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Research Article

To Explore the Effect of Bu Zhong Yi Qi Powder on the Levels of Inflammatory Factors IL-6 and TNF-α in Stable COPD Patients with Spleen-Lung Qi Deficiency Pattern

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Abstract

Objective: Traditional Chinese medicine (TCM) herbal formulae provide valuable therapeutic strategies. However, the active ingredients and mechanisms of action remain unclear for most of these formulae. Therefore, the identification of complex mechanisms is a major challenge in TCM research.

Methods: This study used a network pharmacology approach to clarify the anti-inflammatory and cough suppressing mechanisms of the Chinese patent medicine Buzhong YiQi Powder (BZYQ). Further, we recruited 20 subjects of Chronic Obstructive Pulmonary Disease (COPD) with spleen-lung qi deficiency at stable stage and randomly divided into control group and intervention group. The control group was treated with routine maintenance therapy, and the intervention group was treated with BZYQ in addition to the control group. Both groups were treated for 2 weeks. The differences of TCM syndrome score, lung function improvement, serum inflammatory factors Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were observed between the two groups before and after treatment.

Results: Ten main constituents affected ten predicted core genes, were obtained by network pharmacology analysis, including IL-6, TNF, IL-1β, CASP3, et al., affecting several therapeutic functions: immunoregulation, anti-inflammation, oxidative stress and atherosclerotic signaling pathways. Serum inflammatory factors IL-6 and TNF-α level were significantly decreased after BZYQ treatment.

Conclusion: This novel approach of global chemomics-integrated systems biology represents an effective and accurate strategy for the study of TCM with multiple components and multiple target mechanisms. The molecular mechanism of treatment of COPD may be anti-inflammatory and antioxidant stress as the core, preventing airway remodeling, improving clinical symptoms, and improving lung function of patients.

Keywords: Buzhong yiqi powder; Chronic obstructive pulmonary disease; Network pharmacology; Spleen-lung qi deficiency; Inflammatory cytokines

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a common and frequently occurring disease in clinical practice. It is characterized by incompletely reversible airflow limitation that can develop progressively, and it can lead to respiratory failure in severe cases [1]. Recurrent airway inflammation causes immune dysfunction in COPD patients, and overactive lymphocytes and neutrophils produce and release various inflammatory factors, among which the IL-6 and TNF-α are the most active [2]. Traditional Chinese medicine believes that COPD patients take turbid phlegm and blood stasis as the excess syndrome and the pathogenesis is mostly due to the lung and spleen qi deficiency, and it also affects the kidney. Therefore, COPD patients are mostly of the lung-spleen qi deficiency pattern. The "Lingshu Distension Theory" said that "the people with lung distension will be deficient and full to cough and gasp" [3], and it is believed that the dysfunction of the five internal organs and the poor circulation of qi-blood-body fluid are the main causes of the disease. The main cause is the deficiency of lung, spleen, and kidney. In addition, it is invaded by exogenous pathogens which results in the stasis-phlegm type. Treatment should be given to supplement lung and replenish qi, as well as to support the healthy energy. At present, western medicine treatment of stable COPD mainly adopts western medicine treatment and health support, such as bronchodilators, glucocorticoids, aminophylline, etc., which can improve the ventilation of patients and effectively control the disease, but the adverse reactions are obvious after long-term medication. Traditional Chinese medicine treats the COPD based on syndrome differentiation. For the stable patients, it usually uses the treatment of relieving cough and asthma, replenishing qi and supplementing deficiency, invigorating spleen for eliminating dampness, and reducing phlegm for descending adverse qi to improve patients' clinical symptoms based on the syndrome, which long-term therapeutic effect is better in improving patients' ventilation, alleviating inflammatory response, and reducing toxic and side effects, compared with the glucocorticoid and bronchodilator therapy in western medicine [4].

In Li Dongyuan's "Spleen and Stomach Theory" [5], it is recorded that BZYQ can invigorate spleen and replenish qi to transport and transform the spleen qi to supplement the lungs, playing a role in the treatment of COPD. Previous studies have shown that BZYQ can effectively control the progression of the disease and prevent recurrence in the treatment of COPD patients [6]. At present, the molecular mechanism of BZYQ in the treatment of COPD is still unclear. Therefore, in this study, network pharmacology analysis was combined with clinical application to intervene with BZYQ in

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stable COPD patients with spleen-lung qi deficiency pattern. When observing the therapeutic effect, the changes of inflammatory factors in patients were analyzed to provide a theoretical basis for exploring the optimal application of traditional Chinese medicine preparations in the actual clinical treatment of COPD and exploring the molecular mechanism of therapeutic effects through network pharmacology.

Materials and Methods for Network Pharmacology Analysis

To screen the active ingredients and target genes of BZYQ

The active ingredients in BZYQ were screened in the TCMSP [7], with the components of milkvetch root, dried tangerine peel, *codonopsis pilosula*, *angelica sinensis*, ginger, *Cimicifuga foetida* L, liquorice root, thorowax root, *Atractylodes macrocephala* Koidz and Chinese date. The screening conditions for active ingredients: OB ≥ 30%, the similarity analysis between the compound and the known drugs is ≥ 0.18, and then the target corresponding to the active ingredients of BZYQ was obtained. The English name of the target corresponding to the active ingredient of the drug was standardized through the UniProt database, and the corresponding target gene number was obtained.

To obtain COPD-related genes and screen BZYQ-COPD interaction targets

Using "chronic obstructive pulmonary diseases, COPD" as the key word and the species as "human sapiens", we retrieved the disease dataset from the GEO (https://www.ncbi.nlm.nih.gov/geo/) database. After normalizing and screening the content of the dataset again, we used the dataset GSE11784 expression profile chip as the final research sample and downloaded the expression matrix and related information of the gene expression profile chips. The gene chips obtained were analyzed online by GEO2R, and differential analysis was performed on the expression profile of the normalized GSE11784 chip, taking |log2 FC|>0.05 and P<0.05 as the screening condition for genes with significant difference. The screened results were visualized by the "pheatmap" and "ggplot2" packages of the R software and outputted in the form of volcano plot and heat map [8]. The Draw Venny Diagram online program (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to intersect the medicinal material gene and the disease target gene to obtain the common targets of the medicinal material and the disease, which were the possible targets for medicinal material to treat diseases, and a Venn diagram was drawn.

To construct the network of BZYQ-compound-target-COPD

We sorted out the interaction targets of BZYQ and COPD, and identified the corresponding drug components, gene targets and other attributes, then drawn the network of BZYQ-compound-target-COPD using Cytoscape 3.8.0.

To make a PPI network to screen the hub genes of drug diseases

Targets with certain connections in the network with certain connections between proteins were imported into the STRING database (https://string-db.org/) [9], the species in the retrieval condition was set as "Homo sapiens", free nodes were shielded, the score threshold was >0.900, and the PPI and string-interactions file were exported. The string-interactions file was imported into Cytoscape 3.8.0 software, and the hub genes were obtained using the MCODE plug-in in Cytoscape 3.8.0 software to score.

Target GO enrichment and KEGG pathway analysis

The hub genes were subjected to functional enrichment and main function pathway analysis [10]. On the Metascape website (https://metascape.org/gp/index.html/main/step1), the results of GO (Gene Ontology) enrichment and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis were exported with P<0.01 as the screening condition, which were converted into graphs on the Metascape website [11].

Molecular docking

We found and downloaded the protein structure of the target by searching the PDB protein database, removed water molecules and added hydrogen by using Pymol software, and then calculated the charge of the protein. Then we obtained the 3D structures of the active ingredients in Bu Zhong Yi Qi Wan by searching the PubChem database. The protein and active ingredients were converted into pdbqt format by AutoDock vina 1.5.6 software, and the position of the self-ligand in the protein was defined as the active site, that is, the docking site. We set the coefficient Spacing=0.5, and finally run the software for molecular docking. When the affinity is less than 0, it can be considered that the molecule and protein can bind spontaneously [12].

Clinical Research Materials and Methods

General data

This study adopted a prospective, randomized, and parallel-controlled trial design. The study subjects were 20 stable COPD patients with spleen-lung qi deficiency who were treated in the outpatient department of the First Affiliated Hospital of Guangzhou University of Chinese Medicine from January to June 2022. All subjects were divided into the control group and intervention group using a random number table generated by SAS software, with 10 patients in each group. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (K-2022-019). In the intervention group, there were 8 males and 2 females, and their ages ranged from 62~74 years old, with an average of (68.43 ± 6.71) years old; In the control group, there were 8 males and 2 females, and their ages ranged from 61~73 years old, with an average of (67.71 ± 6.63) years old; There was no significant difference in general data between the two groups of patients (P>0.05), and they were comparable.

Criteria for diagnosis, inclusion and exclusion

Western medicine diagnostic criteria of COPD: It should be in line with the diagnosis criteria in the "Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease" (Revised Version 2013) [13] and the "Clinical Practice Guideline for Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease in Elderly Patients in China" [14] formulated by the Chronic Obstructive Pulmonary Disease Group of the Respiratory Diseases Branch, the Chinese Geriatrics Society. 1) Most of the patients have a history of chronic bronchitis, with symptoms of the recurrent cough and expectoration, chest distress and asthma. In the acute stage, the above symptoms are obviously aggravated, and there is a fever; 2) Hyperresonance appears in the patient's lung when percussion, and the barrel chest appears when visual examination; 3) Wheezing sound can be heard in the lungs; 4) The ratio of forced expiratory volume in the first second to expiratory volume (referred to as 1-second rate, FEV1/FVC) is less than 70% (when bronchodilators are not used).

Traditional Chinese medicine diagnosis criteria of the lung-spleen qi deficiency pattern: It should be in line with the syndrome

differentiation criteria for lung-spleen qi deficiency in the "Guidance Principle of Clinical Study on New Drug of Traditional Chinese Medicine" [15]. Primary symptoms: To be short breath, worse with a little tired, cough with phlegm, which is sticky and white; Secondary symptoms: To be spontaneous sweat, fear of wind, stomach distension, loose stools, poor appetite, vomiting, nausea, weakness and fatigue; Tongue and pulse: The tongue is pale, the tongue coating is thin and greasy, and the pulse is thin and slippery.

Inclusion criteria: 1) It should be in line with the western medicine diagnosis criteria of stable COPD; 2) It should be in line with the traditional Chinese medicine diagnosis criteria (the spleen-lung qi deficiency); 3) Patients aged in 18-75 years old; 4) Patients who can accept this treatment plan and cooperate with the clinical observation; 6) Patients who have not administrated traditional Chinese medicine preparations within one week before enrollment. All the enrolled patients should sign the informed consent form.

Exclusion criteria: 1) Patients with bronchial asthma, bronchiectasis, pneumonia, interstitial lung disease, pulmonary tuberculosis, lung cancer or other lung diseases; 2) Patients with acute COPD; 3) Patients with severe cardiovascular, cerebrovascular, liver and kidney and hematopoietic system diseases; 4) The females who are in the plan for pregnancy, pregnant, and in the suckling period; 5) Patients with allergic constitution or who are allergic to known ingredients in the drug of this study; 6) Patients with mental or legal disabilities.

Treatment methods

Control group: To treat with Seretide (SALMETEROL Fluticasone) powder for inhalation, a Glaxo Welcome Production, France. The registration certificate No. is H20150324, and the specification is 50 μg: 250 μg × 60 blisters/box. 1 inhalation once, 2 times per day, for 2 weeks.

Intervention group: Oral BZYQ is administered based on the control group, with drug specification: 3 g/bag × 9 bags/box, drug approval number: GYZ Zi Z20040120; Manufacturer: Beijing Han Dian Pharmaceutical Co., Ltd. The main ingredients of the drug include: Milkvetch root, *codonopsis pilosula*, liquorice root, *angelica sinensis*, *Atractylodes macrocephala* Koidz, *Cimicifuga foetida* L, thorowax root, dried tangerine peel, ginger and Chinese date. Usage and dose: PO, bid, one bag (3 g/bag) at a time, p.c. for 2 weeks.

Observation indicators and statistical methods

Observation indicators: (1) To compare the TCM syndrome score between 2 groups The symptoms of spleen-lung qi deficiency before and after treatment were scored according to the "Guidance Principle of Clinical Study on New Drug of Traditional Chinese Medicine (Trial)" [16] and combining clinical experience. The score of primary symptoms: 1) Cough. 2 points: Occasional; 4 points: Recurrent, which can be relieved after treatment with drugs for reducing phlegm and relieving cough; 6 points: Recurrent or even persistent, with no relief with drug intervention. 2) Chest distress and asthma. 2 points: Occasional and mild; 4 points: Recurrent, with no three concave signs; 6 points: Persistent, even with three concave signs. The score of secondary symptoms: 1) Spontaneous sweat. 1 point: Occasional, with self-relief; 2 points: Recurrent, which is aggravated after activity; 3 points: Recurrent, both day and night. 2) Soreness and weakness of waist and knees. 1 point: Occasional, self-relief without drug intervention; 2 points: Occasional, with self-relief after the drug intervention; 3 points: Persistent. 3) Weakness. 1 point: Occasional, which can be relieved temporarily or after a temporary rest; 2 points: Recurrent, which can be relieved temporarily after adequate rest; 3 points: Persistent. The changes of the scores of primary symptoms (including chest distress, asthma and cough) and the scores of secondary symptoms (including spontaneous sweat, soreness and weakness of waist and knees, and weakness) in the two groups before and after treatment were observed. (2) To compare the lung function improvement the lung function of the patients was assessed using the COPD Assessment Test (CAT) score. Among them, the CAT score includes 7 items such as climbing stairs, chest distress, expectoration, activity, cough, sleep, and going out. It is divided into 3 grades according to the severity, and the highest item score is 5 points. The higher the score, the more serious the impact on lung function. (3) To compare the inflammatory state of the two groups. 2 mL of venous blood was drawn and centrifuged, and the level of IL-6 was detected by electrochemiluminescence method, and the level of TNF-α was determined by double antibody sandwich enzyme linked immunosorbent assay.

Statistical method: SPSS 22.0 statistical software was used for statistical analysis of data. Measurement data (complying with the normal distribution) were expressed as mean ± standard deviation (± s), the matched samples t-test was used in the comparison before and after treatment, and the two independent samples t-test was used in the comparison between groups; $\bar{x}$ Enumeration data were expressed as rate or constituent ratio, and the χ^2 test was used for comparison between groups. It is statistically significant if $P<0.05$.

Results

Main active compounds of BZYQ and their targets

In this study, 206 main active compounds, in the *codonopsis pilosula*, milkvetch root, liquorice root, dried tangerine peel, *Atractylodes macrocephala* Koidz, *angelica sinensis*, *Cimicifuga foetida* L, thorowax root, Chinese date and ginger, were retrieved from the TCMSP database according to the conditions of OB ≥ 30% and DL ≥ 0.18. And 183 active compounds were obtained after deduplication, including 12 for *codonopsis pilosula*, 20 for milkvetch root, 92 for liquorice root, 5 for dried tangerine peel, 7 for *Atractylodes macrocephala* Koidz, 2 for *angelica sinensis*, 17 for *Cimicifuga foetida* L, 17 for thorowax root, 29 for Chinese date and 5 for ginger.

Potential targets of BZYQ in the treatment of COPD

After $|\log2\ FC|>0.05$ and $P<0.05$, 4855 genes with significant effects on the occurrence and development of COPD were screened, including 895 up-regulated genes and 3961 down-regulated genes. And 3433 genes were obtained by UniProt correction (Figure 1). The Draw Venn Diagram online program was used to intersect the medicinal material gene and the disease target gene to obtain the common targets of the medicinal material and the disease, and a Venn diagram was drawn, in which there were 225 drug targets and 3433 disease targets, and there were 55 intersection targets of BZYQ and COPD disease as shown in Figure 2.

Results of the network of traditional Chinese medicine-compound-target-disease

The network of traditional Chinese medicine-compound-target-disease was drawn by Cytoscape 3.8.0, as shown in Figure 3. The blue nodes represent the main active ingredients of BZYQ, the yellow nodes represent potential targets of COPD, and each line represents the interaction between BZYQ and COPD. The 10 most active compounds were screened using the cytohubba plug-in in cytoscape,

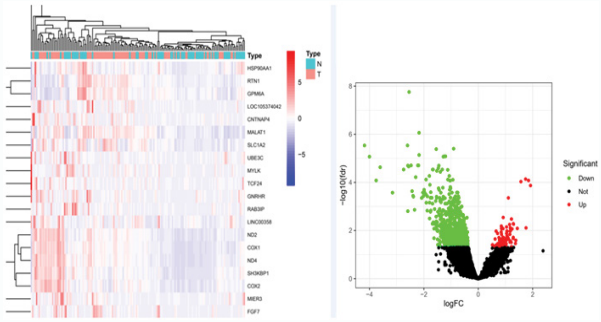

Figure 1: Heat map (A) and volcano map (B) of COPD targets.

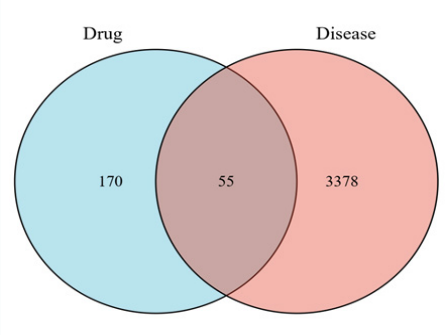

Figure 2: Venn diagram of drug- disease common target of BZYQ for COPD.

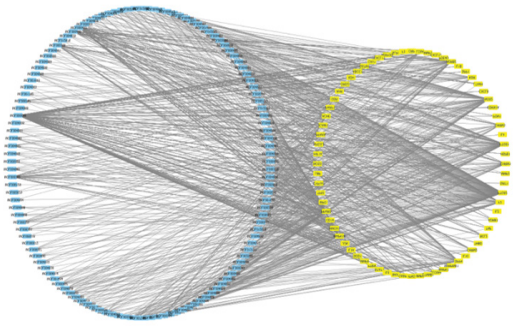

Figure 3: Drug-ingredient-common target-disease network diagram of BZYQ for COPD.

as shown in Figure 4. The results showed that active compounds, such as glycyrrhizin, formononetin, naringenin, quercetin, kaempferol, nobiletin, β-sitosterol, and isomucronulatol, were key roles in the entire network, which may be the core compounds of BZYQ in the treatment of COPD.

PPI network and its core screening

The PPI protein network was obtained through the STRING platform, as shown in Figure 5. The MCODE plug-in in cytoscape software was used for screening to obtain the core genes such as IL-6, TNF, IL-1β, JUN, PTGS2, TP53, MMP9, CASP3, CXCL8, and HMOX1, as shown in Figure 6.

GO enrichment analysis

GO functional enrichment analysis was performed through the Metascape website, and the top-ranked GP entries were plotted as a histogram. The biological process mainly includes the cells' metabolic regulation of reactive oxygen species, response to lipopolysaccharide

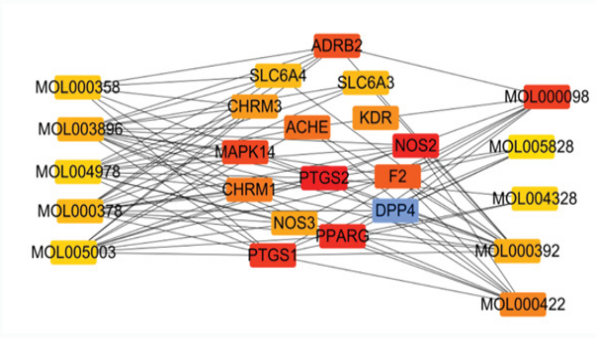

Figure 4: Drug-ingredient-common target-disease network diagram of BZYQ for COPD.

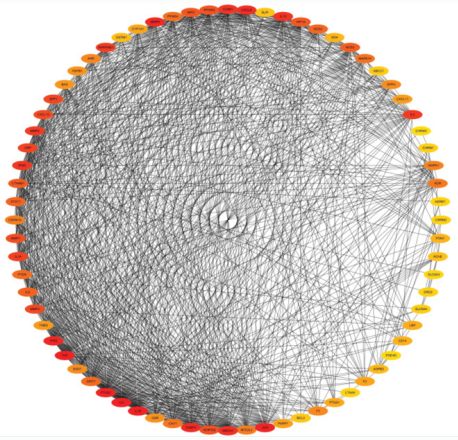

Figure 5: PPI network topology analysis diagram of BZYQ for COPD.

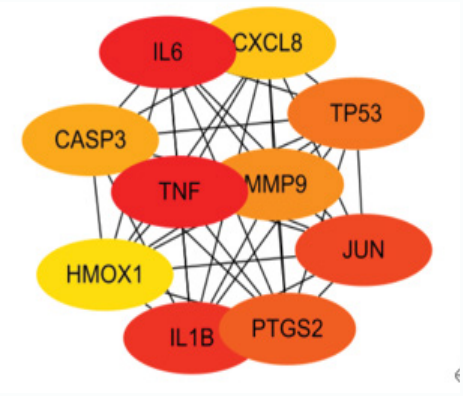

Figure 6: Core gene analysis diagram of BZYQ for COPD.

and apoptosis pathway; Cell components are mainly related to transcription regulator complexes, CDK, holoenzyme complexes, protein kinase complexes, nuclear chromatin, and cell membrane; Molecular functions mainly involve nuclear receptor activity, ligand-activated transcription factor activity, steroid hormone receptor activity, DNA binding, transcription factor binding, phosphatase binding, cytokine receptor binding, cytokine activity, etc., (Figure 7).

Analysis of KEGG enrichment results

The KEGG enrichment results were analyzed through the Metascape website, and 20 pathways with smaller P values were selected to draw a histogram, as shown in Figure 8. It mainly involved

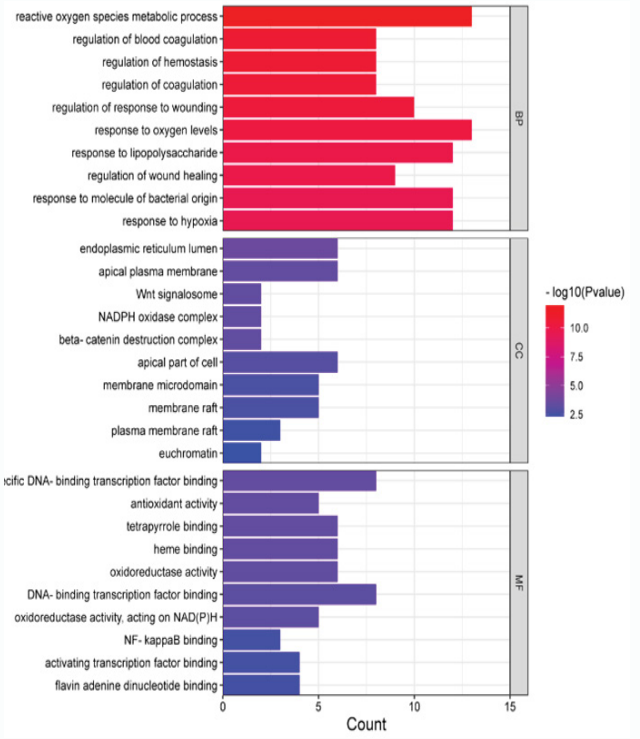

Figure 7: GO function enrichment analysis of BZYQ for COPD.

signaling pathways closely related to inflammation, such as lipids, atherosclerosis, IL-17 signaling pathway, and cancer cascade.

Molecular docking results

The top 10 active ingredients in the degree value were selected for molecular docking with the predicted main targets TNF and IL-6. The molecular docking results indicated that there were 4 compounds with strong binding ability to the target (Affinity <-7 kcal/mol) in the results of the docking with the IL-6 target, among which glycyrrhizin had the strongest binding ability to IL6, with the affinity of -7.5 kcal/mol. There were 6 compounds with strong binding ability to the target (Affinity < -7 kcal/mol) in the results of docking with the TNF target, among which formononetin is the best to TNF, with the affinity of -9.5 kcal/mol. Some molecular docking models are shown in Figure 9.

Comparison of TCM syndrome scores and pulmonary function between the control group and the intervention group before and after treatment

Results in Table 1 showed: Before treatment, there was no significant difference in the scores of primary symptoms (including chest distress, asthma, and cough) and the secondary symptoms (including spontaneous sweat, soreness and weakness of the waist and knees, and weakness) between the two groups (P>0.05). After treatment, the scores of primary symptoms and secondary symptoms in the two groups were significantly lower than those before treatment (P<0.05), and the reduction of scores in the intervention group was significantly better than that in the control group. The differences were statistically significant (P<0.01). Before treatment, there was no significant difference in the CAT score of the pulmonary function evaluation index between the two groups (P>0.05). After treatment, the scores in both groups were lower than those before treatment, and the differences were statistically significant (P<0.05). And the reduction of CAT score in the intervention group was significantly better than that in the control group. The differences were statistically significant (P<0.01).

Comparison of serum IL-6 and TNF-α level between the control group and the intervention group

By t-test, IL-6 and TNF-α in the two groups after treatment decreased compared with those before treatment, and IL-6 and TNF-α in the intervention group after treatment were significantly lower than those in the control group (P<0.001) (Table 2).

Discussion

COPD is a syndrome characterized by airway obstruction and chronic airway inflammation, which can develop into respiratory failure and pulmonary heart disease. The occurrence and development of COPD is mostly related to abnormal inflammatory response, and it has high morbidity, disability and mortality in people over 40 years old [17]. However, its pathogenesis has not been fully clarified. It is generally believed that chronic inflammatory injury, imbalance of pulmonary protease and anti-protease systems, imbalance of oxidative stress system, and accelerated apoptosis of lung epithelial cells and endothelial cells play an important role in the occurrence and development of COPD. And chronic inflammation of airways, lung parenchyma and pulmonary vessels is a characteristic change of COPD [18]. Chronic airway inflammation is mainly caused by the infiltration of T lymphocytes, pulmonary macrophages and neutrophils, which can release a variety of inflammatory mediators and cytokines, destroy the normal tissue structure of the lung and promote the body's inflammatory response. It is the main cause of airflow obstruction and aggravation.

BZYQ has been passed down for several years. It can invigorate spleen-stomach, replenish qi, invigorate splenic yang and raise qi. In the formulation, radix astragali preparata is sweet and slightly warm, in spleen and lung meridians, and can invigorate the spleen-stomach and replenish qi, which is the principle drug; Codonopsis pilosula, radix glycyrrhizae preparata and *atractylodes macrocephala* koidz can invigorate qi and invigorate spleen, which are minister drugs; Blood is the carrier of qi, so angelica sinensis and Chinese date are used to nourish blood and harmonize, and milkvetch root and codonopsis pilosula are used to invigorate qi and nourish blood; *Cimicifuga foetida* L and thorowax root can invigorate splenic yang and raise qi, helping principle drug raising the collapsed qi; dried tangerine peel and ginger can regulate qi-flowing for harmonizing stomach, helping

Table 1: Comparison of TCM syndrome scores and lung function between the two groups of patients with stable COPD before and after treatment ($\bar{x} \pm s$).

Group	Number	Primary symptoms		Secondary symptoms		CAT	
		Before	After	Before	After	Before	After
Control	10	9.18 ± 0.63	4.56 ± 0.34①	4.0 ± 0.35	2.6 ± 0.30①	13.25 ± 1.51	9.75 ± 1.46①
Intervention	10	9.21 ± 0.69	3.0 ± 0.30①②	4.01 ± 0.36	1.89 ± 0.32①②	12.95 ± 1.40	7.10 ± 0.70①②
t value		-0.101	10.898	-0.063	5.123	0.46	5.183
Pvalue		0.92	<0.001	0.951	<0.001	0.651	<0.001

①P<0.05, Compared with before treatment; ②P<0.01, Compared with the control group after treatment

Table 2: Comparison of serum IL-6 and TNF-α level between the two groups of patients with stable COPD before and after treatment ($\bar{x} \pm s$).

Group	Number	IL-6 (ng/mL)		TNF-α (ng/L)	
		Before	After	Before	After
Control	10	9.78 ± 2.71	4.25 ± 1.59①	41.42 ± 1.75	26.58 ± 2.22①
Intervention	10	10.61 ± 1.87	7.52 ± 1.43①②	41.37 ± 1.60	33.59 ± 1.62①②
t value		-0.79	-4.837	0.067	-8.061
P value		0.44	<0.001	0.948	<0.001

①P<0.05, Compared with before treatment; ②P<0.01, Compared with the control group after treatment

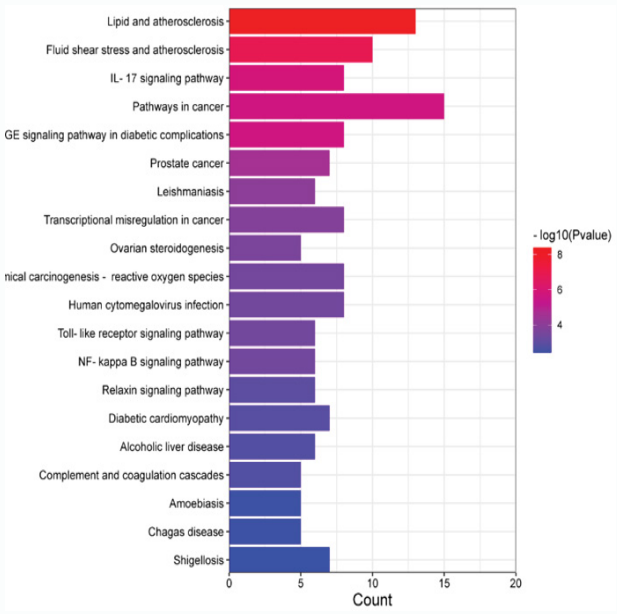

Figure 8: KEGG pathway enrichment analysis of BZYQ for COPD.

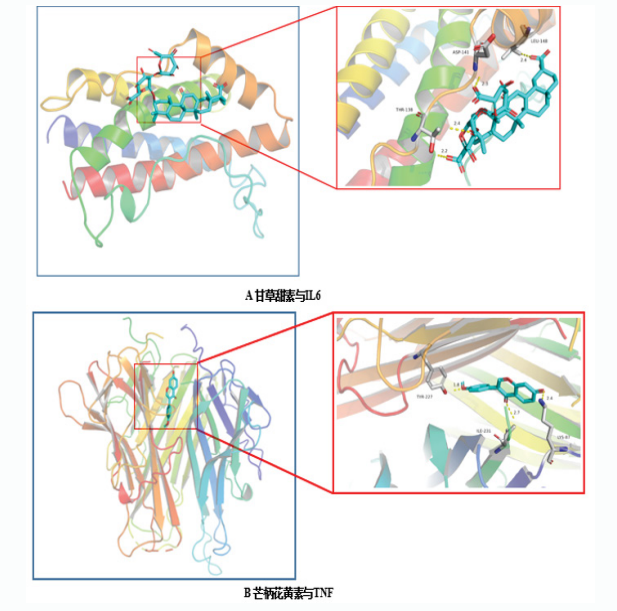

Figure 9: 2D and 3D schematic diagrams of molecular docking between active ingredients and targets of BZYQ for COPD (part).

the drugs nourishing but not greasy; radix glycyrrhizae preparata can coordinate the drug actions, which is the envoy drug. All the drugs are combined to play the roles of invigorating spleen-stomach and replenishing qi [19]. Studies have confirmed that BZYQ can significantly improve the clinical symptoms of cough, asthma, phlegm and pulmonary wheezing in patients with stable COPD, enhance pulmonary ventilation function, and improve the quality of life [20]. However, its effect on inflammatory factors in COPD patients has not been reported.

In the early stage of this study, 225 active ingredients and 55 potential targets were obtained by constructing a network of "Traditional Chinese Medicines for BZYQ-active compounds-action targets-diseases". According to the analysis results of BZYQ components and targets, the molecular mechanism of the formula compatibility was further discussed. Milkvetch root is the principle drug, sweet and warm, and it has the effect of invigorating qi. Its core components include quercetin [21], kaempferol, and formononetin. Quercetin has pharmacological effects such as inhibiting abnormal response of tissue cells during infection, trauma, and allergy, inhibiting virus proliferation, and regulating the immune. It can inhibit the high expression of CASP3 protein in cells that are invaded by pathogens and led to abnormal functions, so as to inhibit the proliferation of pathogens. Kaempferol can significantly inhibit lipid peroxidation induced by NADPH or ferrous ion, activate NF-κB and STAT3 signaling pathways, and reduce the effect of IL-6 on COX-2 expression to play an anti-oxidative stress role. The minister drugs include liquorice root and Chinese date. Liquorice root can invigorate spleen, replenish qi and regulate stomach, and the core ingredients include naringenin [22], Licochalcone A and 7-Methoxy-2-methyl-3-phenyl-4H-chromen-4-one. Naringenin is a dihydroflavone compound with antioxidant, anti-fibrotic, anti-inflammatory and anti-cancer properties. Licochalcone A can't only inhibit inflammation, but also inhibit mitochondrial damage and apoptosis caused by oxidative stress. Chinese date can invigorate qi and nourish blood, and the core components include quercetin, β-carotene, and β-sitosterol [23]. β-carotene can be anti-inflammatory and antioxidant through multiple targets and multiple pathways and inhibit apoptosis and proliferation. *Codonopsis pilosula* can replenish qi, invigorate spleen, engender liquid and moisten lung. It contains polysaccharides, which can inhibit tumor cells and enhance immunity, and triterpenes can promote the proliferation of T cells in mouse and enhance immunity [24]. *Angelica sinensis* can enrich blood and nourish yin, and its active ingredients can promote specific and non-specific immunity [25]. *Atractylodes macrocephala* Koidz can invigorate spleen, replenish qi, remove dampness and turbidity, and its active ingredients can enhance the immune function of tumor model rats, inhibit the proliferation of cancer cells and induce apoptosis [26]. The main active ingredients of BZYQ also include formononetin, nobiletin, isomucronulatol, glycyrrhizin, and β-sitosterol, which also have antioxidant and anti-inflammatory effects. Formononetin can also assist immune cells in the body and have an inhibitory effect on tumor cells. It can be seen from the above that the active compounds obtained by the reasonable compatibility of BZYQ mainly have the functions of inhibiting inflammatory response, inhibiting pathogen proliferation, regulating

immune and inhibiting oxidative reaction in body, which may have a certain intervention effect on the related inflammatory response caused by chronic obstructive pulmonary disease [27].

According to the PPI protein interaction network analysis, the core genes of BZYQ acting on COPD may be IL-6, TNF, IL-1β and so on. As an important inflammatory factor, IL-6 is involved in the occurrence and development of COPD, and is closely related to lung function, which can be used as an auxiliary indicator for COPD stage. The TNF-α co-produced by macrophages and monocytes is a pro-inflammatory cytokine whose broad biological activities can promote the synthesis and release of IL-8 and play a core role in the chronic inflammatory response of COPD.

Through GO analysis, it was found that core genes were enriched in biological processes such as lipopolysaccharide inflammatory response, apoptosis signaling pathway, and cardiovascular oxidative stress. Lipopolysaccharide has immunostimulatory and inflammatory effects, which can cause foot process fusion, cytoskeleton changes, and damage podocytes [28]. It has been reported that BZYQ can obviously inhibit lipopolysaccharide-induced qi deficiency and fever [29]. Physiological apoptosis plays an important role in maintaining the homeostasis of the internal environment and ensuring the health of the body. Pulmonary blood vessels are an important part of the cardiovascular system, and the process of oxidative stress has a great significance to the occurrence and development of pulmonary vascular diseases. Due to the decreased ventilation in patients with chronic obstructive pulmonary disease, the function of blood circulation is affected. BZYQ can invigorate spleen and replenish qi and can enhance the cardiopulmonary function and immunity of COPD patients after using for a long time.

KEGG pathway enrichment analysis found that the process of BZYQ in the treatment of COPD mainly involved IL-17 signaling pathway and atherosclerosis-related signaling pathway. The IL-17 signaling pathway is a classic inflammatory response signaling pathway, and its activation state increases the sensitivity of immune cells to oxidative stress damage and releases pro-inflammatory cytokines, enhancing the inflammatory response. The oxidative stress signaling pathway plays an important role in the body's metabolism, damage repair and other links. Among them, *HIF-1* can sense hypoxia and regulate the expression of target genes to adapt to the hypoxic environment [30]. Studies have shown [31] that the *HIF-1α* gene is highly expressed in hypoxic COPD patients. BZYQ may improve the body's tolerance to hypoxic environment and stabilize COPD through the regulation of oxidative stress-related signaling pathways. In conclusion, BZYQ may play a role in the treatment of COPD through the regulation of inflammation, immunity, hypoxia tolerance and other pathways.

However, network pharmacology analysis has certain limitations. Therefore, based on the network pharmacology analysis, this study recruited subjects through a clinical trial to further explore the effect of BZYQ on the inflammatory factors in COPD. The results of this study showed that the improvement of clinical symptoms of spleen-lung qi deficiency and the improvement of lung function in the intervention group were significantly higher than those in the control group, and the life quality and life ability were also better than those in the control group. After treatment, IL-6 and TNF-α in the two groups decreased compared with those before treatment, and IL-6 and TNF-α in the intervention group were significantly lower than those in the control group ($P<0.01$). It indicates that BZYQ combined with western medicine in the treatment of chronic obstructive pulmonary disease is better than the simple western medicine, which can reduce the level of inflammation and improve the immune function of patients.

In the early stage of this study, the network pharmacology analysis was used as the carrier to comprehensively analyze the components, targets, and pathways. And combining with the existing literatures, it discovered that the molecular mechanism of BZYQ in the treatment of COPD involves anti-inflammatory and anti-oxidative stress processes. A randomized, parallel controlled trial design was adopted in the later study. The results showed that BZYQ combined with western medicine in the treatment of stable COPD patients with spleen-lung qi deficiency pattern can replenish qi and supplement deficiency, improve the symptoms of shortness of breath and asthma, and improve the life quality and life ability. It can effectively regulate the body's inherent immunity while reducing the airway inflammation and it is safe in clinical application. This study has certain reference significance, but due to the normalization of management and control, the sample tracking is limited, and research samples are also limited, thus more accurate conclusions still need to be verified by subsequent large-scale clinical studies.

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Authors' contributions

Conceived and designed the experiments: Xueqing Chen, Jie Jia. Performed the experiments: Yingchun Zhou. Analyzed the data: Qionghua Tang. Wrote the paper: Xueqing Chen. All authors read and approved the final manuscript.

Ethical approval

The study protocol was approved by the Ethics Committee of First Affiliated Hospital of Guangzhou University of Chinese Medicine. The Ethics Committee of our institute approved the method of obtaining consent. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (K-2022-019).

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References

1. Mingsheng L, Zhang Yi, Zhang Di, et al. Review of the Research on the Mechanism of Multi-Target Intervention of Traditional Chinese Medicine for Chronic Obstructive Pulmonary Disease. J Tradit Chin Med. 2019;60(19):1697-700.

2. Wang C, Xu J, Yang L, Xu Y, Zhang X, Bai C, et al. Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross- sectional study. Lancet. 2018;391(10131):1706-17.

3. Liang X, Li H, Li S. A novel network pharmacology approach to analyse traditional herbal formulae: the Liu-Wei-Di-Huang pill as a case study. Mol Biosyst. 2014;10(5):1014-22.

4. Zongwei L, Xiuyan S. Control Effect of Spleen Polypeptide Combined with Buzhong Yiqi Pill on Acute Exacerbation of Chronic Obstructive Pulmonary Disease. Chin Arch Tradit Chin Med. 2019;37(12):3034-7.

5. Sheng W, Haiyong Y, Chen Y, Hongyan J, Chundong Z, Zuo R, et al. TCM Syndrome Distribution in 302 Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease. J Beijing University Tradit Chin Med. 2015;38(1):57-62.

6. Huiyan W, Duanming H, Yanqiong J. Analysis of the Correlation between FEV1% Value and Inflammatory Factors, Dysbacteriosis in Patients with Stable Chronic Obstructive Pulmonary Disease. J Pract Med. 2019;35(12):1939-43.

7. Jing W, Shuyang Z, Jiechen Z, et al. Assessment of Early Readmission for the Serum Hypersensitive C-reactive Protein/Albumin Ratio in Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease Combined with Respiratory Failure. Sci Tech Eng. 2019;19(17):128-32.

8. Haiying X, Hongwei Z. Overview of Research on TCM Constitution in Chronic Obstructive Pulmonary Disease. J Southwest Med University. 2017;40(6):608-9.

9. Liu CX, Liu R, Fan HR, Xiao XF, Chen XP, Xu HY, et. Network pharmacology bridges traditional application and modern development of traditional Chinese medicine. Chin Herb Med. 2015;7(1):3-17.

10. Fang Z, Lu BX, Liu MY, Zhang M, Yi Z, Wen C, et al. Evaluating the pharmacological mechanism of chinese medicine Si-Wu-Tang through multi-level data integration. PLoS One. 2013;8(11):e72334.

11. Maogang C. Efficacy of Shenling Baizhu San on Chronic Obstructive Pulmonary Disease in Stable Period of the Feipi Qixu Type [J]. Clin J Chin Med. 2018;10(23):34-5.

12. Guoyu M, Zhen L, Jialiang Z. Clinical Observations of Pingchuan Guben Decoction and Bufei Decoction in Treating Patient with Lung and Kidney Qi Deficiency Syndrome of COPD. Guangming J Chin Med. 2019;34(10):1536-7.

13. Chronic Obstructive Pulmonary Disease Group, Chinese Thoracic Society. Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease (Revised in 2013). Chin J Tuberculosis Respiratory Dis. 2013;36(4):255-64.

14. Chronic Obstructive Pulmonary Disease Group, Respiratory Branch of Chinese Geriatrics Society. Clinical Practice Guideline for Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease in Elderly Patient. Chinese J Tuberculosis Respiratory Diseases. 2020;9(2):100-19.

15. Jihong H. Traditional Chinese Medicine Intervention and Treatment of COPD Stable Period. Practical Clinical Journal of Integrated Traditional Chinese and Western Medicine. 2014;14(1):14-5.

16. Youyu Z. Guidelines for Clinical Research of New Traditional Chinese Medicines (trial). Beijing: China Medical Science Press; 2002.p.243.

17. Jinping Z. Interpretation of the 2019 Global Initiative for Chronic Obstructive Pulmonary Disease (Drug Therapy). Chinese Journal of Tuberculosis and Respiratory Diseases. 2019;42(2):157-60.

18. Yujia W, Yaqian L, Qiaomin W, et al. Pathogenesis and Treatment of Senile Chronic Obstructive Pulmonary Disease Based on Oxidative Stress Mechanism in Traditional Chinese and Western Medicine. Beijing J Traditional Chinese Med. 2020;39(4):354-7.

19. Ping C, Guanglan X, Qian W. Effect of Modified Buzhong Yiqi Decoction on the Quality of Life in Patients with Stable Stage of Chronic Obstructive Pulmonary Disease. Liaoning J Traditional Chinese Med. 2012;39(9):1812-3.

20. Huilian X, Dandan H, Chunfeng M, et al. Effect of Buzhong Yiqi Decoction on Nutrition Dtatus of Patients with Chronic Obstructive Pulmonary Diseases. Chinese J Gen Pract. 2015;13(3):466-8.

21. Ping W, Zhibin C, Chun'e W, DaZhi L, Qiang C. Protective Function and Mechanism of Quercetin in Chronic Obstructive Pulmonary Disease. Chin J Immunol. 2019;35(21):2570-5.

22. Luzhi Y. Pharmacodynamics and Clinical Research Progress on Anti-inflammatory Effect of Licorice. Guangming J Chin Med. 2017;32(19):2895-8.

23. Cong L, Qiang H, Yanxiang Z, et al. Research Progress on the Pharmacological Activity of Quercetin. Hubei J Tradit Chin Med. 2018;40(6):63-6.

24. Shaojun H, Rumei F. Mechanism of Peitu Shengjin Decoction for Airway Remodeling in Patients with Chronic Obstructive Pulmonary Disease at Stationary Phase. Chin J Exp TraditMed Formulae. 2018;24(1):174-9.

25. Xi L, Lihong Z, Xiaoxiao W, et al. Chemical Composition and Pharmacological Research Progress of Angelica Sinensis. J Chin Med Materials. 2013;36(6):1023-8.

26. Ying Y, Mengxin W, Yaoye W, et al. Research Progress on Extraction Separation, Chemical Constitution and Pharmacological Activities of Polysaccharide Extracted from Atractylodes Macrocephala. Chin Herbal Med. 2021;52(2):578-84.

27. Shanshan M, Fangfang L, Xiaoguang H, et al. Inhibition of Naringenin on Airway Mucous Hypersecretion Induced by A2 Strain of RSV. J Wenzhou Med University. 2015;45(09):631-5.

28. Yubin L, Hui W, Xiaofeng T, et al. Evaluation of Effect of Buzhong Yiqi Tang on Rats of Qi-deficiency with Fever by Grey Relational Clustering Method. J Guangdong Pharma University. 2012;28(04):450.

29. Zizhen T, Wenfeng W, Yiran W, et al. Application of the Idea of "Preventive Treatment" in the Prevention and Treatment of Diabetic Retinopathy Mediated by AGE-RAGE Signaling Pathway. China's Naturopathy. 2021;29(10):11-3555/r.2021.1003.

30. Luan H, Zhang Q, Wang L, Wang CX, Zhang M, Xu X, et al. OM85-BV induced the productions of IL-1 beta, IL-6, and TNF-alpha via TLR4-and TLR2-Mediated ERK1/2/NF-kappa B pathway in RAW264.7 Cells. J Interferon Cytokine Res. 2014;34(7):526-36.

31. Govindaraju V, Michoud MC, Ferraro P, Arkinson J, Safka K, Valderrama-Carvajal H, et al. The effects of interleukin-8 on airway smooth muscle contraction in cystic fibrosis. Respir Res. 2008;9(1):76.