen Baidu Powder(RSBDP), which originated in the Tang dynasty, is a famous Chinese medicine prescription that has been used for nearly 1400 years. The Chinese medicine preparation of RSBDP composed of ten TCMs: *Panax ginseng* C. A. Mey. (Renshen in Chinese), *Notopterygii Rhizoma Et Radix* (Qianghuo in Chinese), *Radix Angelicae Biseratae* (Duhuo in Chinese), *Radix Bupleuri* (Chaihu in Chinese), *Platycodon Grandiflorus* (Jiegeng in Chinese), *Aurantii Fructus* (Zhike in Chinese), *Chuanxiong Rhizoma* (Chuanxiong in Chinese), *Peucedani Radix* (Qianhu in Chinese), *Poria Cocos*(Schw.) Wolf. (Fuling in Chinese), and *licorice* (Gancao in Chinese). Many clinical studies have suggested that RSBDP is used to treat UC across the Chinese Mainland¹⁶⁻¹⁷. Also, many experiments have shown that RSBDP can promote gastrointestinal motility function, improve the immune function of the body, repair intestinal mucosal damage¹⁸.

Network pharmacology is an emerging method that combines pharmacology, bioinformatics, chemoinformatics and systems biology. Nowadays, the scope of network pharmacology is expanding, including the study of the preparation of TCM and the exploration of drugs and diseases. TCM formula is a multi-target, multi-pathway, and multi-link system. Network pharmacology methods have been used to analyze the complexity between biological systems, drugs and diseases¹⁹⁻²⁰. This study illustrates the mechanism of action of RSBDP in the treatment of UC from the perspective of network pharmacology. A flowchart of the study design is illustrated in Fig. 1.

Materials And Methods

Screening of the active compounds in RSBDP. The active compounds in RSBDP have been researched and collected from the TCM Systems Pharmacology Database (TCMSP, <http://tcmspw.com/tcmssp.php>), which is a useful tool based on systematic pharmacology for Chinese herbal medicines, including TCM compounds, targets, diseases and ADME-related properties²¹. ADME includes absorption, distribution, metabolism and excretion, and is an indispensable step in determining the pharmacokinetics of candidate drugs²². Among them, oral bioavailability (OB) and drug-like degree (DL) were selected as pharmacokinetic parameters. OB, the oral absorption and permeability through pharmacological activity, is usually used to evaluate the development of drug candidates²²⁻²³. DL, the qualitative properties of

chemicals, is widely used in the early stages of drug discovery and can predict drug-like molecules²⁴⁻²⁵. According to recent literature, $OB \geq 30\%$ and $DL \geq 0.18$ are essential indicators for screening active compounds.

Targets Prediction. For the large number of the active compounds obtained above, we also summarized the relevant targets of RSB DP from TCMSP database. Besides, the UC-related targets come from multi-source databases, including DrugBank database (<https://go.drugbank.com/>)²⁶, Therapeutic Target Database (TTD, <http://bidd.nus.edu.sg/group/cjttd/>)²⁷, Online Mendelian Inheritance in Man database (OMIM, <https://www.omim.org/>)²⁸, DisGeNET database (<https://www.disgenet.org/>)²⁹, and using “ulcerative colitis” as a keyword to obtained disease targets. Then, the intersection of compound-target network and UC-related target network was integrated by the Venny 2.1.0 software (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>)³⁰. Finally, the candidate targets were mapped to the UniProt database (<http://www.uniprot.org/>)³¹, and duplicate, and non-corresponding genes were eliminated.

Protein-protein interaction (PPI) Data. The data of PPI have come from the String database (<https://string-db.org/cgi/input.pl>)³², which is a database for the exploration and analysis of the protein-protein association, including direct physical and indirect functional interactions between proteins. We set the organism as “Homo sapiens (human),” set the required interaction score at “high confidence (0.7),” and build a PPI network.

GO and KEGG Pathway Enrichment Analyses. GO biological function and KEGG pathway enrichment analysis were performed by The Database for Annotation, Visualization, and Integrated Discovery database (DAVID, <https://david.ncifcrf.gov/>)³³, a web-based portal that provides gene list annotation and analysis resource. We set the genetic species to “Homo sapiens.” And the Bar and Bubble Charts were plotted using the bioinformatics platform (<http://www.bioinformatics.com.cn/>)³⁴.

Network Construction. Network construction was performed by Cytoscape 3.7.1 (<http://www.cytoscape.org/>)³⁵, which is an open software project used to integrate the interaction network and status of biomolecules. Each node represents a compound, target or pathway, and edge indicates the interaction between nodes. Then, the following networks were constructed, including Herbs-Compounds network (H-C networks), Compounds-Targets network (C-T network), PPI core targets network, and Target-Pathway network (T-P network).

Results

Potential Active Compounds and Herb-Compound network. A total of 1546 compounds of RSB DP were retrieved from TCMSP. Then, the following criteria were used for screening: $OB \geq 30\%$ and $DL \geq 0.18$, and deleted the duplicate compounds, a total of 181 active compounds were obtained as a result.(Table 1). We was constructed the H-C network by Cytoscape 3.7.1, and it contained 169 nodes (including 10 herbs and 159 compounds) and 181 edges (Fig. 2). Among 159 compounds, 12 of them provided a high degree

(degree $\geq$ 2). According to the descending order of degree, the compounds were beta-sitosterol (MOL000358, Degree=5), Sitosterol (MOL000359, Degree=4), quercetin (MOL000098, Degree=3), kaempferol (MOL000422, Degree=3), Imperatorin (MOL001941, Degree=3), isoimperatorin (MOL001942, Degree=3), nodakenin (MOL004792, Degree=3), isorhamnetin (MOL000354, Degree=2), Stigmasterol (MOL000449, Degree=2), Phellopterin (MOL002644, Degree=2), naringenin (MOL004328, Degree=2), (+)-Anomalin (MOL004653, Degree=2).

Targets Prediction and Compound-Target Network. In this progress, a total of 266 predicted targets of 181 active compounds were obtained. Then, we have integrated various disease gene databases, including DrugBank, TTD, OMIM, DisGeNET, and obtained 857 UC-related targets. Finally, the collected UC targets and RSB DP targets were intersected, and a total of 92 RSB DP-UC targets were obtained(Fig. 3a,b).

Based on the above research, the RSB DP C-T network was constructed by Cytoscape 3.7.1, which contained 233 nodes(including 141 compounds and 92 RSB DP-UC targets), and 1145 edges (Fig. 4). These network show that multiple compounds can regulate targets, and indicate the active compounds and the targets interaction.

PPI Network analysis. To further obtain the PPI network, 92 RSB DP-UC targets were input into the String database, and removed the unconnected nodes. Then, the result was imported into Cytoscape3.7.1, and obtained the network which contained 85 nodes and 704 edges (Fig. 5). To analyze the core PPI network, we were screened out with the following topological characteristics, including “ Degree Centrality(DC) $\geq$ 20, Closeness Centrality (CC) $\geq$ 0.48 and Betweenness Centrality (BC) $\geq$ 0 ”. Finally, we were selected the top 30 targets of the PPI network (Table. 1), and obtained 30 nodes and 318 edges (Fig. 6a,b).

GO Biological Processes (BP) enrichment analysis. In order to further identify whether the enrichment of the biological processes of the above candidate targets were related to UC. GO-BP enrichment analysis was performed by DAVID, and we were obtained the top 20 significant GO BPs(Fig. 7). GO BP terms are mainly enriched in inflammatory response, regulation of apoptotic process and regulation of transcription, such as “inflammatory response,” “negative regulation of apoptotic process,” “aging,” “positive regulation of transcription, DNA-templated” and “positive regulation of transcription from RNA polymerase II promoter.”(Table. 2).

KEGG pathway enrichment analysis. In this progress, KEGG pathway enrichment analysis was used to further reveal the mechanism of UC remission. The pathways related to UC mainly included TNF signaling pathway (hsa04668), Toll-like receptors signaling pathway (hsa04620), HIF-1 signaling pathway (hsa04066), NOD-like receptors signaling pathway (hsa04621), T-cell receptor signaling pathway (hsa04660) and so on (Fig.8). In addition, a T-P network was constructed according to the target of RSB DP on each pathway (Fig. 9).

Discussion

TCM has the advantages of reducing side effects, improving therapeutic efficacy, and have low treatment cost. Nowadays, network pharmacology provides a new approach to study TCM formulas and the advantages of multi-compound, multi-target and multi-pathway characteristics of TCM formula.

Based on the ADEM properties, we have screened out 181 active compounds of RSB DP. Then, the H-T network of RSB DP had 159 active compounds, including β -sitosterol, Sitosterol, quercetin, kaempferol, Imperatorin, isoimperatorin, nodakenin, isorhamnetin, Stigmasterol, Phellopterin, naringenin, (+)-Anomalin, and the compounds as mentioned above can act on 2-5 Chinese herbal medicines simultaneously, and were divided into three major categories, including Phytosterols, Flavonoids and Coumarins. Phytosterols, the plant analogs of cholesterol, including β -sitosterol, Sitosterol and Stigmasterol. Recently, many studies have shown that phytosterols have anti-inflammatory functions³⁶⁻³⁷. β -sitosterol has shown many biological functions, including lowering cholesterol level, anti-inflammatory and anticancer effects. Oral β -sitosterol (20 mg/kg) may significantly inhibit the expression of cytokines TNF- α , IL-1 β , and IL-6 in the colon of male C57BL/6 mice with TNBS-induced colitis and simultaneously inhibits the expression of cyclooxygenase (COX)-2 and the phosphorylation of p65³⁸. β -sitosterol can inhibit the activation of NF- κ B by inhibiting the binding between lipopolysaccharides (LPS) and toll-like receptor (TLR)4 to express anti-inflammatory effects³⁹. Furthermore, Feng et al. studies have shown that dietary phytosterols, β -sitosterol and stigmasterol inhibited NF- κ B activation in mice with DSS-induced colitis⁴⁰. Flavonoids are widely distributed in the plant kingdom, including quercetin, kaempferol, isorhamnetin and naringenin. Quercetin has multiple biological functions, like anti-inflammatory, anticancer, antioxidant and anti-hypertensive. Quercetin regulates epithelial inflammatory genes associated with LPS-induced models, which down-regulated C/EBP- β , and Lcn-2, and up-regulated Hmox1, and Fpn1⁴¹. The most important properties of kaempferol are anti-inflammatory, can be used to treat numerous diseases caused by acute and chronic inflammation. Park et al. have been confirmed that kaempferol down-regulated the colonic mRNA expression levels of the inflammatory cytokines (i.e., TNF- α , IL-1 β , and IL-6) in the colons of female C57BL/6J mice with DSS-induced colitic and inhibited the activation of myeloperoxidase (MPO)⁴². Coumarins are plant-derived products, including Imperatorin, isoimperatorin, nodakenin, Phellopterin and (+)-Anomalin. The antioxidant, anticancer and anti-inflammatory effects have been recognized in many *in vivo* and *in vitro* studies⁴³⁻⁴⁴. Imperatorin (IMP) can effectively reduce the increase of IL-1 β and TNF- α levels in LPS-induced mice⁴⁵. Luo et al. reported that IMP inhibited TNF- α and IL-6 in TNBS-induced colitis mice and alleviated the symptoms of UC by regulating the Nrf-2/ARE/HO-1 pathway⁴⁶.

According to the active compounds obtained above and matched with related UC targets, we were obtained 92 RSB DP-UC targets, and constructed the C-T network and PPI network. Cytokines such as TNF, IL-1, IL-6, IFN- γ , IL-2, and IL-8 (CXCL8) were produced by Th1 cells, which mediate cellular immune responses to pathogens and participate in promoting the development of CRC and CAC. In contrast, IL-4 and IL-10 were produced by Th2 cells, which are related to anti-inflammatory response⁴⁷. If the balance between Th1 and Th2 is lost, inflammation of the tissues and viscera will occur, leading to the occurrence and development of diseases. TNF is a well-known pro-inflammatory cytokine, which is mainly secreted

by monocytes or macrophages. Signaling pathways and transcription factors (i.e., NF- κ B) were triggered by the activation of TNF- α ⁴⁸⁻⁴⁹. Like TNF- α , IL-1 plays a crucial signal in the initial alarm, causing a series of up-regulation and anti-inflammatory responses to be activated. Among the IL-1 family, IL-1 α and IL-1 β are cytokines that have been widely studied. And evidence suggested that IL-1 β can indirectly activate endothelial cells and angiogenesis by up-regulating pro-inflammatory effects and pro-angiogenic molecules. Moreover, the findings have shown that the activation blockage of IL-1 β reduces tumorigenesis in a murine CAC model by impairing macrophage-dependent IL-6 secretion. These findings suggested that IL-1 has a significant effect on the initiation of colonic inflammation⁵⁰⁻⁵¹.

IL-6 is essential for long-standing inflammation. It is used to inhibits the effect of regulatory T cells, and involved in activating STAT3, a major oncogenic transcription factor. STAT1 and STAT3 as a downstream targets of IL-6 signaling, which can prolong the survival period of CRC patients. The inhibition of colitis activity is related to the inhibition of STAT3 activation, the induction of T cell apoptosis and the reduction of pro-inflammatory cytokines^{49-50,52-53}. Gordziel et al. have shown that the expression of STAT1 and STAT3 are correlated respectively with IL-6, and the significantly expression of IL-6 in CRC biopsies tissues are both shown to a tendency for association with longer survival⁵⁵.

Then, the T-P network were constructed and performed enrichment analysis, and the results reflected that 85 targets could be mapped to 179 pathways. Five pathways of the top 20 pathways, including "TNF signaling pathway," "Toll-like receptors signaling pathway," "NOD-like receptors signaling pathway," and "HIF-1 signaling pathway," were closely related to inflammatory and immune function. TNF signaling pathway acts as an important role in a regulator of inflammation. TNF has two primary receptors, such as TNF receptors 1 and 2 (TNFR1 and TNFR2). In UC, high tissue levels of TNF and cytokines (i.e., IFN- γ , IL-17 and IL-12) can cause a series of symptoms such as diarrhea. Bradford et al. studies have shown that TNF promotes the recovery of colitis mucosa by acting on the colonic epithelial stem and progenitor cell populations⁵⁵. Pattern recognition receptors (PRPs) are essential for regulating the homeostasis of the intestinal immune system and are mediators of inflammation in the gastrointestinal tract, including Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-like receptors (RLR)⁵⁶. TLRs signaling pathway mainly control immune function and can act as a risk modifier for UC and CAC. It has been found that TLRs expressed in the intestinal epithelium are related to the long-term existence of mucosal inflammation, result in a high degree of morbidity. Activation of the TLR signal transduction pathway induces pro-inflammatory cytokines, and transcription factors like NF- κ B to target invaders⁵⁷. Once activated, TLRs trigger the subsequent activation of downstream signaling cascades and induce the activation of NF- κ B. TLR mutations and dysregulation are the main factors in the predisposition and susceptibility to IBD⁵⁸⁻⁵⁹. NLRs signaling pathways are involved in the regulation of inflammatory and apoptotic responses. A pivotal role of NOD2 in the inhibition of intestinal inflammation and tumorigenesis. Kurzawski et al. reported the NOD2 might be a risk factor for CRC⁶⁰. Udden et al. studies *in vivo* have shown that NOD2-mediated protection against colorectal tumorigenesis, and the inhibition of TLR-mediated activation of NF- κ B pathways and MAPK pathway, and promotion of the inflammatory cytokines⁶¹.

However, recent studies have provided evidence that a comparison of chronic inflammation and normal tissues found that hypoxia mainly exists in the former. The adaptive responses to hypoxia are mediated via the Hypoxia Inducible transcription Factors (HIFs)⁶². HIF-1 signaling pathway has been shown to have a protective effect in colitis *in vivo*. The HIF pathway is regulated by a family of oxygen-sensitive HIF prolyl hydroxylases. The expression and transcriptional activity of HIF-1 was inhibited by hydroxylases⁶²⁻⁶³. Cummins et al. studies *in vivo* and *in vitro* have demonstrated that the pan-hydroxylase inhibitor, dimethyloxalylglycine (DMOG), can induce the activation of HIF-1 in cultured intestinal epithelial cells, and accelerate recovery in DSS-induced female C57BL/6 mice, and promote the pro-inflammation cytokines in DMOG-treated mice⁶⁴.

These results reveal that RSB DP not only can regulate pro- and anti-inflammatory, immune and anti-hypoxic effects but also can prevent the occurrence and development of tumors. Moreover, the occurrence and development of UC were related to the imbalance of cytokines and downstream mediators. These networks in this study illustrate the features of RSB DP multi-compounds, multi-targets and multi-pathways therapy of UC with the complicated and varied action mechanisms.

Conclusion

In summary, we have identified 181 active compounds that mediate 85 UC-related targets to play a synergistic effect. The multiple signal pathways and biological processes can enrich same target, indicating the prescription has a multi-target and multi-pathway synergistic effect on UC. At the same time, this research remains has limitations, one of them is based on the prediction results obtained from existing databases. Simultaneously, this research only provided a preliminary hypothesis for scientific research in TCM, which requires systematic experimental research to further confirm.

Declarations

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 325020015).

Author contributions

X.Y.T. wrote the manuscript; X.Y.T. and X.L.L. edited and improved the manuscript; X.Y.L. were responsible for coordinating this study. X.L.L. and B.J. directed the research and proposed changes to the manuscript, and all authors reviewed the manuscript and approved the final version of the manuscript.

Additional Information

Competing interests The authors declare no competing interests

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Figures

Figure 1

Framework of network pharmacological of RSBDP based on an integration strategy

(a)

(b)

Figure 3

Venn diagram of the targets in UC and RSB DP.

Figure 4

Compound-Targets network (C-T network) of RSBDP.

Figure 6

The progress of screening for the PPI network.

Figure 7

The GO BPs analysis of targets of RSBDP. Enrichment results were obtained for biological processes; only the top 20 GO terms with $P < 0.05$ are displayed for each category.

Figure 8

The top 20 KEGG enrichment pathway analysis.

Figure 9

Targets-Pathways Network (T-P Network).