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Research on the mechanism of berberine in the treatment of COVID-19 pneumonia pulmonary fibrosis using network pharmacology and molecular docking

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A B S T R A C T
Purpose Pulmonary fibrosis caused by COVID-19 pneumonia is a serious complication of COVID-19 infection, there is a lack of effective treatment methods clinically. This article explored the mechanism of action of berberine in the treatment of COVID-19 (Corona Virus Disease 2019, COVID-19) pneumonia pulmonary fibrosis with the help of the network pharmacology and molecular docking.

Methods We predicted the role of berberine protein targets with the Pharmmapper database and the 3D structure of berberine in the Pubchem database. And GeneCards database was used in order to search disease target genes and screen common target genes. Then we used STRING web to construct PPI interaction network of common target protein. The common target genes were analyzed by GO and KEGG by DAVID database. The disease-core target gene-drug network was established and molecular docking was used for prediction. We also analyzed the binding free energy and simulates molecular dynamics of complexes.

Results Berberine had 250 gene targets, COVID-19 pneumonia pulmonary fibrosis had 191 gene targets, the intersection of which was 23 in common gene targets. Molecular docking showed that berberine was associated with CC2, IL-6, STAT3 and TNF-α. GO and KEGG analysis reveals that berberine mainly plays a vital role by the signaling pathways of inflammation, inflammation and immune response.

Conclusion Berberine acts on TNF-α, STAT3, IL-6, CCL2 and other targets to inhibit inflammation and the activation of fibrocytes to achieve the purpose of treating COVID-19 pneumonia pulmonary fibrosis.

Introduction
Since the end of 2019, the epidemic of COVID-19 has brought serious harm to the world, being the cause of pneumonia and subsequent pulmonary fibrosis that is characterized by lung scarring (tissues scar and thicken over time), making it harder to breathe. A meta-analysis containing 50,466 embodiment COVID-19 inpatients showed that 14.8% of COVID-19 patients with acute respiratory distress syndrome (ARDS) result in epithelial and endothelial injury, proliferation of fibroblasts out of control, eventually leading to pulmonary fibrosis (Crisan-Dabija et al., 2020; Sun et al., 2020; Tale et al., 2020). After normal alveolar tissue was damaged, then interstitial secrete collagen repair, if repair in excess serve fibroblast hyperproliferation and large accumulation of the extracellular matrix, it will lead to structural abnormalities formed into a scar. The pathogenesis of most patients with pulmonary fibrosis is still unclear. The underlying mechanism of COVID-19 pneumonia pulmonary fibrosis may be that lung macrophages are polarized into M1 macrophages and secrete interleukin 12 (IL-12) under the induction of γ-interferon secreted by Th1 and tumor necrosis factor TNF-α and induced nitric oxide synthase (NOS), TNF-α, IL-6 and other.

Abbreviations: ARDS, acute respiratory distress syndrome; BP, biological process; CC, cellular component; CCL2, chemokine ligand2; COVID-19, corona virus disease 2019; ECM, extracellular matrix; EMT, epithelial-mesenchymal cell transformation; FOXM1, forkhead box M1; Fsp1, fibroblast-specific protein 1; GO, gene ontology; HIF-1, hypoxia inducible factor; IBD, inflammatory bowel disease; IL-12, interleukin 12; IL-6, interleukin 6; JAK, Janus kinase; KEGG, Kyoto encyclopedia of genes and genomes; LR-MSCs, mesenchymal stem cells; MF, molecular function; MMP14, matrix metalloproteinase 14; MMP7, matrix metalloproteinase 7; NF-κB, nuclear transcription factor; NOS, nitric oxide synthase; OTUB1, deubiquitinase; PAI-1, plasminogen activator inhibitor 1; PPI, protein-protein interaction; sIL-6R, interleukin 6 receptor; STAT3, transcription activator; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; α-SMA, α-smooth muscle actin.

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pro-inflammatory active substance, to further promote fibroblasts prolif-erate and secrete collagen, increased pulmonary fibrosis (Shamaei et al., 2018b). However, the mechanism of COVID-19 pneumonia pul-monary fibrosis is still unclear, and there is a lack of effective drugs that can treat symptoms in the clinic.

Berberine is a low toxicity natural isoquinoline alkaloid. In the Novel coronavirus infection pneumonia treatment program (Trial Eighth Edition) (Xie, 2020), many traditional Chinese medicine compounds such as Huanglian Jiedu Decoction (HLJDD) contain Coptis chinensis rhizome, of which the main active ingredient is berberine (Yin et al., 2020). Berberine shows a great biochemical and pharmacological activity in clinic, having a hypolipidemic (Wang et al, 2018), anti-inflammatory (Najaran et al., 2019), anti-cancer (Liu et al., 2019) anti-bacterial (Peng et al., 2015) and antiviral and other characteristics. Berberine is able to penetrate most of the cells (Shamaei et al., 2018a). At the same time, berberine has strong antiviral activity against different viruses. The antiviral impact of berberine to herpes virus, influenza virus and respiratory syncytial virus has been proven scientifically. Studies have indicated that, in the model of pulmonary fibrosis berberine inhibition of rapamycin target protein mTOR activated to enhance cell autophagy and inhibits fibrosis (Chitra et al., 2015). But the suppressive mechanism of berberine for COVID-19 pneumonia pulmonary fibrosis still remains unclear.

In this study, with bioinformatics analysis, the core target genes between drugs and diseases were obtained, and GO, KEGG and PPI were used to analyze the associations between target genes to explore their mechanism of action and potential pathways. It provides a theoretical basis for the mechanism of berberine in the treatment of COVID-19 pneumonia pulmonary fibrosis. The workflow for studying the effect of berberine on COVID-19 pneumonia pulmonary fibrosis based on network pharmacology is shown in Workflow Abstract.

Material and methods

Predicting berberine gene targets and COVID-19 pneumonia pulmonary fibrosis gene targets

We used Pubchem (https://pubchem.ncbi.nlm.nih.gov/) database to obtain berberine base 3D structure, and utilized Pharmmapper (http://www.lilab-ecust.cn/pharmmapper/index.html) and SwissTarget (http://www.swisstargetprediction.ch/) database to predict the protein targets of berberine. Lastly, converted prediction protein targets into prediction gene targets by UniProt (https://www.uniprot.org/). Through the GeneCards (https://www.genecards.org/v3/), DisGenET (https://www.disgenet.org/) database, "COVID-19 pneumonia", "COVID-19" and "SARS-CoV-2" as the keyword and "pulmonary fibrosis" as the keyword to get the disease gene targets. The disease gene targets and the drug prediction gene targets were combined through the venny website (https://bioinfogp.cnb.csic.es/tools/venny/index.html) to obtain common gene targets.

Berberine treatment of COVID-19 pneumonia pulmonary fibrosis target protein interactions (Protein-protein interaction, PPI) network building

We logged STRING database (http://string-db.org/), input common gene targets, set for the species “human (Homo sapiens)”, select the highest confidence level (0.9, remaining parameters remain the default), obtain protein targets interaction data, construct a PPI network, and screen for core target genes.

Enrichment analysis

The common target genes were used in DAVID database (https://david.ncifcrf.gov/) for gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, obtain the molecular function (MF), cellular component (CC) and related biological process (BP) of the target protein or gene through GO enrichment, and carry out various aspects of the function of the target gene description and qualification. Through the KEGG pathway enrichment of the signal pathways involved in the target, the target gene screening was performed under the condition of $p < 0.05$, and the main signal pathway and biological process of berberine on COVID-19 pneumonia pulmonary fibrosis were analyzed. The Omicshare Tools platform (https://www.omicshare.com/tools/Home/Soft/roc) was used to visualize the results of GO enrichment and KEGG enrichment.

Network diagram of "Disease-core target gene-drug"

Cytoscape 3.7.1 network map software was used to construct a disease-core target gene-drug network and conduct topological analysis. The core target genes can be screened based on the node degree value greater than 2 times the median.

Component target molecular docking and validation of the docking protocol

We used the PDB database (http://www.rcsb.org) to obtain 3D structures of large molecules and 3D structures of small molecules (http://zincdocking.org/) and used AutoDockTools-1.5.6 to pre-process the molecule with water removal, hydrogenation and ligand setting, and then perform molecular docking. PyMOL displayed the 3D structure, protein binding bonds and residues of small molecules and proteins. We used the original crystal ligand of the target protein as a positive reference, and analyzed and compared the binding posture of the original crystal ligand and protein, the chemical bond length and the chemical bond angle by re-docking the original crystal ligand and protein. Finally, the consistency of the binding mode can indicate the correctness of the molecular docking protocol.

Molecule dynamics

All-atom molecular dynamics simulations were performed based on the small molecule complexes obtained by docking as initial structures, and the simulations were performed using AMBER 18 software (Lee et al., 2020a). The charge of the small molecule was calculated in advance by the antechamber module and the Hartree–Fock (HF) SCF/6-31G* of the gaussian 09 software before the simulation. Afterwards, small molecules and proteins were described using the GAFF2 small molecule force field and the ff14SB protein force field, respectively (Wang et al., 2004). Each system used the LeaP module to add hydrogen atoms to the system, added a truncated octahedral TIP3P solvent box at a distance of 10 Å, and added Na+/Cl− to the system to balance the system charge (Harrach, 2014). Finally, the simulated topology and parameter files were exported.

Molecular dynamics simulations were performed using AMBER 18 software (Lee et al., 2020b). Before the simulation, the system was energy optimized, including 2500-step steepest descent method and 2500-step conjugate gradient method. After the energy optimization of the system was completed, the system was heated for 200 ps under a fixed volume and a constant heating rate, so that the temperature of the system slowly increased from 0 to 298.15 K. Under the condition that the system maintains a temperature of 298.15 K, a 500 ps NVT (isothermal system maintains a temperature of 298.15 K, a 500 ps NVT (isothermal...
and (isovolumetric) phylogenetic simulation was performed to further uniformly distribute the solvent molecules in the solvent box. Finally, in the case of NPT (isothermal and isobaric), a 500 ps equilibrium simulation of the entire system is performed. 50 ns NPT phylogenetic simulations were performed for the two composite systems under periodic boundary conditions, respectively. In the simulation, the non-bond cutoff distance was set to 10 Å, the Particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions, the SHAKE method was used to limit the length of hydrogen bonds, and the Langevin algorithm was used for temperature control, the collision frequency $\gamma$ is set to 2 ps$^{-1}$, and the system pressure is set to 1 atm (Larini et al., 2007).

**MMGBSA binding free energy calculation**

The binding free energies between proteins and ligands for all systems were calculated by the MM/GBSA method (Chen et al., 2020; Rastelli, et al., 2010). In this study, the molecule dynamics trajectory of 45-50 ns was used for calculation, and the specific formula is as follows:

$$\Delta G_{\text{bind}} = \Delta G_{\text{complex}} = \Delta G_{\text{receptor}} + \Delta G_{\text{ligand}}$$

$$= \Delta E_{\text{internal}} + \Delta E_{\text{VDW}} + \Delta E_{\text{elec}} + \Delta G_{\text{GB}} + \Delta G_{\text{SA}}$$

In the formula, the internal energy is expressed, the van der Waals interaction and the electrostatic interaction are expressed. The internal

**Fig. 1.** Common targets and common targets-active ingredient networks. Targets of the intersection of berberine and COVID-19 pneumonia pulmonary fibrosis.

**Fig. 2.** Protein-protein interaction (PPI) network. (A) PPI network of target protein, (B) PPI network of core target protein (confidence > 0.9).
energy includes bond energy ($E_{bond}$), angular energy ($E_{angle}$), and torsional energy ($E_{torsion}$); $\Delta G_{GB}$ and $\Delta G_{GA}$ are called the solvation free energy, where $G_{GB}$ is the polar solvation free energy and $G_{SA}$ is the non-polar solvation free energy. For this paper, the GB model developed by Nguyen was used for calculation ($\iota_{gb} = 2$) (Nguyen et al., 2013). The non-polar solvation free energy ($G_{SA}$) was calculated based on the product of surface tension ($\gamma$) and solvent accessible surface area (SA), $G_{SA} = 0.0072 \times SA_{15}$. The entropy change is ignored in this study due to high computational resource consumption and low precision.
**Results**

*Obtained common gene targets by intersection*

We obtained 250 berberine target genes and 191 COVID-19 pneumonia pulmonary fibrosis target genes. A total of 23 common gene targets were processed by Venny, shown in Fig. 1.

*Core target screening and PPI network diagram*

Through STRING database analysis of 23 mapping of the intersection genes of COVID-19 pneumonia pulmonary fibrosis, construct the PPI network interaction map of the target protein of berberine in the treatment of COVID-19 pneumonia pulmonary fibrosis, shown in Fig. 2 A. 16 core genes such as CCL2, IL-6, STAT3, TNF-α, TLR4, MTOR, etc. are obtained by setting the interaction score (confidence degree > 0.9), and use the 16 core genes to reconstruct the core gene PPI, shown in Fig. 2 B.

*GO and KEGG enrichment analysis*

The 23 common gene targets were imported into the DAVID database for enrichment analysis. Under the condition of $p < 0.05$, the GO enrichment analysis yielded a total of 186 GO entries, including 146 BP entries, 18 CC entries, and 22 MF entries. According to the number of targets contained, the first 20 BP compressions, the top 10 CC and MF compressions are screened, and the results are visualized using the Omicshare tool platform. The results show that in biological processes, biological regulation is highly correlated with metabolic processes, mainly involving the positive regulation of nitric oxide synthesis, inflammation, positive regulation of gene expression, and positive regulation of protein kinase B signal transduction. Among cell components, extracellular space, extracellular area, cell surface, endoplasmic reticulum cavity, and membrane rafts account for a relatively large amount. In molecular functions, cytokine activity, protease binding, and growth factor activity are relatively high, shown in Fig. 3 A–F. KEGG pathway analysis yielded 53 pathways with $P < 0.05$. According to the number of targets contained, the first 20 pathways were screened, and the results were visualized using the Omicshare Tools platform. The results show that the enriched pathways involve multiple pathways related to immune response and inflammation, mainly influenza A, hypoxia inducible factor (HIF-1), inflammatory bowel disease (IBD) and rheumatoid joints inflammation and other signaling pathways, shown in Fig. 3 G, H.
Molecular docking

The results of screening docking between ligands and receptors are shown in Table 1.

| Ligand | Binding energy (kcal/mol) | Inhibitory constant | Internal energy | Electrostatic energy |
|--------|--------------------------|---------------------|----------------|---------------------|
| CCL2   | -6.31                    | -0.25               | 23.61          | -6.91               | 0.40               |
| STAT3  | -6.15                    | -0.25               | 31.08          | -6.75               | -0.22              |
| FN1    | -6.86                    | -0.27               | 9.37           | -7.46               | 0.33               |
| GDF15  | -6.79                    | -0.27               | 10.62          | -7.38               | -0.09              |
| BSG    | -6.57                    | -0.26               | 15.30          | -7.17               | -1.40              |
| HSAP5  | -5.00                    | -0.20               | 218.00         | -5.59               | -0.27              |
| MTOR   | -6.99                    | -0.28               | 7.56           | -7.58               | -0.20              |
| VEGFA  | -6.72                    | -0.27               | 11.78          | -7.32               | -0.52              |
| INS    | -6.04                    | -0.24               | 37.15          | -6.64               | -0.32              |
| DPP4   | -4.32                    | -0.17               | 678.90         | -4.92               | -0.86              |
| EGFR   | -4.91                    | -0.2                | 251.65         | -5.51               | -0.61              |
| IL-6   | -4.94                    | -0.2                | 239.57         | -5.54               | -0.45              |
| F3     | -5.21                    | -0.21               | 150.58         | -5.81               | -0.77              |
| TNF-α  | -6.24                    | -0.25               | 26.48          | -6.84               | -0.63              |
| TLR4   | -5.75                    | -0.23               | 61.41          | -6.34               | -0.64              |
| F2     | -6.46                    | -0.26               | 18.49          | -7.05               | 0.19               |
| CXCL8  | -5.71                    | -0.23               | 65.79          | -6.30               | 0.08               |
| IL2RA  | -7.54                    | -0.30               | 2.95           | -8.14               | -0.52              |
| NFkB1  | -4.94                    | -0.20               | 238.09         | -5.54               | -0.84              |
| IL1B   | -6.79                    | -0.27               | 10.53          | -7.39               | -0.24              |
| IFNα2  | -5.03                    | -0.2                | 206.14         | -5.62               | 0.24               |
| CD4    | -4.25                    | -0.17               | 762.23         | -4.85               | 0.28               |
| TNFβ   | -5.72                    | -0.23               | 64.53          | -6.31               | -0.26              |

**Hydrogen bond analysis**

The results showed that the number of hydrogen bonds in the STAT3/berberine and TNF-α/berberine systems was more in the early molecular dynamics stage. In the late molecular dynamics period, which is the stable period, TNF-α/berberine formed the most hydrogen bonds. The number of hydrogen bonds formed by CCL2/berberine and STAT3/berberine is less. The results are shown in Fig. 7.

**Discussion**

In this study, the mechanism of action of berberine in the treatment of COVID-19 pneumonia pulmonary fibrosis was investigated using network pharmacology and molecular docking. Firstly, berberine through TNF-α inhibited inflammatory reaction and reduce the activation of inflammatory factors. Secondly, berberine inhibited the synergistic effect between IL-6 and STAT3, and reduced the inflammatory response. Lastly, berberine inhibited the chemotaxis of CCL2 to fibroblasts and reduces inflammation.

Pulmonary fibrosis is a serious complication of end-stage COVID-19 characterized by proliferation and accumulation of fibrotic tissue. The clinical manifestations are dry cough and progressive dyspnea. As the disease and lung injury worsen, the patients’ respiratory function will continue to deteriorate. However, there is still a lack of effective drugs for the treatment of pulmonary fibrosis.

Traditional Chinese medicine has significant impact on COVID-19 treatment and recovery prognosis (Chen et al., 2022). Berberine is a quaternary ammonium alkaloid isolated from the traditional Chinese medicine Coptis Rhizoma. Clinical studies have shown that berberine has anti-inflammatory and enhanced leukocyte phagocytic effects. Although Chitra has shown that berberine attenuates pulmonary fibrosis injury by inhibiting Smad and non-Smad signaling cascades (Chitra et al., 2015), the mechanism by which berberine treats pulmonary fibrosis remains unclear (Li et al., 2019). This study can get rid of experimental techniques limits to realize the research on the mechanism of berberine in pulmonary fibrosis from a macroscopic to a microscopic perspective. Since the effect of berberine on pulmonary fibrosis depends on the network of signaling pathways, this study can more comprehensively study the relationship between different signaling factors, and provide multiple research directions for follow-up research. Chowdhury (2021) used tools of molecular docking to find that berberine can inhibit the function of the 3CLpro protein, thereby controlling viral replication. Compared with the traditional Computer Simulation Analysis, this study achieves from gene expression to protein regulation to molecular interaction. Experimental results verifies that berberine can act on relevant core targets in regulating pulmonary fibrosis. The use of network pharmacology to construct a PPI network can explain the mechanism of core protein targets in the treatment of COVID-19 pneumonia pulmonary fibrosis with berberine. Since GO enrichment analysis and KEGG enrichment analysis can prove the biological mechanism of key targets in the disease, we carried out GO and KEGG enrichment analysis on the core target genes in the PPI network. The main signaling...
pathways include influenza A, signaling pathways such as hypoxia-inducible factor-1 (HIF-1), inflammatory bowel disease (IBD) and rheumatoid arthritis. This suggests that berberine may exert its therapeutic effects by modulating immune responses and inflammatory activation processes.

Potential target of berberine against of COVID-19 pneumonia pulmonary fibrosis

Berberine inhibits COVID-19 pneumonia pulmonary fibrosis by acting on tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), activator

Fig. 5. Molecular docking of active ingredients and hub targets. TNF-α, (B) IL-6, (C) STAT3, (D) CCL2.
of transcription (STAT3) and chemokine ligand 2 (CCL2).

TNF-α causes pulmonary fibrosis by promoting the activation of fibroblasts. TNF-α is a monokine, mainly produced by monocytes and macrophages, and it plays an important role in promoting cell proliferation and differentiation and regulating the immune system. Analysis of protein interaction network PPI suggested that TNF-α was closely related to inflammatory targets such as IL-6. TNF-α is a monokine, mainly produced by monocytes and macrophages, and it plays an important role in promoting cell proliferation and differentiation and regulating the immune system. Analysis of protein interaction network PPI suggested that TNF-α was closely related to inflammatory targets such as IL-6.

KEGG pathway analysis found that TNF-α played a role in influenza and other pathways. Molecular docking showed that the binding energy of TNF-α and berberine reached -6.24. Molecular dynamics simulation found that the binding of berberine to TNF-α was very stable, and the movement process of small molecule berberine and TNF-α entered a stable state 10 ns before the simulation. The binding energy of berberine to TNF-α reached -17.88 ± 1.35 kcal/mol, which suggested that their binding strength was the highest, and TNF-α/berberine formed the most hydrogen bonds during the molecular dynamics simulation. Therefore, berberine and TNF can bind tightly and remain stable.

Inflammatory responses mediated by IL-6 and STAT3 can activate fibroblasts and lead to pulmonary fibrosis. IL-6 is an important member of the interleukin family, which plays an anti-infective immune role by regulating the growth and differentiation of a variety of cells. The transcriptional activator STAT3 is a protein that can bind to DNA. It can regulate gene transcription and expression by entering the nucleus and binding to specific sites in the promoter sequence of target genes. Among PPIs, IL-6, STAT3, and NF-kB are highly correlated, and together they play important roles in the inflammatory response. KEGG pathway analysis showed that IL-6 and STAT3 exist in hypoxia-inducible factor (HIF-1) and inflammatory bowel disease (IBD) and other pathways. Molecular docking showed that the binding energies of IL-6 and STAT3 to berberine were -4.94 and -6.15, respectively. Molecular dynamics results suggested that the stability of berberine to STAT3 and IL-6 were relatively low, which may be because their protein system greatly affects the binding of berberine. The binding free energies of berberine to IL-6 and STAT3 were -15.30 ± 0.41 kcal/mol and -12.54 ± 0.89 kcal/mol, the affinity between them is high. The number of hydrogen bonds of STAT3/berberine in the early stage of molecular dynamics simulation is relatively large, and the number of hydrogen bonds of IL-6 remains relatively stable during the process of molecular dynamics simulation.

As a pro-inflammatory chemokine, CCL2 can promote chemotaxis and inflammatory responses of fibroblasts, increasing the risk of pulmonary fibrosis. PPI results suggest that CCL2 is closely related to IL-6 and CXCL8. KEGG pathway analysis found that CCL2 played a key role in rheumatoid arthritis, influenza A and other pathways. Molecular docking showed that the binding energy of CCL2/berberine in the early stage of molecular dynamics simulation is relatively large, and the number of hydrogen bonds of IL-6 remains relatively stable during the process of molecular dynamics simulation.

![Fig. 6. Variation of complex root mean square deviation (RMSD) difference over time during molecular dynamics simulations.](image-url)
protein itself. Their binding free energy was $-13.78 \pm 0.88$ kcal/mol, and the number of hydrogen bonds formed by the CCL2/berberine system is also less. Berberine can inhibit the circulation of CCL2 chemotactic fibroblasts to the alveolar interstitium, further reducing the risk of pulmonary fibrosis.

A summary of the mechanisms of action of berberine in the treatment of COVID-19 pneumonia pulmonary fibrosis is shown in Graphical Abstract.

**Fig. 7.** Changes in the number of hydrogen bonds between ligands and protein receptors in each complex system during molecule dynamics.

**Flowchart abstract.** Flowchart of exploring the mechanism of berberine for COVID-19 pulmonary fibrosis. The targets of COVID-19 for pulmonary fibrosis and berberine were obtained from multiple databases. The drug active ingredient target and disease target were intersected to obtain a common target. The common target protein interaction PPI network was constructed, and the common target genes were analyzed by GO and KEGG. To explore the mechanism of berberine in the treatment of COVID-19 pulmonary.
The mechanism of action of berberine against COVID-19 pneumonia pulmonary fibrosis

Berberine inhibits fibroblast activation

TNF-α is an important pro-inflammatory factor and also plays an important role in the inflammatory response of pulmonary fibrosis (Lundblad et al., 2005; Piquet et al., 1990; Piquet et al., 1993). Firstly, berberine inhibits the activation of nuclear transcription factor (NF-κB) by acting on TNF-α, reduces the production of fibrotic cytokines, and inhibits the differentiation of mesenchymal stem cells (LR-MSCs) into myofibroblasts, thereby reduces type I collagen and alpha-smooth muscle actin (α-SMA) in myofibroblasts (Wang et al., 2017; Pandey et al., 2008; Hou et al., 2018). Second, berberine can modulate Wnt/β-catenin by acting on TNF-α, preventing β-catenin from entering the nucleus and binding to matrix metalloproteinase 7 (MMP7) and matrix metalloproteinase 14 (MMP14), reducing fibronectin. Berberine can also affect fibrocyte-specific protein 1 (Fsp1) and plasminogen activator expression inhibitor 1 (PAI-1) and other fibrosis-related genes, directly or indirectly inhibiting the formation of pulmonary fibrosis (Zhang et al., 2021; Blavier et al., 2006; Valenta et al., 2012; Tan et al., 2014). Thirdly, berberine inhibits COVID-19 pneumonia pulmonary fibrosis by preventing TNF-α from acting on transforming growth factor-β (TGF-β). TNF-α increases the expression of TGF-β and type I and II TGF-β receptors, and stimulates downstream Smad3 phosphorylation (Voloshenyuk et al., 2011). On the contrary, berberine can also inhibit Smad2/3 to reduce the fibrotic effects of TGF-β and TNF-α (Tew et al., 2020). Therefore, TNF-α induces lung fibroblasts to differentiate into myofibroblasts, reduces extracellular matrix (ECM) accumulation and epithelial-mesenchymal cell transformation (EMT) in the lung (Zhao et al., 2016; Kabel et al., 2016; Song et al., 2013). Interestingly, another study found that berberine can also promote HGF entry into the circulation system through intestinal production of PPAR-γ. HGF further promotes epithelial and endothelial cell survival, and quiescence of fibroblasts, thereby reduces the extracellular matrix to prevent fibrosis (Guan et al., 2018). Therefore, berberine inhibits fibroblast activation in the treatment of COVID-19 pneumonia pulmonary fibrosis.

Berberine inhibits inflammatory response to COVID-19

In the study of pulmonary fibrosis, IL-6 and STAT3 mediated inflammation that can activate fibroblasts and lead to fibrosis (O’Donoghue et al., 2012; Saito et al., 2008; Kobayashi et al., 2015; O’Reilly et al., 2014; Barnes et al., 2011). There are two mechanisms for berberine to inhibit the activation of fibroblasts by inhibiting IL-6-mediated phosphorylation of STAT3. Firstly, the IL-6/JAK2/STAT3 signaling pathway activates the downstream related Janus kinase (JAK) through the binding of IL-6 to the interleukin 6 receptor (IL-6R) on inflammatory cells, activates tyrosine kinase, then phosphorylates STAT3. Phosphorylated STAT3 activates NF-κB and up-regulates the expression of inflammatory cytokines such as IL-6 and TNF-α, thereby enhancing inflammation, cell damage and fibrosis. In addition, berberine can also inhibit the secretion of IL-6, iNOS and TNF-α through the NF-κB pathway and reduce inflammation (Wang et al., 2020; Chitra et al., 2013). Secondly, phosphorylated STAT3 can also regulate fibrosis by promoting the expression of deubiquitinase (OTUB1) and Forkhead box M1 (FOXM1) to activate TGF-β signaling (Bisserier et al., 2020; Evans et al., 2002). Studies have shown that by targeting the upstream components of the JAK/STAT signaling pathway, the expression of fibrotic genes induced by TGF-β can be reduced (Pedroza et al., 2016; Milara et al., 2018; Sallam et al., 2018). Therefore, we believe that IL-6/STAT3-mediated inflammation is closely related to COVID-19 pneumonia pulmonary fibrosis.

Berberine inhibits chemotaxis of fibroblasts

In COVID-19 pneumonia, berberine inhibits the chemotaxis of fibroblasts and reduces cytokine storms by reducing the expression of cell CCL2. Studies showed that alveolar epithelial cells in pulmonary fibrosis will express a large amount of chemokine CCL2 (Mercer et al., 2009), CCL2 with the high affinity to the chemokine receptor CCR2 which is highly expressed in fibroblasts. As a chemokine, CCL2 can promote the chemotaxis, induction and survival of fibroblasts (Wang et al., 2021). Therefore, fibroblasts are tended from the peripheral circulation to the alveolar interstitium, which increases the risk of pulmonary fibrosis (Ekert et al., 2011; Wolters et al., 2014b; Murray et al., 2010). Studies have also shown that by inhibiting or attenuating CCL2, pulmonary fibrosis can be inhibited (Moore et al., 2005; Moore et al., 2006; Murray et al., 2008). In addition, CCR2 can induce fibroblasts to proliferate and differentiate into myofibroblasts, and the survival of fibroblasts also depends on the expression of IL-6 induced by CCL2, on the other hand, IL-6 enhances the inflammatory response and further aggravates pulmonary fibrosis (Wolters et al., 2014a; Liu et al., 2007).

Conclusion

This study uses of network pharmacology to explain the potential molecular mechanism of berberine in the treatment of pulmonary fibrosis through multiple targets and multiple pathways, and analyzes the molecular dynamics and binding free energy of the complex through integrated molecular docking. The experimental results show that berberine can regulate cell proliferation, metabolism, mainly through tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), activator of transcription (STAT3) and chemokine ligand 2 (CCL2) and survival, thereby exerting a therapeutic effect on COVID-19 pneumonia pulmonary fibrosis. These results suggest the potential of berberine to become a clinical drug for COVID-19 pneumonia pulmonary fibrosis. The pathogenesis of COVID-19 pneumonia pulmonary fibrosis is very complex, and there is a dose-response relationship between drugs and disease, which is difficult to quantify with current network pharmacology techniques. Due to the research based on network pharmacology is still in the stage of static network analysis, body function is a continuous and dynamic process; the process of disease occurrence, drug development and curative effect is also dynamic. It is hoped that future research can further analyze the pharmacological effects of berberine in the treatment of pulmonary fibrosis, as well as the targets and pathways of active ingredients for further verification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyu.2022.100252.

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