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An integrative analysis of Qingfei Paidu Decoction for its anti-HCoV-229E mechanism in cold and damp environment based on the pharmacokinetics, metabolomics and molecular docking technology

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\textbf{ABSTRACT}

\textbf{Background:} The novel coronavirus pneumonia (COVID-19) has spread rapidly around the world. As a member against the epidemic, Qingfei Paidu Decoction (QFPDD) has been approved for the treatment of COVID-19 in China. However, its antiviral mechanism was still largely unclear.

\textbf{Purpose:} An integrated strategy was used to explore the antiviral mechanisms of QFPDD in cold and damp environment, including pharmacokinetic (PK), network pharmacology, metabolomics and protein verification.

\textbf{Methods:} Firstly, the pharmacokinetic study of the prototype absorbed ingredients were analyzed by UHPLC-QqQ-MS. Secondly, the metabolomics analysis of the endogenous constituents was carried out. Based on the aforementioned results, an integrated network was constructed to identify the curative components, crucial endogenous differential metabolites and related pathways. Finally, the validation tests were implemented by molecular docking and western blotting (WB).

\textbf{Results:} According to the pharmacokinetic behaviors analysis of 31 components in vivo, the flavonoids presented more longer residence time and higher exposure compared with the other compounds. The efficacy and antiviral mechanism of QFPDD were verified by the poly-pharmacology, metabolomics, molecular docking and WB. For the occurrence of metabolic disorder, the change of amino acid transporters should not be neglected. Afterward, 8 curative compounds, 6 key genes and corresponding metabolic pathways were filtered by compound-reaction-enzyme-gene network. The molecular docking verified that the active ingredients bound to the relevant targets well.

\textbf{Conclusion:} In the present study, an in vivo comprehensive pharmacokinetic behaviors of QFPDD was analyzed for the first time. The results illustrated that QFPDD could exhibit immune regulation, anti-infection, anti-inflammation and metabolic disorder to perform a corresponding therapeutic effect. Moreover, our findings highlighted the roles of amino acid transporters in the coronavirus infection situation.

\textbf{Abbreviations:} ACHE, Acetylcholinesterase; ALOX5, Polyunsaturated fatty acid 5-lipoxygenase; CE, Collision energy; COMT, Catechol O-methyltransferase; CRS, Cytokine storm; COVID-19, The novel coronavirus pneumonia; DP, Declustering potential; DNMT, DNA (cytosine-5)-methyltransferase; GO, Gene ontology; GM-CSF, Granulocyte-macrophage colony stimulating factor; KEGG, Kyoto Encyclopedia of Genes and Genomes; KP, Kynurenine pathway; LC-MS, Liquid chromatograph mass spectrometer; MCP-1, Monocyte chemotactic protein-1; OPLS-DA, Orthogonal partial least squares discrimination analysis; PVDF, Polyvinylidene Fluoride; PDE4D, cAMP-specific 3',5'-cyclic phosphodiesterase 4D; PK, Pharmacokinetic; PTGS1, Prostaglandin G/H synthase 1; PCA, Principal component analysis; QFPDD, Qingfei Paidu Decoction; TCA, Tricarboxylic acid cycle; TNF-\textalpha, Tumor necrosis factor; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; VEGF, Vascular endothelial growth factor.

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Introduction

COVID-19 has caused millions of deaths because of the characteristics of strong pathogenicity and rapid transmission (Luo et al., 2020). QFPDD, a recommended prescription for the treatment of COVID-19 in China, which is principally derived from four classic prescriptions in the Shang-Han Lun. And the Qingfei Paidu Granule was officially listed in 2021. According to the clinical reports, QFPDD could significantly relieve the clinical symptoms and reduce the adverse reactions (Zhao et al., 2021). The chemical composition and the in vivo process of QFPDD has been reported preliminary (Liu et al., 2021a). However, only a few components were included in the analysis because of the absence of references. To ensure its efficacy and application safety, the therapeutic mechanism and the process of QFPDD in vivo should be further discussed.

The patients with COVID-19 presented characteristic changes in clinic, involving amino acids, organic acids, nucleotides, glycerides, phospholipids and fatty acids (Xu et al., 2021; Wu et al., 2020). Compared with the healthy control group, the tricarboxylic acid cycle (TCA) and glycolysis pathway were all significantly down-regulated in mild and severe patients (Thomas et al., 2020). Additionally, tryptophan, one of the essential amino acids, was classically associated with the kynurenine pathway (KP). The circulating kynurenine were significantly increased in SARS-CoV-2 infection, in correlation with levels of severity (Anderson et al., 2021). As reported, the supplement of the metabolites as arginine, tryptophan metabolism inhibitor or purine metabolism related inhibitor mycophenolic acid could effectively regulate the release of pro-inflammatory cytokines from PBMC induced by COVID-19 (Xiao et al., 2021). The majority of the cytokines were related to “cytokines storm” (CRS).

The clinical manifestations of COVID-19 are mostly superficial symptoms such as fever, dry cough, muscle pain, fatigue and so forth. In order to coordinate with the traditional Chinese medicine (TCM) treatment of COVID-19 and provide laboratory data support, the mouse model was constructed by HCoV-229E in cold and dampness environment, which has been awarded the B grade animal model by Chinese Association for Laboratory Animal Sciences.

Hence, a more comprehensive dynamic process in vivo of QFPDD needed to be discussed. Then, based on the viral model in cold and damp environment, a compound-reaction-enzyme-gene network would be constructed to build the interactions among the differential endogenous metabolites, components and the relevant metabolic pathway. The target verification would be performed by the WB and molecular docking.

Material and methods

Reagents and chemical

Herbal medicines in QFPDD were all purchased from Anguo Medicine Market (Hebei, China). The medicinal materials were identified by professor Zhao Haiyu of China Academy of Chinese Medical Sciences. The chemical reference substances for PK behaviors analysis were obtained from Beijing Salibaicao Technology Co., Ltd. (Beijing, China). The amino acids reference substances were bought from Bailingwei Technology Co., Ltd. (Beijing, China). The TCA cycle reference substances were offered by Zhongkehuaxian Technology Co., Ltd. (Beijing, China). The KP reference substances were supplied via Yuanye Biotechnology Co., Ltd (Shanghai, China). The primary antibodies used in western blot analysis were as follows: xct, asct2 (Cell signaling technology, USA). The QFPDD lyophilized powder was made and stored in the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (Beijing, China).

Animals and group

The authorization of all animal operations was granted by Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (2020D025, September 10, 2020). And all animals were in a normally controlled breeding room (temperature: 25±2 °C, humidity: 60±5%, 12 h dark/7 light cycle) with standard laboratory food and water prior to the experiments.

Sprague Dawley rats (220 to 250 g) were normally raised by the Experimental Center of China Academy of Traditional Chinese Medical Sciences (Beijing, China). After the adaptation period for 7 days before the experiment, they were stochastically classified and fasted for 12 h before administration with drugs, but had free access to water. Rats (n = 24) were randomly divided into high and low dose groups, which were administered with QFPDD (3.34 g lyophilized powder/kg and 10 g lyophilized powder/kg were dissolved in saline, separately.). At each time point of 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 h after administration, a 0.3 ml blood sample of each rat was collected into a heparinized EP tube.

BALB/c mice (10 to 15 g) for metabolomic research were randomized into control, model, positive drug group (chloroquine), high-dose and low-dose groups, which were normally raised by the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. Except for the control group, the other mice were placed in an artificial climate chamber with 90±3% relative humidity, temperature of 4±2 °C and removed after stimulation for 4 h each time for 7 consecutive days. Then, the other mice were slightly anesthetized with ethyl ether on the 5th day of cold and dampness stimulation and infected with 100 TCD50 HCoV-229E intranasally, 50 µl per mouse. Meanwhile, on the fifth day, the treatment groups were given the QFPDD by oral administration, with volume of 0.2 ml/10 g body weight. After weighing on the 4th day of infection, two classes bio-samples were collected, including the blood and the lung tissues.

PK behaviors analysis

100 µl plasma sample was added with 1% ascorbic acid 10 µl, 300 µl methanol, including IS (Internal standard) (cinobufagin in positive mode, gliburnide in negative mode), vortexed for 3 min, ultrasonic extraction for 3 min and centrifuged for 15 min (12000 rpm, 4 °C). Next, the supernatant was evaporated to dryness under 37 °C N2. The residue was redissolved with 100 µl 70% methanol, vortexed (3 min), centrifuged (12000 rpm, 4 °C, 10 min). Then, 3 µl supernatant was taken for the PK behaviors analysis (The detailed chemical reference substances, instrumental conditions and method validation were shown in the supplemental file S1).

DAS 3.2.0 software program (Chinese Pharmacological Society) was applied to calculate PK parameters (including T_{max}, C_{max}, T_{1/2}, Vz/F, CLz/F, AUCo, and AUC(0–ω)).

Network pharmacology analysis

COVID-19 has been identified as a typical viral infection in cold and dampness environment by experts. In order to explore the curative components and potential molecular targets of COVID-19 treated by QFPDD, we conducted network pharmacological analysis. First, the plasmatic prototype compounds were identified. Next, the components targets were predicted on SwissTargetPrediction (http://www.swisstargetprediction.ch/). Then, the disease targets were filtered on TTD (http://db.idrblab.net/TTD/), DrugBank (https://go.drugbank.com/) and Gene Card (https://www.genecards.org/). Only the common targets closely related to COVID-19 were retained by Venny 2.1 (https://bioinfo.cnb.csic.es/tools/venny/). Finally, we performed the Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis by cytoscape (3.8.2, USA).
Therapeutic effect of QFPDD on the mouse model of pneumonia in cold and dampness environment

Pulmonary wet/weight ratio, viral load, HE pathological staining, inflammatory factors and the daily status of the mice was observed to evaluate the efficacy of QFPDD.

Sample preparation: The inflammatory factors, viral load of the lung tissues were detected by Mouse Cytokine Array Q1 kit (QAM-CYT-1-2, Beijing Bioway Biological Technology Co., Ltd., China) and Human Coronavirus (HCoV-229E) Real Time RT-PCR kit (P20191201, Shanghai ZJ Bio-Tech Co., Ltd., China), thus the samples were prepared according to the instructions.

HE staining process: Before tissue staining occurred, the paraffin wax needed to be removed. The tissues were successively put into three new xylens for 15 min, followed by ethanol absolute twice (5 min each time), 95% ethanol (5 min) and 85% ethanol (5 min), separately. Then, the tissues were rinsed with running water. Afterwards, the tissues were processed in proper sequence by hematoxylin stain solution (1-2 min), differentiation solution, ammonia solution, running water, eosin Y solution (2-3 min), ethanol absolute (3 times, 5 min each time) and xylene (5 min). Finally the tissues were sealed by neutral balsam to detect. All reagents were purchased from Wuhan Hundred Thousand Degree Biotechnology Co., LTD (Wuhan, China).

Global non-targeted metabolomic analysis by UHPLC-ESI-Orbitrap-MS preparation of untargeted metabolomics samples

Serum sample preparation: 50 µl serum mixed with 200 µl acetonitrile (containing 0.2% formic acid) was placed in a 0.5 ml centrifuge tube, vortexed (3min), centrifuged (12000 rpm, 4 °C, 15 min), 5 µl supernatant was taken for analysis.

Lung tissue samples preparation: a certain amount of lung tissue was added with 2.5 times saline for homogenization for 3 min. Then, 2.5 times acetonitrile (containing 0.2% formic acid) was added twice, followed by ultrasonic extraction for 10 min and centrifugation for 15 min (12000 rpm, 4 °C). 90 µl supernatant was taken for analysis.

UPLC-ESI-orbitrap MS analysis

UPLC-ESI-Orbitrap MS analyses were performed by an UHPLC system (Ultimate 3000, Thermo Fisher Scientific, USA) with a UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 µm, Waters) coupled to LTQ Orbitrap Velos Pro (Thermo Fisher Scientific, USA). The mobile phase A was acetonitrile and the mobile phase B was 0.1% formic acid in water. The elution gradient was set as follows: 0 min, 5% A; 2 min, 55% A; 10 min, 95% A; 15 min, 95% A. The flow rate was 0.3 ml/min. The injection volume was 10 µl (serum samples) or 5 µl (lung tissue samples).

LTQ Orbitrap Velos Pro was combined with UHPLC via an ESI (Electric spray ion source) interface. The analysis was performed both positive and negative ion modes. The acquisition software (Xcalibur 3.0, Thermo) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on pre-selected criteria. ESI source conditions were set as following: sheath gas flow rate as 40 psi, aux gas flow rate as 10 psi, capillary temperature as 350°C, ion spray voltage as ± 3.5 kV, full mass resolution as 30000, the MS2 and MS3 experiments were set as data-dependent scans.

Analysis of untargeted metabolomics data

The acquired raw files were preprocessed by Progenesis QI software (Waters, USA) and then imported into SIMCA 14.1 software (Umetrics, Sweden) for further analysis. First, the principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were screened by VIP >1.5, FC>2 and p <0.05, differential metabolites were treated as markers contributing to the model. Next, xcalibur software was used to extract and verify the chromatographic peaks. Then, the obtained compounds were compared with KEGG, HMDB databases and the accurate molecular weight with the error limit of 5 ppm. Finally, we conducted the GO and KEGG enrichment analysis to analyze the related pathway by the metaboanalyst 5.0 (https://www.metaboanalyst.ca/).

Targeted metabolomics analysis was performed using UPLC-AB Sciex Triple Quad™ 6500 + mass spectrometer

Combined the previous non-targeted metabolism results with literature, amino acid metabolism and TCA cycle were confirmed playing a critical role in the antiviral mechanism of QFPDD. The Mean±SEM of all experimental values were calculated and the statistical analysis was performed by Graphpad Prism 8.0 (GraphPad Software, USA).

Preparation of targeted metabolomics samples

Serum sample preparation: 50 µl serum, 10 µl IS solution (tryptophan-d5 in positive mode, puerarin in negtive mode) and 200 µl acetonitrile (containing 0.2% formic acid) was mixed in a 0.5 ml centrifuge tube, vortexed (3 min), centrifuged (12000 rpm, 4 °C, 15 min), 5 µl supernatant was taken for analyzing the amino metabolism.

The other 150 µl supernatant was blow-dried in a gentle nitrogen water bath at 37 °C, then 50 µl water was added for redissolution, vortexed (3 min), centrifuged (12000 rpm, 4 °C, 15 min), 10 µl supernatant was taken for analyzing the TCA metabolites.

Lung tissue samples preparation: lung tissue was added with 2.5 times saline for homogenization for 3 min, then 2.5 times acetonitrile (containing 0.2% formic acid) was added twice, followed by ultrasonic extraction for 10 min and centrifugation for 15 min (12000 rpm, 4 °C). 90 µl supernatant and 10 µl internal solution (as serum samples) were mixed for the determination of the TCA metabolites.

Another 50 µl supernatant and 200 µl acetonitrile were added in a 0.5 ml centrifuge tube, vortexed (3 min) and centrifuged (12000 rpm, 4 °C, 15 min). 90 µl supernatant was used to analyze the amino acid metabolism.

Quantitative LC-MS/MS analysis

LC conditions were as follows: column: ACQUITY UPLC® HSS PFP column (2.1 mm × 100 mm, 1.8 µm, Waters), mobile phase: A (acetonitrile) and B (water with 0.1% formic acid). The elution gradient was set as follows: 0 min, 2% A; 4 min, 2% A; 6 min, 98% A; 10 min, 98% A. The flow rate: 0.3 ml/min; column temperature: 35 °C. Mass spectrum conditions were as follows: electric spray ion source; multiple response monitoring (MRM) mode; air curtain pressure (N2) was 35 psi, impact air pressure (N2) was 9 psi, atomization gas pressure (N2) was 55 psi, auxiliary gas pressure (N2) was 55 psi; spray voltage was 5500 V; atomization temperature was 550 °C. The specific reference substances and method validation were shown in supplemental file S2 and literature (Zhou, et al., 2020).

Western Blot Analysis

Primary antibodies used in western blot analysis were as follows: ASCT2 (V501) Antibody (1:1000, #5345, Cell Signaling Technology, USA), xCT/SLC7A11 Antibody (1:1000, #98051, Cell Signaling Technology, USA), Anti-GAPDH polyclonal antibody (1:1000, Solarbio, China), Goat anti-rabbit IgG-HRP (1:10000, Solarbio, China). Sample preparation: After being dissected, the lung tissues were homogenized in ice-cold RIPA lysis buffer (Solarbio, China) supplemented with complete protease inhibitor cocktail (Solarbio, China). The homogenates were centrifuged (12,000 r/min, 4 °C) for 10 min and the supernatant was transferred to a new EP tube for later assay. Then, the protein quantification would be balanced by the Pierce BCA protein assay kit (Thermo Fisher Scientific, USA). 100 µg total protein lysate was...
subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and resolved on 12.5% polyacrylamide gels. The proteins were then electrophoretically transferred onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, USA). Primary antibodies were used to detect the corresponding protein level in the lung. GAPDH served as loading control of total protein. Immunoreactive bands were detected with species-specific HRP-conjugated secondary antibodies. The signals were visualized using the ECL advance reagent (Solarbio, China) and

Fig. 1. Mean plasma concentration-time profiles for the analytes in rats after oral administration of QFPDD (n = 8). (A) 18 quantitative indexes. (B) 13 semi-quantitative indexes.
The differential endogenous metabolites and putative genes closely related to COVID-19 treatment of QFPDD were imported into metscape. Y. Zhang et al.

Table 1

| Parameters | Dose | AU0(t) (ng. h/ml) | AU0∞ (ng. h/ml) | MRT0-t (h) | MRT0-∞ (h) | T1/2 (h) | Tmax (h) | Vz/F (L/kg) | CLz/F (L/h/kg) | Cmax (ng/ml) |
|------------|------|------------------|-----------------|------------|------------|---------|---------|-------------|---------------|-------------|
| Wogonin    | Low  | 40.01±16.08      | 44.75±16.48     | 3.89       | 4.864      | 2.22    | 3.30    | 1865.81     | 9.37±2.78     | 686.68      |
|            | High | 86.15±24.51      | 104.50±32.79    | 4.95       | 6.02       | 4.13    | 5.55    | 3490.48     | 24.46±7.00    | 357.20      |
|            | ±0.58 | ±0.121           | ±0.80          |          | ±1.40      |         |         | ±42061.4    |               |             |
|            | ±0.47 | ±0.73            | ±2.56          |          | ±2.17      |         |         | ±6206.10    |               |             |
|            | ±1.11 |                | 11.12±3.64     |          |           |         |         | ±1351.58    |               |             |

Molecular docking

The 3D structure of compounds was obtained from PubChem (https://www.ncbi.nlm.nih.gov/ pe Compound structural of...
targets were acquired from the RCSB Protein Data Bank (https://www.rcsb.org/). Six protein targets were Acetylcholinesterase (ACHE, PDB ID: 6N2W), Catechol-O-methyltransferase (COMT, PDB ID: 3A7E), Prostaglandin G/H synthase 1 (PTGS1, PDB ID: 3TUH), DNA (cytosine-5)-methyltransferase (DNMT, PDB ID: 3SWR) and CAMP-specific 3',5'-cyclic phosphodiesterase 4D (PDE4D, PDB ID: 1XOQ). All coordinates were converted from their native formats into pdbqt formats with AutoDockTools 1.5.7. The structures were optimized by deleting water molecules and adding hydrogen atoms. Then, the molecular docking study was performed using Autodock 4.0.

Results and discussions

Pharmacokinetic behaviors analysis results

Since the clinical utilization of QFPDD was oral administration, the therapeutic effects was relied on gastrointestinal absorption. Retained as potential effective components group in QFPDD, 31 candidates were analyzed, covering Atractylodes macrocephala Koidz, Glycyrrhiza uralensis Fisch., Pinellia ternata, Prunus armeniaca L.var.ansu Maxim, Tussilago farfara L, Zingiber officinale Roscoe, Bupleurum chinense DC., Citrus reticulate Blanco, Pogostemon cablin (Blanco) Benth., Aster tataricus L. f., Citrus aurantium L., Scutellaria baicalensis Georgi, Belamcanda chinensis (L.) DC., and Ephedra sinica Stapf. Totally, 18 quantitative and 13 semi-quantitative indexes were detected, which contained flavonoids, lactones, organic acids, saponins terpenoids and so forth. As shown in Fig. 1, the trends of average blood concentration-time curves of 31 detectable analytes were diagrammed. Specific PK parameters related to absorption, distribution and elimination were meaningfully different (Table 1). According to the AUC0-t (and/or AUC0-∞) and the MRT0-t (and/or MRT0-∞), the compounds (Baicalin, Prunasin, Wogonoside, Enoxolone) from 3 Herbs (Glycyrrhiza uralensis Fisch, Prunus armeniaca L.var.ansu Maxim, Scutellaria baicalensis Georgi) displayed higher levels and longer residency time in vivo than those of others in QFPDD. With regard to Tmax, the components could be sum-marily divided into three clusters. They reached the Tmax generally at 1, 3, 6 h, individually. The organic acids, dihydroflavonols and alkaloids reached time-to-peak before 1 h. Except for ephedrine, the residence time of them was about 4 h. The flavonoids and isoflavonols reached concentration peaks about 3 h. The average resident time of them was about 7 h. After 6 h, the secondary absorption of baicalin, wogonoside and the secondary metabolites were observed.

In a word, the flavonoids had analogous pharmacokinetic behaviors because of their similar structures. And the flavonoids demonstrated secondary absorption phenomenon, higher exposure and longer residency time compared with the other compounds. The secondary absorption was mainly observed in flavonoids, which was related to hepatenteral circulation. The flavonoids also showed strong activities on antiviral. Baicalin and baicalein could significantly inhibit the replication of SARS-CoV-2 and its 3C-like protein in vitro with small dose (Su et al., 2020). While the alkaloids, organic acids and dihydroflavonoids performed rapider uptakes and shorter residence time. To sum up, it was obvious that the average elimination time of components in QFPDD was about 11 h. Therefore, it is reasonable to apply QFPDD twice a day.

Network pharmacology construction and analysis

Based on the absorbed components in plasma, 376 putative targets were identified which met the condition of “Probability ≥ 0.1”. And the 916 candidate targets were screened by the keywords of “COVID-19”. The overlaps targets between QFPDD and COVID-19 were shown in the Fig. 2A. There were 52 genes were selected as the potential COVID-19
target genes. Next, the ClueGO APP of Cytoscape was applied to perform the biological process (BP) enrichment of potential targets and KEGG analysis was set as \( p < 0.01 \). Exactly as the Fig. 2B showed, the GO enrichment analysis revealed strong activation of immune-related biological processes, which included the regulation of B cell activation and the leukocyte proliferation. The inflammatory related pathways including positive regulation of chemokine production, stress-activated MAPK cascade, regulation of lymphocyte proliferation and oxidative functional disorder. Then, HIF-1 signaling pathway, EERBB signaling Pathway, Toll-like receptor signaling pathway, IL-17 signaling pathway as well as other immune pathways were exhibited in the Fig. 2C according to the KEGG enrichment analysis.

**Poly-pharmacology verification**

HCoV-229E was a coronavirus which mainly infects the respiratory tract and mucosal surfaces of the intestine. Respiratory tract infection mainly caused mild respiratory tract infection symptoms, typical manifestations were runny nose, sore throat, cough, headache, fever and so on (Gidding et al., 2018). These symptoms were similar with the conditions after COVID-19 infection. In order to evaluate the effect of drugs, the animal model was established to discuss the antiviral mechanism of QFPDD. The purpose was to simulate the performance of relevant clinical syndromes. Herein, the daily experience of groups was recorded including the behavior state, activity level, skin and hair state and stool state. As the Fig. 3A shown, the mice in the model groups appeared moist skin and hair from the second day, but there was no significant difference. On the 4th day, the scores of stool status in all groups increased without the control group. Simultaneously, the scores of skin and hair status significantly increased and the behavior status scores decreased. These situations lasted until the end of modeling. It was evident that the behavior status scores of the model group were significantly lower than the control group. And the abnormal conditions such as moist skin and hair and sticky stools were significantly presented, which was consistent with the literature reports of cold and dampness syndrome (Wu et al., 2022). Surprisingly, these abnormal conditions were significantly mitigated after intragastric administration with QFPDD.

The nucleic acid load is one of the most intuitive indicators of virus infection. After virus invasion, the nucleic acid expression increased greatly. As shown in the Fig. 3B, the intervention of QFPDD was able to decrease the virus load significantly. In order to further quantify the capillary permeability and the anti-edema, the pulmonary wet/weight ratio was calculated. Just as the Fig. 3C presented, the wet/weight ratio down-regulated after administration with QFPDD, evidently. And the pulmonary HE staining was performed to verify the pathological injury caused by viral infection (Fig. 3D). HCoV-229E infection induced pathological phenomena, including interstitial edema, incrassate alveolar septa and extensive inflammatory cell infiltration, which were alleviated after administration with QFPDD.

Simultaneously, in consideration of the impact of leukocytes infiltration on inflammatory cytokines secretion, the levels of 20 cytokines were determined. As reported, the novel coronavirus infection induces skin and hair from the second day, but there was no significant difference. On the 4th day, the scores of stool status in all groups increased without the control group. Simultaneously, the scores of skin and hair status significantly increased and the behavior status scores decreased. These situations lasted until the end of modeling. It was evident that the behavior status scores of the model group were significantly lower than the control group. And the abnormal conditions such as moist skin and hair and sticky stools were significantly presented, which was consistent with the literature reports of cold and dampness syndrome (Wu et al., 2022). Surprisingly, these abnormal conditions were significantly mitigated after intragastric administration with QFPDD.

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an excessive immune response, blocking the airway and exacerbating hypoxia, causing systemic acid-base disturbances (Subbarao et al., 2020). While the inflammation spread, the excessive inflammation cytokines, oxygen free radicals and other inflammatory mediators would damage the organ’s function. The vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-α), IL5, IL9, IL12P, monocyte chemotactic protein-1 (MCP-1) and granulocyte-macrophage colony stimulating factor (GM-CSF) were significantly up-regulated in the

Fig. 4. Non-targeted metabolomic analysis. (A) PCA diagrams between the detected samples and QC samples. Green and blue represented the determinands and the QC samples, respectively. (B) OPLS-DA diagrams between the control group and the model group. Green and blue corresponded the model group and the control group, respectively. (C) Distribution of the serum differential metabolites in each group. (D) Distribution of the lung tissue differential metabolites in each group. (E) KEGG pathways significantly enriched with the 31 differential metabolites.

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model group (Fig. 3E). By integrating COVID-19 with drug targets, the pathways have been found to be closely related to immune system responses and inflammatory responses. As reported, VEGF took a vital role in capillary integrity, vascular permeability and edema, thus exhibiting an indirect pulmonary protection manner from inflammatory injury (Cremer et al., 2021; Wang et al., 2020). TNF-α was a cytokine produced naturally by macrophages in response to bacterial infection (Liu et al., 2021b). GM-CSF might be a mediator of the hyperactive inflammatory response associated with respiratory failure and death. Blocking GM-CSF could subdue the activation of granulocytes and reduces downstream cytokine production, including MCP-1, IL5, IL9, IL12P70, etc (Vassilara et al., 2018). They played the indispensable roles in the body’s inflammatory response.

**Global non-targeted metabolomic analysis**

Furthermore, the intervention of QFPDD on endogenous variation in blood and lung during modeling was investigated. To ensure the accuracy of data, the principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) diagrams were depicted. As shown in Fig. 4A, the quality control samples (QC) were all gathered. In the Fig. 4B, the intergroup separation could be apparently depicted. As shown in Fig. 4A, the quality control samples (QC) were all gathered. In the Fig. 4B, the intergroup separation could be apparently observed. After the filtration by the conditions of VIP ≥ 1, fold change (FC) ≥ 1.2, p < 0.05 and comparison to original data, a total of 32 compounds were remarkably influenced after treatment with QFPDD. The concrete information of differential metabolites was displayed in the Fig. 4C-D and Table 2. Then, the metaboanalyst was implemented to visualize and appraise the metabolic pathways affected by QFPDD. According to the Fig. 4E, we should pay attention to the arachidonic acid metabolism, TCA cycle, arginine biosynthesis, aminoacyl-tRNA biosynthesis, galactose metabolism, alanine, aspartate and glutamate metabolism and so on. The results were consistent with the literature on COVID-19 (Jeong et al., 2021).

**Targeted metabolomics analysis**

The amino acid metabolism and the TCA cycle played a critical role in the mechanism exploration of QFPDD in accord with the previous non-targeted metabolomic tests. As reported, the virus infection was often accompanied by the inflammation, while the inflammation response could significantly activate the KP (Collier et al., 2021). Thus, the kynurenine pathway was included in our investigation. The results showed that there were 25 and 24 endogenous differential metabolites in the serum and the lung tissue, respectively. Surprisingly, the exact opposite results were true between the two biosamples. Comparing the model groups with the control groups, the content of the metabolites in peripheral blood were down-regulated and the metabolites in the lung tissue were up-regulated. For the sake of indepth understanding the abnormal conditions, 12 identified metabolites were filtered under the conditions of FC ≤ 0.8 or FC ≥ 1.2. Excluding histidine, ornithine and serine with low abundance, there were seven differential metabolites were finally illustrated in the Fig. 5A-C, namely arginine, glutamic acid, glutamine, tyrosine, tryptophan, citrulline and malic acid. According to the available reports, the variation of the metabolites such as glutamine, glutamic acid, arginine and tryptophan were closely related to the disease process. The increase of metabolites could alleviate the abnormal symptoms caused by inflammatory storm (Jiroski et al., 1997).

The metabolome is the terminal downstream product of the genome and proteome which aims to measure a wide breadth of small molecules in the context of physiological, stimuli or in disease states. In the previous studies, an interesting phenomenon occurred. For serum samples, the levels of metabolites in the model groups down-regulated significantly, which was consistent with the literature (Shen et al., 2020). Nevertheless, the levels of other metabolites except for malate and tyrosine were up-regulated significantly in the lung tissue, which was completely opposite to the results of serum. As we knew, lung is not the organ that produces amino acids. Why did the above phenomenon occur?
occur? The ACE2 was the main receptor to enter host cells for COVID-19. Moreover, it could alleviate inflammatory and other cardiopulmonary diseases (Guney, et al., 2021). Meanwhile, the intestinal ACE2 receptor was related to neutral amino acid transporter B0AT1. ACE2 was necessary for the expression of B0AT1 on the lumen surface of intestinal epithelial cells (Sharma, et al., 2020). A heterodimer composed of ACE2 and B0AT1 could affect neutral amino acid transport, inhibit systemic and intestinal inflammation, which played a direct effect on the lung or indirect effects through the ACE2 dependent gut-lung axis (Dang and Marsland, 2019). Consequently, a hypothesis came to our attention. The inconsistency of amino acid levels might be partly owing to the amino acid transporters.

**Western blot analysis**

In order to verify the results related to amino acid transporters, ASCT2 and XCT protein were selected to perform the experiment based on seven key substrates. As we all saw in the Fig. 5D, the ASCT2 and the XCT were up-regulated in the model group. ASCT2 was the most crucial transporter for glutamine uptakes and mediating peripheral naive T-cell homeostasis, activation and differentiation, especially for Th1 and Th17 cells (Ren et al., 2017). The naive T cell activation was coupled with rapid glutamine uptaking, which was also depended on the ASCT2. ASCT2 deficiency impaired the induction of T helper 1 (Th1) and Th17 cells and attenuated inflammatory T cell responses in the mouse models through immunity and autoimmunity. XCT was highly specific for cysteine and glutamate. In this system, designated Xc(-), the anionic form of cysteine was transported in exchange for glutamate. Glutamate was further converted into glutamine in the body. As reported, there was a certain correlation between the metabolic disorder and disease degree (Krishnan et al., 2021). Part of the pathogenesis of COVID-19 lay in the inflammatory factors and the overactivation immune system. Cellular uptake and the utilization of nutrients were closely related to the T-cell fate decision and function. The variation of amino acid meant that the body’s activities were frequent. More amino acids were needed to maintain homeostasis. After modeling, XCT and ASCT2 were up-regulated which promoted the body’s inflammatory response and autoimmunity. While the intragastric administration with QFPDD induced the decrease of XCT and ASCT2 to a certain extent. It was suggested that QFPDD might down-regulated the level of amino acid transporters to relieve the abnormal excessive immunity situations. However, there was no significant difference existed. Presumably, we could get the answer from the severity of the disease. Moreover, the results were consistent with the prediction of pharmacology, suggesting that the anti-viral mechanism of QFPDD was related to IL-17 signaling pathway, Th17 cell differentiation and T-cell receiver signaling pathway.

**Compound-reaction-enzyme-gene network construction**

To obtain a comprehensive view of the antiviral mechanisms of QFPDD, an interaction network was constructed based on metabolomics and network pharmacology in the Fig. 6. By matching the potential genes, the curative compounds and the differential endogenous metabolites in metscape, 6 key targets, including ALOX5, ACHE, PTGS1, PDE4D, DNMT and COMT were selected. The related key metabolites were serine, ADP, taurine, L-methionine, tryptophan, 5,6-EET, L-tyrosine, s-adenosyl homocysteine, norepinephrine and adrenaline. The curative compounds were hesperidin, baicalin, liquiritigenin, wogonoside, baicalin, scutellarin, isoliquiritigenin, 6-gingerol, nobiletin and irinotecan. And the affected pathways were glycerophospholipid metabolism, purine metabolism, methionine and cysteine metabolism, arachidonic acid metabolism, tyrosine metabolism and tryptophan metabolism.
Molecular docking result

The molecular docking studies was implemented to further investigate the interaction possibility between the curative components in QFPDD and the key targets. As shown in the Table 3 and the Fig. 7, the binding mode with the best docking score was displayed. The docking score represents the affinity between the protein receptor and the docking ligands. The lower docking score reflected the better affinity. The docking analysis of ACHE showed that baicalein, isoliquiritigenin made hydrogen-bonding interactions with HIS-447, TYR-133, GLU-202, ASN-87, ASP-74 and SER-203 at the active site. Baicalein and isoliquiritigenin might exert a corresponding cellular immune function by regulating ACHE to interfere with glycophoripid pathway in the abnormal cholinergic system (Hampel et al., 2018). In addition, isoliquiritigenin also made hydrogen-bonding interactions with PTGS1 by LYS-191, LYS-41, GLU-196, THR-36. In the interaction with COMT, an important target in the hypertension and asthma, umbelliferone made hydrogen-bonding interaction with GLU-90, LYS-144 and HIS-142. PDE4D made hydrogen-bonding interaction with enoxolone by SER-226, which attenuates MAPK/ERK signaling in a CRAF-dependent manner (Marbach-Breitrück et al., 2021). And ALOX5 made hydrogen-bonding interaction with 6-gingerol, nobiletin, irisflorentin, baicalein, isoliquiritigenin and enoxolone. ALOX5 was the key enzyme in the biosynthesis of inflammatory leukotrienes and an asthma predisposition factor (Cao et al., 2022). Once again, the results verified that QFPDD had the positive effects on antiviral by intervening inflammation and immunity response.

Conclusion

Based on the PK analysis, the network pharmacology and the metabolomics, an integrated multi-systemic research strategy of QFPDD was developed. A total of 31 compounds behaviors were analyzed in vivo, respectively, which provided guidance for the clinical application of QFPDD and the basement for network construction. The integrated analysis revealed 8 representative curative compounds and 5 potential genes as well as related metabolites and pathways. Furthermore, the variation of amino acid transporters brought us a glimpse for the metabolic disorders of coronavirus infection.

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.
CRediT authorship contribution statement

Yan Zhang: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Xinru Gu: Investigation. Yanyan Zhou: Investigation. Nan Si: Writing – review & editing, Supervision. Wenya Gao: Writing – review & editing, Supervision. Bo Sun: Writing – review & editing, Supervision. Jing Sun: Investigation. Tao Li: Investigation. Linna Wang: . Xiaolu Wei: Writing – review & editing, Supervision. Shanshan Guo: Writing – review & editing, Supervision. Xiaolan Cui: Investigation. Baolin Bian: Funding acquisition. Hongjie Wang: Visualization. Liang Wang: .

Declaration of Competing Interest

The authors declare no competing financial interests that could inappropriately influence the outcome of this study.

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