Study on the mechanism of Astragalus-Panax notoginseng in the treatment of gastric ulcer based on network pharmacology

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Abstract. Objective: To explore the possible mechanism of Astragalus Panax notoginseng in the treatment of GU by using network pharmacology. Methods: combined with the network pharmacology technology, the active components were obtained with the help of tcmp database, OB ≥ 30% and DL ≥ 0.18 were set, the potential active components were screened, and the drug target information was obtained; After screening the genes, disenem and other important targets in the database; Using venny2 1 software, draw Wayne diagram and obtain drug disease common targets; Using Cytoscape 3.7.2 software, construct the "drug component target disease" network diagram, screen the core genes, and conduct go analysis and KEGG analysis. Results: 27 potential active components, 488 drugs targets and 118 drug disease common targets were got. Go enrichment analysis showed that the intersection gene set was enriched to 1948 biological processes, 45 cell component expression processes and 106 processes related to molecular function. KEGG enrichment analysis is mainly based on 146 related signal pathways. The signal pathways of Astragalus Panax notoginseng in the treatment of Gu mainly involve proteoglycans in cancer, prostate cancer, EGFR tyrosine kinase inhibitor resistance and other signal pathways. Conclusion: Astragalus-Panax notoginseng drug pair can treat gastric ulcer through multiple components acting on multiple targets and multiple signal pathways, which preliminarily reveals its mechanism of action and provides a support for further empirical research.

Keywords: network pharmacology; Astragalus membranaceus; Panax notoginseng; gastric ulcer; mechanism.

Gastric ulcer(GU) is a common digestive system disease characterized by epigastric pain, nausea and acid regurgitation. It has the characteristics of being difficult to cure, easy to relapse, high incidence rate, long onset period, and many complications. It has a serious impact on patients' psychology, spirit and health. After many years of clinical practice, the current treatment of Gu is mainly western medicine, and the drugs mainly include proton pump inhibitors, H2 receptor antagonists, antibiotics, gastric mucosal protective drugs, etc. Many studies show that traditional Chinese medicine(TCM) has unique curative effect on the treatment of GU and has a broad research and Application prospect[1,2].In TCM, Gu belongs to the categories of "epigastric pain", "gastric carbuncle", "acid swallowing", "noisy" and "ruffian syndrome". The etiology and pathogenesis can be divided into external evil guest stomach, diet injury stomach, internal injury of seven emotions, weakness of spleen and stomach, blood stasis obstruction of collaterals. Good results have been achieved by dialectical treatment of TCM combined with acupuncture and moxibustion. The achievements of TCM on GU are not only reflected in the protective effect of gastric mucosa and ulcer healing, but also in reducing the recurrence rate of gastric ulcer. TCM treatment has significance in the regulation and protection of gastric ulcer[3]. However, there are also some problems, such as the experimental gastric ulcer animal model is still the main research in the treatment research, the evaluation system of the combination of disease and syndrome model is not perfect, the research depth is not enough, and the research on the action mechanism is relatively backward[4].

Li Shao of Tsinghua University put forward the hypothesis related to TCM and biomolecular network in 1999. Combined with the theory of system biology, he explored the research methods with the characteristics of TCM from the perspectives of bioinformatics, big data and AI. After
Astragalus and Panax notoginseng have achieved good curative effect in the treatment of Gu [8-10]. With the help of network pharmacological methods, this study studies the effect of Astragalus Panax Notoginseng on gastric ulcer, hoping to reveal the possible mechanism of Astragalus Panax notoginseng on gastric ulcer, and provide theoretical reference for relevant research in future.

1. Materials and methods

1.1 Material

Swiss target prediction database, tcmsp database, OMIM database, PubChem database, genecards database, diskenet database, string database, Venny2.1, Cytoscape3.7.2, R3.6.1 software, etc.

1.2 Drug chemical composition and target screening and prediction

The chemical components of Astragalus membranaceus and Panax notoginseng were searched by using the Chinese herbal medicine names "Astragalus membranaceus" and "Panax notoginseng" in tcmsp database. Combined with the pharmacokinetic parameters, two thresholds of oral bioavailability (OB) and drug likeness (DL) were set to screen the potential active components in which OB ≥ 30% and DL ≥ 0.18. Obtaining the SDF structure of the above components, importing it into Swiss target prediction database, taking the targets with prediction score greater than 0 as drug targets.

1.3 Disease targets screening

The relevant target information is obtained by searching with "gastric ulcer" as the keyword in database of OMIM, diskenet, genecards. After merging and eliminating the repeated targets, the disease target is obtained.

1.4 Drug disease common targets screening

Select venny2.1 online software mapping tool, input the screened drug target and disease target into the software, draw the Wayne diagram, and obtain the target after taking the intersection.

1.5 Construction and analysis of TCM component target disease network

Import the above potential chemical components and target information of Astragalus and Panax notoginseng into the Cytoscape 3.7.2, construct the "drug component target disease" network diagram, and use the function of network analyzer to conduct topological analysis on the main active components of Astragalus and Panax notoginseng.

1.6 Construction of PPI

The targets of Astragalus membranaceus, Panax notoginseng and GU diseases were input into the string database for retrieval. The minimum interaction threshold was 0.4 and the protein type was set as "Homo sapiens". The PPI network was constructed. The top 30 were the core targets according to the degree. The PPI network is imported into cystoscap 3.7.2. It is sorted by degree according to degree, betweenness centrality, average shortest path length and closeness centrality. Select the core targets genes with whose scores greater than the average score, draw the bar graph of the first 30 targets with R3.6.1.

1.7 GO and KEGG enrichment analysis

The biological function of core targets was analyzed. Based on R software, Bioconductor bioinformatics software package was used to analyze the function enrichment of key target genes go
2. Results

2.1 Screening results of components and targets of Astragali Panax notoginseng

Screen the chemical components of Astragalus membranaceus and Panax notoginseng in tcmsp database (OB ≥30% and DL ≥0.18), screen the components of Astragalus membranaceus and Panax notoginseng, and obtain 27 potential active components. (Table 1). 488 drug targets were got.

<table>
<thead>
<tr>
<th>No.</th>
<th>Active Ingredient</th>
<th>OB (≥30%)</th>
<th>DL (≥0.18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hoelen</td>
<td>50.47</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>Polygal酸</td>
<td>50.47</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>Aristolochic acid</td>
<td>50.47</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>Morindine</td>
<td>40.55</td>
<td>0.36</td>
</tr>
<tr>
<td>5</td>
<td>Glycyrrhetin</td>
<td>50.47</td>
<td>0.36</td>
</tr>
</tbody>
</table>

The top 20 results were chosen for mapping and output in the form of bargraph.

2.2 Drug-disease target screening results

The potential targets were searched in OMIM, disgenet, genecards and other databases with GU as the keyword. After screening, 715 disease targets were got. Compared with the drug targets screened in the previous Swiss target prediction database, 488 drug targets and 715 disease targets were input into venny2.1, and 118 drug-disease targets were got (Fig 1). The results suggest that Astragalus membranaceus and Panax notoginseng may play an anti ulcer role through the synergistic effect of 118 joint targets.

2.3 TCM-Composition-target-disease diagram

27 potential active components from Astragalus and Panax notoginseng and 118 drug-disease targets were input into Cytoscape software, deleting the isolated components without intersection with the target, and drawing the network diagram of "drug component target disease" interaction (Fig 2). In the figure, purple means drugs, green means 26 active components in Astragalus Panax notoginseng (one active component target has no intersection with the disease target), blue means 118 joint targets, red means diseases.
2.4 Construction and analysis of PPI network

118 joint targets obtained from Wayne diagram were imported into the string database for retrieval. The network relationship data of target interaction was got and imported into Cytoscape software to draw the protein interaction network diagram, as shown in Fig 3, in which the size of nodes The color and its variation in depth represent the magnitude of the degree value. The mapping is carried out according to the conditions in 1.6, and the results are shown in Figure 4.

2.5 GO enrichment analysis

118 joint targets were imported into David database for go enrichment analysis, and three parameters of go analysis were checked: biological process (BP), cellular component (CC) and molecular function (MF). The results showed that the intersection gene set was enriched into 1948 BP, It mainly includes peptidyl serine modification, muscle cell promotion, peptidyl serine phosphorylation, positive regulation of response to external stimulus, etc; The cross gene set was enriched to 45 CC, mainly including membrane microdomain, membrane raft, membrane region, vesicle lumen, etc; The intersection gene set was enriched to 106 MF processes, mainly including protein serine/threonine kinase activity, protein tyrosine kinase activity, serine type peptidase activity, serine type endopeptidase activity, serine hydrolase activity and endopeptidase activity. The top 20 results of padjust are plotted to obtain the bar graph of BP, CC and MF enrichment (Fig. 5). It suggests that Astragalus-notoginseng can treat GU through a variety of targets.
Fig. 5 go enrichment analysis of Astragalus and Panax notoginseng on treating GU (from top to bottom: BP, CC and MF)

2.6 KEGG enrichment analysis

Using David database for KEGG enrichment analysis, setting P<0.05 and Q<0.05. After 118 common targets are run in R language, 146 KEGG pathways are got. The top 20 of significance form a bar graph of KEGG function enrichment (Fig. 6). P_adjust represents the significance of enrichment. The redder the color, the higher the significance. The results showed that the signal pathways of Astragalus notoginseng in the treatment of Gu mainly involved proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, prostate cancer, PI3K Akt signaling pathway, VEGF signaling pathway, etc.

Fig.6 KEGG enrichment analysis of Astragalus and Panax notoginseng on treating GU. P represents the importance of enrichment (The redder the color, the higher the importance)

3. Discussion

27 active components of Astragalus Panax notoginseng were got through tcmsp database and literature search, mainly including quercetin, mairin, jaranol, hederagenin, mandenol, etc. 488 corresponding active components targets were got by using Swiss target prediction database, and the intersection was taken with the GU disease-related targets obtained from Disgenet, OMIM and Genecards databases. 118 drug-disease joint targets were got, suggesting that Astragalus Panax notoginseng may treat GU through 27 active ingredients and 118 targets. The network analysis of Astragalus and Panax notoginseng active ingredient-gastric ulcer-target was constructed. The results showed that 26 of the chemical constituents of the drug could be connected with at least 3
targets, and 9 ingredients could be connected with more than 27 targets, including 5 components jaranol (galangin, derived from Huangqi) DFV (lipotrophin, from Sanqi), isorhamnetin (isorhamnetin, from Huangqi), kaempferol (kaempferol, from Huangqi), quercetin (quercetin, from Huangqi and Sanqi) were connected with more than 33 targets and preliminarily determined as the main components. By constructing the protein PPI network, the top 30 targets were chosen as the heart targets. Among the core targets, degree ranked first mainly in VEGFA, AKT1, EGFR, CASP3, STAT3, mapk1, TNF, SRC, HRAS, etc. The go analysis results of these 118 targets obtained 1948 biological process pathways, 45 cell component expression process pathways and 106 molecular function related process pathways. KEGG analysis obtained 146 related signal pathways, mainly involving proteoglycans in cancer, prostate cancer, EGFR tyrosine kinase inhibitor resistance and other pathways. It can be seen that Astragalus-Panax notoginseng drug pair plays the role of treating gastric ulcer through multi-components, multi-targets, multi-channels, and multiple pathways, and its mechanism may be related to the above main components, core targets and relevant pathways.

Quercetin is the main chemical component of Astragalus membranaceus and Panax notoginseng. Quercetin is a kind of natural flavonoid widely existing in nature. Studies have shown that quercetin has multiple pharmacological effects such as anti-oxidation, antiviral, anti-inflammatory, antitumor and so on. It can be used to treat liver, heart, spleen, lung, kidney, orthopedic diseases, nervous system diseases and so on in cell and animal experiments[11]. Yang Dengyu et al. reviewed the main pharmacological activities and potential applications of quercetin in clinical medicine, clarified the antioxidant mechanism and broad-spectrum antibacterial and antiparasitic properties of quercetin, and summarized its potential applications in zoology, cardiovascular protection and anti-immunosuppressive therapy, which is expected to provide some enlightenment for the further study of quercetin and its properties[12]. Many studies have also shown that quercetin can protect indomethacin induced gastric ulcer in rats, histamine induced gastric ulcer in male guinea pigs and experimental peptic ulcer in rats caused by aspirin, and has a good effect of protecting and treating GU[13-15].

KEGG analysis showed that Astragalus-Panax notoginseng may treat GU through cancer proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, prostate cancer and so on. Among the top five signal pathways, there are 34 targets enriched in PI3K Akt signal pathway, including 18 core targets such as KDR, map2k1, mTOR, PIK3CA, CDK4, CCND1, rps6kb1, MCL1, EGFR, IL2, MDM2, mapk1, IGF1R, AKT1, HRAS, bcl2l1, VEGFA and FGF2. PI3K Akt signaling pathway participates in the processes of cell differentiation, proliferation and apoptosis, and has a variety of physiological activities. Based on the theory of "simultaneous treatment of different diseases" in TCM, He Ru-pu and others revealed the spleen strengthening effect of Codonopsis pilosula on different gastric diseases, indicating that Codonopsis pilosula acts on GU, gastritis and gastric cancer mainly through the overall adjustment of PI3K Akt signal channel[16]. Chen Yongxiang and others explored the mechanism of Lizhong Decoction in the treatment of gastric ulcer rats based on PI3K/Akt pathway. The experiment shows that Lizhong decoction can significantly reduce the serum inflammatory level of gastric ulcer rats and down regulate the expression of PI3K and Akt proteins in rat gastric tissue, which may be related to the regulation of PI3K/Akt pathway[17].

The top three core targets VEGFA (vascular endothelial growth factor a), AKT1 and EGFR are also more studied targets. VEGF gene was located on chromosome 6p21.3, is a relative molecular weight of (34~45)×103 is a highly glycosylated basic protein with a relative molecular weight of (17~22)×103. In vivo, it can specifically promote endothelial cell division, increase capillary permeability, induce endothelial cell migration and angiogenesis. VEGF A is a very important subtype of VEGF. It can activate PI3K, ERK, Akt and other signal pathways by combining with VEGFR1 on the cell membrane to promote the migration and proliferation of endothelial cells and form neovascularization[18-19]. Epidermal growth factor (EGF) has a wide range of biological effects inside and outside the human body. It can inhibit gastric acid secretion, promote epithelial cell proliferation, tissue repair and cell protection. It activates regulatory protein through the
specific receptor EGFR acting on the target cell membrane, and plays a biological effect through enzymatic reaction to accelerate the proliferation of endothelial cells. Promote mucosal repair and reconstruction, protect the integrity of gastric mucosa, promote the repair of ulcer wound, accelerate ulcer healing, so as to alleviate the occurrence and development of ulcer and help patients with gastric ulcer recover [20-21].

The paper preliminarily analyzed the mechanism of Astragalus Panax notoginseng on treating gastric ulcer with the help of network pharmacological methods and means, and speculated that the drug may play a synergistic role in the treatment of GU through multi-ingredients, multi-targets and multi-ways. The possible components are jaranol (warfarin), DFV (lipoid), isorhamnetin (isorhamnetin), kaempferol (kaempferol) Quercetin, etc. possible targets include VEGFA, AKT1, EGFR, CASP3, STAT3, mapk1, etc. possible pathways include cancer proteoglycans in cancer, PI3K Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, etc. However, the clear mechanism needs to be further tested and verified. In short, TCM has obvious advantages in treating diseases compared with western medicine with a single component and a single target. With the progress of computer technology, omics technology, network analysis and other technical means, it will help to reveal the mysterious veil of TCM in the treatment of diseases, accelerate the process of modernization and internationalization of traditional Chinese medicine, give full role to the characteristics of TCM and protect human health.

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References


