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Research article

Model exploration for discovering COVID-19 targeted traditional Chinese medicine

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ABSTRACT

In terms of treatment, a particularly targeted drug is needed to combat the COVID-19 pandemic. Although there are currently no specific drugs for COVID-19, traditional Chinese medicine(TCM) is clearly effective. It is recommended that through data analysis and mining of TCM cases (expert experience) and population evidence (RCT and cohort studies), core prescriptions for various efficacy can be obtained. Starting from a multidimensional model of regulating immunity, improving inflammation, and protecting multiple organs, this paper constructs a multidimensional model of targeted drug discovery, integrating molecular, cellular, and animal efficacy evaluation. Through functional activity testing, biophysical detection of compound binding to target proteins, multidimensional pharmacodynamic evaluation systems of cells (Vero E6, Vero, Vero81, Huh7, and caca2) and animals (mice infected with the new coronavirus, rhesus macaques, and hamsters), the effectiveness of effective preparations was evaluated, and various efficacy effects including lung moisturizing, dehumidification and detoxification were obtained. Using modern technology, it is now possible to understand how the immune system is controlled, how inflammation is reduced, and how various organs are protected. Complete early drug characterization and finally obtain effective targeted TCM. This article provides a demonstration resource for the development of new drugs specifically for TCM.

1. Introduction

Globally, COVID-19 has become a pandemic [1, 2]. A novel coronavirus (SARS-CoV-2) was reported in January 2020; the World Health Organization (WHO) later designated it as COVID-19 [3]. 526 million confirmed cases and more than 6 million fatalities had been reported globally as of 29 May 2022 [4]. There are currently three new coronavirus epidemics in humans, including COVID-19, Severe Acute Respiratory Distress Syndrome (SARS), and Middle East Respiratory Disease (MERS). SARS-genome SARS-CoV-2 has 80% of its similarities with the original SARS-CoV, which first appeared in humans more than ten years ago, and roughly 97% of its similarities with the bat coronavirus (CoV RaTG13), indicating that it likely evolved from bat viruses [5]. The positive genome of the single-stranded RNA virus SARS-CoV-2 has 29, 903 nucleotides (NCBI reference sequence: NC-045512) [5]. The coronavirus needs the S protein to enter the host cell, creating the necessary circumstances [6]. The S protein of SARS-CoV-2 has a greater affinity for the ACE2 receptor than SARS-CoV [7], which may be the key factor for COVID-19 to spread so quickly (which is more infectious). COVID-19 has been curtailed in some ways by the emergence of vaccines. WHO declared the COVID-19 vaccines to be on its "emergency list" for China in June 2021. It is, however, characterized by rapid transmission, high infectivity, and general population susceptibility, and there are currently no specific targeted drugs available. New drugs that are specifically targeted are required to address this problem on a therapeutic level. There is growing evidence that COVID-19 can be effective using traditional Chinese medicine (TCM). The benefits of TCM in the treatment of COVID-19 have been shown in a review of 40 representative clinical trials [8]. TCM can reduce the time of symptom recovery, delay the development of severe disease, protect multiple organs damage;

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improve lung characteristics; and improve laboratory indicators, including $CD4^{+}T$, $CD8^{+}T$, CRP, IL-6, TNF-A, ESR, and thrombogenic-associated pulmonary embolism levels [8]. TCM can also delay the onset of severe disease and protect against damage to multiple organs. The outcomes demonstrate that TCM is effective in treating COVID-19. For COVID-19, there are more specialized, effective, and targeted TCM treatments available. This article explores new targeted TCM treatments for COVID-19 using a multi-dimensional efficacy evaluation system. Firstly, the core medications with strong efficacy evidence are combined in accordance with the various efficacy and pharmacological effects of TCM. Secondly, pharmacodynamic evaluation is performed using the pharmacodynamic evaluation method from the perspectives of immunity, inflammation, and organ protection based on the features of viruses and medications. Thirdly, explain the mechanism. Finally, complete the evaluation of the medicinal properties.

2. Current methods of targeted drug exploration and potential therapeutic drugs

2.1. Current research methods of targeted drugs

The development of targeted drugs based on protein structure has been studied. Spike protein (S), membrane (M), envelope (E), nucleocapsid (N), and Nsp15 protein are the four structural proteins found in coronaviruses. It has been suggested to use virtual screening to investigate potential drugs. Paritaprevir and Elbasvir have some of the lowest free binding energies when compared using molecular docking techniques to identify potential Nsp15 inhibitors for COVID-19 [9]. Through molecular modeling, docking, and kinetic simulations, the study created a model of the viral protein RNA-dependent RNA polymerase (RdRp) and assessed the drug affinities. SARS-CoV-2 RdRp was combined with Sofosbuvir, Ribavirin, Galidesivir, Remdesivir, Favipiravir, Cefuroxime, Tenofovir, and Hydroxychloroquine [10]. Several well-characterized diacylpyrimidine analogs have been computationally identified as inhibitors that may disrupt hACE2 surface spike protein (S) interactions. The pyrimidine derivative AP-NP may be a powerful inhibitor of the hace2-S complex, as was later discovered using molecular docking, molecular dynamics (MD) simulations, and confinement-free energy calculations [11]. Target drug studies are also being conducted using molecular simulation approaches. Based on network pharmacology, a chemical-target network is built to forecast core medications and identify Lianqiao-Jinyinhua core drugs [12]. There have been reports of potential drugs for COVID-19 being found using molecular docking, molecular dynamics (MD), and structure-activity relationship (SAR) simulations. At the same time, some studies combine molecular docking with molecular dynamics. Ramipril, Delapril, and Lisinopril were screened to bind with ACE2 receptor and [SARS-CoV-2/ACE2] complex more effectively [13]. To calculate suitability scores, some studies use molecular dynamics software and machine learning algorithms. In addition, suture algorithms have been used to predict network pharmacology involving multiple active drug candidate pathways, suggesting that cordycepin can be reused in COVID-19 [14].

In some studies, compounds are screened first, followed by in vitro enzyme-inhibition activity screening of SARS-CoV-2 clinical isolates and in vitro culture assays using Vero cells. Finally, two compounds with dual activity were screened, which showed good application prospects by verifying the computational model and toxicity of the compounds. In a study using an integrated approach to identify more drug molecules targeting SARS-CoV-2 proteins, molecular interactions determined by molecular dynamics simulations revealed the role of catechins during the virtual screening of 75 drugs, and the binding of catechins made it easier to participate in key molecular interactions, in addition, catechins and package, stability was mainly achieved through hydrophobic interactions, indicating that catechins have the potential to be developed as multi-target drugs against COVID-19 [15]. Currently, targeted drugs are derived from viral proteins or computer simulations, which lack a basic description of their therapeutic effects. Multidimensional validation at the molecular, cellular, and animal levels is necessary to make targeted drugs more effective in clinical treatment. Based on this, we propose a targeted drug exploration model. Therefore, this paper constructs a multi-dimensional comprehensive efficacy evaluation system of molecules, cells, and animals to improve inflammation, regulate immunity, protect organs, and explore and discover targeted TCM for the treatment of COVID-19. Table 1 summarizes current approaches to exploring targeted drugs, mainly based on computer simulations and protein structure(Table 1).

2.2. Potential therapeutic drugs

Antivirals, antibiotics, antimalarials, and immunomodulatory drugs may be effective in combating COVID-19 with the onset of the epidemic. Monoclonal antibody drugs include Bamlanivimab (LY-CoV555), which acts against the SARS-CoV-2 spike protein and may prevent and treat COVID-19 [16,17]. Etesevimab (also known as JS016 or LY-CoV016) can specifically bind to the receptor binding domain (RBD) of the SARS-CoV-2 surface protein [16], and many studies have shown that Etesevimab has positive significance for the treatment of COVID-19 [18, 19]. Sotrovimab (Xevudy, GlaxoSmithKline and Vir Biotechnology, Inc.) binds to a highly conserved epitope on the RBD of the SARS-CoV-2 S protein and can delay the clinical progression of COVID-19 [20]. Casirivimab (IgG1-κ) and imdevimab (IgG1-λ) bind to non-overlapping epitopes of the SARS-CoV-2 spike protein RBD [21], and clinical trials have shown that REGN-CoV can reduce viral load [22, 23]. Tocilizumab

Table 1. summarizes the current methods of exploring targeted drugs, which are mainly based on computer simulation and protein structure.

basic method	research methods	result	reference
protein structure	docking techniques to identify potential Nsp15 inhibitors	Paritaprevir and Elbasvir	[9]
protein structure	establish a viral protein RNA-dependent RNA polymerase (RdRp) model through molecular modeling, docking, and kinetic simulations	Sofosbuvir, Ribavirin,Galidesivir, Remdesivir, Favipiravir, Cefuroxime, Tenofovir, and Hydroxychloroquine	[10]
protein structure	identify inhibitors that may disrupt hACE2 surface spike protein (S) interface interactions and through molecular docking, molecular dynamics (MD) simulation	pyrimidine derivative AP-NP	[11]
molecular simulation techniques	construct a chemical-target network based on network pharmacology	Lianqiao-Jinyinhua	[12]
molecular simulation techniques	molecular docking with molecular dynamics	Ramipril, Delapril, and Lisinopril	[13]
molecular simulation techniques	molecular dynamics and machine learning algorithms to calculate suitability scores	cordycepin	[14]
comprehensive method	compounds were firstly screened out, and then SARS-CoV-2 clinical isolates were screened for their in vitro enzyme inhibitory activity, and vero cells were used for in vitro culture detection	catechins	[15]

(RoActemra, Roche Pharma AG) specifically binds to soluble and membrane-bound receptors (sIL-6R and mIL-6R) of IL-6 [24]. A meta-analysis by Malgie et al showed that the use of tocilizumab was associated with reduced mortality [25]. Remdesivir can inhibit viral replication [26], effectively achieve early clinical improvement and reduce early mortality [27]. Baricitinib can reduce the expression of IL-6 [28] and reduce the mortality of COVID-19 [29]. Application of autophagy and UPR in SARS-CoV-2 infection, Paxlovid and the SARS-CoV-2 covalent 3CL protease inhibitor PF-07,321,332 [30] can reduce mortality compared with placebo [31]. Molnupiravir (Lagevrio) can neutralize pathogens and exert antiviral effects [32], which has a positive significance for the treatment of COVID-19 [33]. Regdanvimab (Regkirona), which blocks cell invasion and SARS-CoV-2 infection [34], is used to treat COVID-19 in adults who do not require supplemental oxygen and those at increased risk of severe disease [35]. Anakinra (Kineret) inhibits the biological activity of interleukin 1. A meta-analysis by Kyriazopoulou et al. showed a significant improvement in survival with Anakinra [36]. Sotrovimab (Xevudy, also known as VIR-7831 and GSK4182136), which can adsorb the S protein of SARS-CoV-2, is an active monoclonal antibody against COVID-19 [37], which can delay disease progression [38]. Tixagevimab and cilgavimab (Evusheld) were designed to attach to different sites of SARS-CoV-2 spike protein 2, reducing the risk of disease progression and mortality [39]. Other drugs with potential efficacy against COVID-19 infection, such as hydroxychloroquine (HCQ) [40, 41, 42, 43, 44, 45, 46], Colchicine [47, 48, 49], convalescent plasma [50, 51, 52, 53, 54], Amantadine [55, 56, 57, 58, 59], Ivermectin [60, 61, 62, 63, 64, 65, 66], Niclosamide (NIC) [63, 67, 68, 69], Sarilumab (Kevzara) [70].

3. Search for targeted drugs

3.1. Find core prescriptions based on data mining

Clinical experience can be accumulated, and valuable experience can be provided for follow-up treatment to improve the accuracy of COVID-19 treatment by digging out core prescriptions for COVID-19 treatment from case experiences (expert experiences) and group evidence (RCTs and cohort studies) and applying the mined prescriptions to follow-up evaluation and exploration, which has an important role.

Through the analysis and mining of expert treatment experiences and whole case data, the core prescriptions with different efficacy sets were obtained. First, electronic medical records (EMR) can be mined [71]. The data processing process of EMR generally includes five processes: data acquisition, data preprocessing, data mining, evaluation, and knowledge application [72, 73, 74]. Text mining mainly includes four steps: information retrieval, information extraction, knowledge discovery, and knowledge application [6]. There are three main data mining algorithms: classification, clustering, and association [75]. Core drugs for COVID-19 treatment by experts were searched based on literature collection, and COVID-19 treatment plans issued by the authorities of provinces, municipalities, and autonomous regions were collected. Meanwhile, core prescriptions were retrieved according to COVID-19 diagnosis and treatment protocols released by The National Health Commission of China. *Novel Coronavirus Protocol for Diagnosis and Treatment of Pneumonia (Trial)* was released on 16 January 2020, followed by *Novel Coronavirus Protocol for Diagnosis and Treatment of Pneumonia (Trial Second Edition)*. The *Novel Coronavirus Diagnosis and Treatment Protocol for Pneumonia (Trial Version* 3) were released on 22 January 2020. *Novel Coronavirus Diagnosis and Treatment protocol for Pneumonia (Trial Edition 4)* was released on 27 January 2020. *Novel Coronavirus Diagnosis and Treatment protocol for Pneumonia (Trial Edition 5)* was released on 5 February 2020. *COVID-19 Diagnosis and Treatment Protocol (Revised Trial Fifth Edition)* was released on 8 February 2020. *Novel Coronavirus Diagnosis and Treatment protocol for Pneumonia (Trial Version 6)* was released on 18 February 2020. *Novel Coronavirus Diagnosis and Treatment protocol for Pneumonia (Trial Version 7)* was released on 4 March 2020. *Novel Coronavirus Protocol for diagnosis and Treatment of Pneumonia (Trial Version 8)* was released on 18 August 2020. *Novel Coronavirus Diagnosis and Treatment Protocol for Pneumonia (Revised Trial Edition 8)* was published on 14 April 2021.

According to the recommendation of the eighth edition, the disease stage was divided into the medical observation stage and the clinical treatment stage. Huo Xiang Zhengqi capsule, Jinhua Qinggan granule, Lianhua Qingwen capsule, and Shufeng Jiedu capsule are recommended during the medical observation period. Qingfei Paidu decoction is recommended for clinical treatment. Mild cold and dampness syndrome of stagnation of the lung is Maxing Shigan decoction and damp-heat syndrome of lung storage is Houpo Wenzhong decoction. The common type of dampness and dampness syndromes of lung stagnation are Xuanfei Baidu decoction, and cold dampness and lung obstruction syndromes are Cangzhu, Chenpi, Houpo, HuoXiang, Caoguo, shengMahuang, Qianghuo, Shengjiang, and Binglang. The severe epidemic virus closed lung syndrome for Huashi Baidu decoction, Qi and yin deficiency pattern for Qingwen Baidu decoction. For severe cases of internal closure and external withdrawal, Renshen, Heishun pian, and Shanzhuyu should be taken the Suhexiang pill or Angong Niuhuang pill [76].

3.2. Evaluate core prescriptions

3.2.1. Evaluate the efficacy of core prescriptions at the molecular level

According to the functional activity mechanism and substrate binding characteristics of each protein, in silico virtual screening methods such as molecular docking (molecular docking can predict ligand-target interactions at the molecular level and can determine the therapeutic value of drugs) [77], molecular modeling, molecular dynamics (MD) simulation and binding free energy calculation, structure-activity relationship (SAR). Functional activity detection methods, such as enzyme activity detection methods based on fluorescence resonance energy transfer [78] (the energy of fluorescence resonance energy transfer is transferred from the excited state of the donor fluorophore to the adjacent ground state biophysical method by dipole-dipole coupling to detect the binding of compounds to target proteins, such as protein thermal stability tests). The main methods include isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), time-resolved energy transfer (TR-FRET), and enzyme-linked immunosorbent assay (ELISA). High throughput screening (HTS) technology is based on molecular or cellular-level experimental methods, using microplates as experimental tool carriers, supporting the overall operation of the technical system through program control, sample detection, and corresponding database systems [79]. HTS, such as fluorescence polarization, is usually linearly proportional to the percentage of a conjugate, from which the IC50 value is quantified. Mainly studying protein-molecule (ligand) interactions, protein-protein interactions, nucleic acid hybridization, etc., almost all types of proteins can be studied [80]. ELISA can detect and quantify proteins and other substances [81]. Among other methods used, are surface plasmon resonance, nuclear magnetic resonance, and acoustic fog ionization-mass spectrometry [82]. Biophysical assays were performed, including surface plasmon resonance (SPR), biofilm interferometry interaction (BLI), tri-dependent fluorescence intensity change method (TRIC), fluorescence thermal drift experiment (TSA), and isothermal titration calorimetry (ITC). Based on the P2/P3/P4 laboratory, the Homo-Trimeric spike glycoprotein (S protein), the Nucleocapsid (N) protein, of the SARS-CoV-2 virus, is the first anchor of the virus in the host cell [83], enveloping the viral RNA into a helical ribonucleic capsid (RNP) and interacting with other structural proteins as the virus assembles, resulting in the genome being encased [81, 82, 83]. Helicase, RdRp (Multi-subunit RNA-dependent RNA polymerase), 3CLpro (3C-like protease, named after the 3C protease of the Picornaviridae), and Stimulator of Interferon Genes (Sting) are used as protease targets in the Picornaviridae, combining effective prescriptions and their effective compounds, monomers, determine antiviral, immunomodulatory, and anti-inflammatory effects, evaluate effective prescriptions and their

effective compounds, antiviral, modulative, anti-inflammatory activity, and other pharmacological effects of monomers. Through preliminary screening, various cold and temperature properties and efficacy groups were obtained.

3.2.2. Evaluate the efficacy of core prescriptions at the cell level

The monkey kidney cell lines Vero E6, Vero and Vero81 are frequently used to culture viruses lacking interferon (itf) secretion. Human lung adenocarcinoma cell Calu3. This cell is readily available and reproducible, differentiates into polarized monocytes with different phenotypes, expresses airway mucus secretion, tight junctions, and microvilli, and can transport proteins and metabolic functions [84], human liver cells Huh7, a permanent cell line [85]. Cancers of colorectal adenoma Caco2 can spontaneously differentiate into single cells, which can form between adjacent cells and are closely related [86]. RAW264.7 cells are typically selected from macrophage cell lines to induce bone marrow-derived macrophages. Thus, during SARS-CoV-2 infection, cell death is induced by stimulating the release of pro-inflammatory cytokines [87]. Using the cell model of fluorescently labeled immune activation pathway as a screening platform, the immune activation characteristics of antiviral, immunomodulatory, and anti-inflammatory activities of different TCM prescriptions were compared. Different outbreak mutants were tested for antiviral, immunomodulatory, and anti-inflammatory effects, and IC50/IC90 values were used to evaluate antiviral effects. In macrophages and other cell lines, differences in the expression of inflammatory factors such as IL-6 and IL-10 within and between groups were tested, and the anti-inflammatory activity of TCM was evaluated. High Content screening (HCS) models are mainly established at the cellular level by observing the various functional effects of samples on the morphology, growth, differentiation, migration, apoptosis, metabolism, and signaling of fixed or dynamic cells. Targets involved include cell membrane receptors, intracellular components, and organelles [88]. Cell-level experiments can be performed to screen COVID-19-targeted drugs by inhibiting cell proliferation, inducing cell differentiation, inducing cell apoptosis, reversing drug resistance, and inducing autophagy. The biological activity test methods at the cellular level mainly include MTT, Cell Counting Kit-8 (CCK-8), and Sulforhodamine B (SRB). Combined with the pharmacodynamic evaluation and screening results at the molecular level, different cold-temperature drug properties and pharmacodynamic groups were determined.

3.2.3. Evaluate the efficacy of core prescriptions at the animal level

In HFH4-hACE2 transgenic mice, K18hACE2 transgenic mice, and hACE2-transgenic mice, the release of inflammatory cytokines such as interferon caused by SARS-CoV-2 can increase ACE2 expression and enhance infection [89, 90]. ACE2 can be released from cells into circulation. In addition, the mouse genome is highly homologous to the human genome, and the mouse genome modification method has matured. Rhesus monkeys and rhesus monkeys are genetically and physiologically similar to humans, and the golden hamster infection model is used to test the efficacy evaluation indicators of each component, including anti-inflammatory, immunomodulatory, antiviral, and visceral protection. SARS-CoV-2, lipopolysac-charides (LPS), Toll-like receptors (TLRs) agonists, and superantigens (SAgs) can be used to induce novel coronavirus models. The effectiveness of this active formula and monomer in the prevention and treatment of immune, viral, and inflammatory abnormalities caused by COVID-19 virus infection was further validated in hACE2 transgenic mice and other models. It was determined that the treatment was efficacious when administered pre-infection and post-infection. The protective effect of the drug was confirmed by detecting changes in body weight, death protection status, changes in lung viral titers, pathological changes, inflammatory cell infiltration, and levels of inflammatory factors in animal models. Infection models have also been established, including in gold hamsters. Golden hamsters are also known to experience weight loss, lung pathological damage, and viral replication after infection, which can be used to evaluate antiviral drugs. Animal models of COVID-19 were constructed and administered in animal models (drug administration group and drug administration methods were set). Animal models were used for pharmacodynamic evaluation, and animal models reflecting pharmacological effects were selected. The clinically recommended dosing method was used for reasonable grouping, and the main pharmacodynamic indexes and secondary pharmacodynamic indexes were detected. Combined molecular and cellular pharmacodynamic evaluation, different cold and temperature characteristics were screened and obtained, and the efficacy was grouped.

According to the pathological processes of COVID-19 virus recognition of host cells, virus replication, and host immune inflammation regulation, the existing efficacy evaluation system was improved in multiple dimensions, and the simulation of different pathological types and the selection of pharmacodynamic evaluation models were strengthened (Table 2). Recombinant protein expression and purification platforms for COVID-19 virus recognition of host cell receptors to S proteins, key hydrolases 3CLpro and PLpro, cGAS and Sting, and key target proteins in other native immunomodulatory pathways were proposed. Many high-quality protein samples with high purity, high activity, and uniform morphology were obtained for viability testing and complex structural analysis. Furthermore, according to the physiological characteristics and functional modules of the cell model, and according to the species categories and associations of Vero E6, human lung adenocarcinoma cell Calu3, human colorectal adenocarcinoma cell Caco2 and other cell lines, immune cell unit, virus-cell unit and inflammatory cell unit were added through cell line database screening and in vitro experiments. Based on existing animal models such as mice, hamsters, rhesus macaques, and targeted ACE2 genes, animal species and targeted genotyping were further expanded by host global and local selective gene knockout technology. Multi-dimensional analysis of antiviral active ingredients and immunomodulators in TCM prescriptions. To clarify the material basis and mechanism of action of TCM formulas in the treatment of COVID-19, comprehensively use modern biology, immunology, laboratory zoology, and other multidisciplinary technologies to analyze key scientific issues such as antiviral active ingredients and immune function

Table 2. shows the model and methodology for evaluating core prescriptions.

Evaluate core prescriptions	Models	Methods
molecular level	Homo-Trimeric spike glycoprotein (S protein), Nucleocapsid (N) protein, RdRp (Multi-subunit RNA-dependent RNA polymerase), 3CLpro (3C-like protease, named after the 3C proteases of the Picornaviridae), Stimulator of Interferon Genes (Sting)	molecular docking, molecular modeling molecular dynamics (MD), structure-activity relationship (SAR), enzyme activity detection method, protein thermal stability tests, isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), time-resolved energy transfer (TR-FRET), enzyme-linked immunosorbent assay (ELISA)
cell level	The monkey kidney cell lines Vero E6, Vero and Vero81, human lung adenocarcinoma cell Calu3, human liver cells Huh7, colorectal adenomas Caco2	High Content screening (HCS), inhibition of cell proliferation, induction of cell differentiation, induction of apoptosis, drug resistance reversal, induction of autophagy
animal level	HFH4-hACE2 transgenic mice, K18hACE2 transgenic mice, hACE2 transgenic mice, rhesus monkey models	SARS-CoV-2, lipopolysac-charides(LPS), Toll-like receptors (TLRs) agonists, and superantigens (SAgs) can be used to induce the novel coronavirus model. animal models of COVID-19——drug administration was performed in animal models—pharmacodynamic evaluation

components of TCM formulas from molecular, cellular, animal, and other dimensions. The molecular mechanisms of the interaction between active ingredients in TCM prescriptions and antiviral proteins and host immune pathways were analyzed, and their effectiveness was verified at the cellular and animal levels. To provide the material basis and mechanism of action for the treatment of COVID-19, guide the better application and optimization of TCM formulas, and make emergency reserves for dealing with new infectious diseases.

3.3. Mechanism interpretation

3.3.1. Study on the mechanism of core prescription regulating host immunity

Patients with COVID-19 exhibit an abnormal immune response, causing a cytokine storm that leads to acute respiratory distress syndrome (ARDS) and ultimately death [91]. Taking NK cells, macrophages, T cells, plasma cells, and other immune cells as in vitro research objects, innate immune cells include granulocytes (neutrophils, eosinophils, basophils, mast cells), monocytes/macrophages, dendritic cells (DC), natural killer cells (NK), T lymphocytes and innate lymphocytes (ILCs) [92]. Il-6, TNF-α, macrophage inflammatory protein 1-α (MIP-1α), MCP3, GM-CSF, IL-2, IP-10, and chemokines (IP-10, CCL/MCP1, CXCL1, CXCL5) are also elevated in SARS-CoV-2 [93,94,95,96]. Patients with severe COVID-19 had significantly fewer CD4+, CD8+, B cells, and NK cells, and fewer monocytes, eosinophils, and basophils. The total number of T cells, including helper and suppressor T cells, in COVID-19 patients, was significantly reduced [97]. In vitro studies, ACE2 series transgenic mice, COVID-19-infected monkeys, and golden hamster infection models were used to find effective formulations to modulate host immunity. Using multidisciplinary technologies such as single-cell sequencing, flow cytometry, immunofluorescent labeling, protein imprinting, immunohistochemistry, etc., the culture system, immune cell activity, lethality rate, protein genes in immune factors, immune-related genes, etc. were quantified, and stereotyped, and the effectiveness and critical path of monomer components and the intrinsic mechanism of host immunomodulation was verified by preventing and treating the most drugs.

3.3.2. Study on anti-inflammatory mechanism of core prescription

The inflammatory response of infected cells may further induce immune cell infiltration into the lungs, leading to the overproduction of pro-inflammatory cytokines and severe lung damage, and multiorgan dysfunction [98]. After SARS-CoV-2 infection, the expression of p38 MAPK, NF-kB, IL-6, IL-8, and other inflammatory factors changes [99]. Patients with severe COVID-19 have many activated pro-inflammatory cytokines and chemokines, including IL-2, IL-6, IL-10, TNF-α, GSCF, and MCP-1 [100]. Spike proteins promote the 1-mediated signaling cascade by activating MAPK and increasing IL-6 release, inducing transcriptional regulatory molecules NF-KB and AP-1/C-FOS [101]. To study the anti-inflammatory effect of effective prescription, lipopolysac-charides-induced RAW 264.7 cells, BV2 cells, and other cell lines were used as in vitro research objects, and lipopolysac-charides -induced inflammation was taken as animal models such as Wistar and SD rats as in vivo research objects, through effective prescription, preventive administration, and therapeutic administration. In each group, HE staining was used to observe the thickness of the alveolar and bronchial walls, inflammation, hyperlipidemia, and edema. Enzyme-linked immunosorbent assay, Western blot, and flow cytometry were used. Expression of IL-6, IL-10, TNF-α, IL-1 β, and other inflammatory factors, as well as expression of key targets of genes, proteins, and related pathways suggested by various omics results, as well as molecular mechanisms of inflammation.

3.3.3. Mechanism of core prescription protecting multiple organs

Multi-organ failure is the leading cause of death in people with COVID-19. Pathologic examination of biopsy specimens from patients with COVID-19 suggests that inflammatory cell infiltration is common in the lungs, heart, kidneys, liver, and other organs [102]. The impact of the COVID-19 lung injury model suggests that ACE2 experimental cells (respiratory epithelial cilia and type 2 alveolar cells) are lost and ACE2 is under-vascular, resulting in endothelial cell damage, vasoconstriction, thrombosis, and interstitial edema, affecting multiple organs of the body [103]. In this study, a mouse model of lipopolysac-charide-induced inflammation, a mouse model of bleomycin-induced pulmonary fibrosis, an animal model of humanized ACE2, a novel coronavirus-infected monkey, and a golden hamster infection model were used in vivo to study the effective prescription mechanism of multi-organ protection. Histopathology, imaging, molecular biology, and other methods were used to test the efficacy of TCM. Multiple organs were stained by pulmonary function tests (resting ventilation, airway resistance, lung volume), liver and kidney function tests, blood-brain barrier, visceral ultrasound, internal microcirculation, etc. Cellular edema, cell degeneration injury, organ fibrosis, edema, and other tissue damage were evaluated. Combining inflammatory factors and immune factors, the results of multi-recombination suggested the key targets of the expression of genes, proteins, and related pathways, and interpreted the molecular mechanism of organ damage caused by effective protective agents of COVID-19 from multiple levels and angles.

The multilayered mechanisms of COVID-19, including pathogenic mechanisms, evolution, and organ damage, are still being studied. Quantification and high-throughput detection of multiple omics can further clarify the mechanism of occurrence and evolution of COVID-19, as well as the mechanism of action of TCM from planar to stereological, from static to dynamic, and from morphological to functional. Animal models such as ACE2 transgenic mice, COVID-19 virus-infected monkeys, and golden hamsters were selected for studies. An effective formulation was used as an intervention, and the concentration of the intervention drug was determined by the equivalent dose conversion between animals and humans. High-throughput proteomics, transcriptomics, genomics, and other omics tests were performed on serum, feces, and lung tissues. After clarifying the host associated with COVID-19 virus infection, the differential expression of proteins, lipids, and genes in the baseline, middle, and post-Liao groups expanded the mechanism spectrum from different levels, laying a biological foundation for in-depth interpretation of the mechanism of TCM (Figure 1).

3.4. Evaluation of drug properties

By establishing a multi-dimensional drug evaluation system that integrates molecular, cellular, and animal characteristics of core formulas, effective formulas were evaluated, different drug characteristics and efficacy groups were obtained, and specific and targeted innovative TCM formulas were us the treatment of COVID-19 were determined. Explain its mechanism with modern technology and complete early drug evaluation. The evaluation of medicinal properties includes five points, (1) Molecular structure characteristics, hydrogen bond binding, PSA, lipophilicity, SHAPE, molecular weight, pKa. (2) Physical and chemical properties, solubility, permeability, and chemical stability. (3) ADME. (4) PK characteristics, clearance, half-life, bioavailability. (5) Toxicity, LD50, DDI, hERG, genotoxicity. The basic principles for evaluating the medicinal properties of Chinese medicines in the new target are absolute effectiveness and reasonable safety. For new approaches based on theory or research results, the effectiveness and safety of their use should be emphasized. For traditional formulas that have entered clinical application, such as classical formulas and prescriptions, attention should be paid to their medicinal properties and efficacy verification, because their effectiveness has been clinically verified. Many new techniques and theories can be used for pharmacodynamic evaluation, including high-throughput screening, high-intention screening, proteomics, genomics, metabolomics, epigenetics, epipharmacology, phenotypic screening, computer, and transgenic technologies. In addition, there is computer modeling technology, network pharmacology technology, bioinformatics, systems biology technology, model biotechnology, biochip

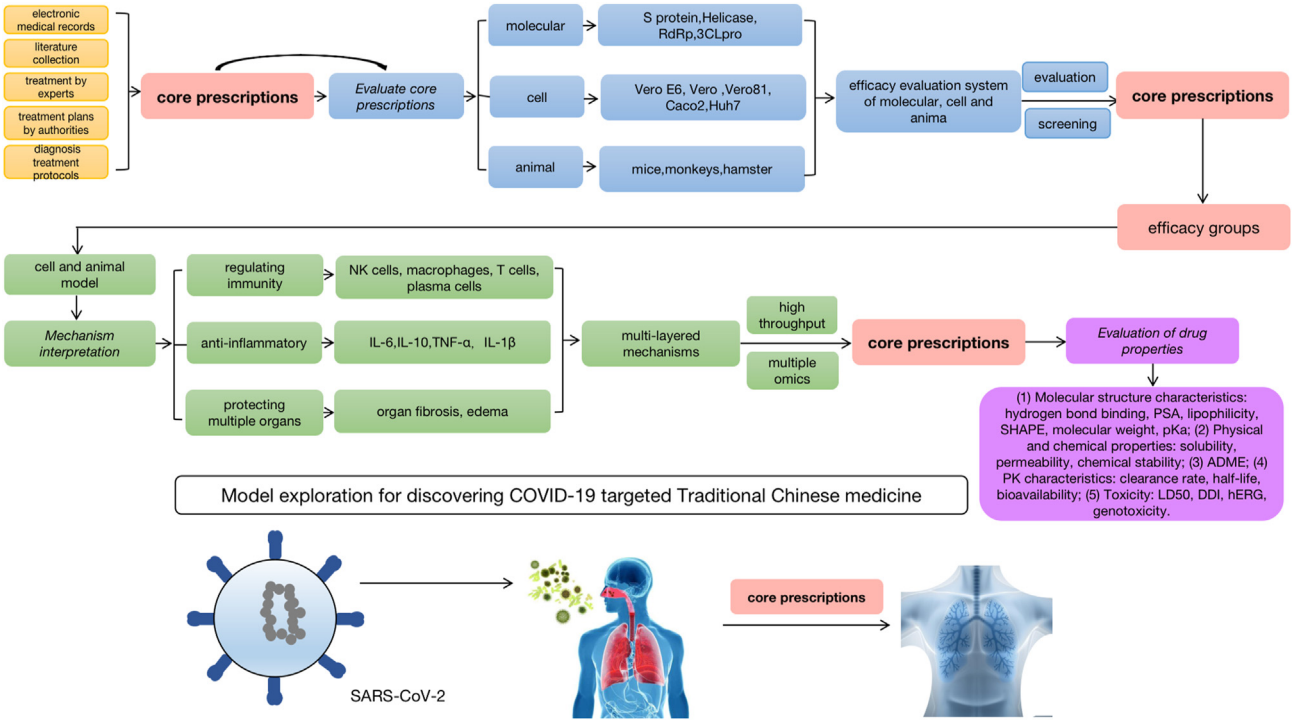

Figure 1. presents a multi-dimensional comprehensive therapeutic system based on molecules, cells and animals, exploring targeted TCM that can improve inflammation, regulate immunity, and protect organs for the treatment of COVID-19.

technology, molecular biotechnology, cell biotechnology, comprehensive target technology, and so on.

4. Discussion

4.1. Advantages and disadvantages compared to existing models

Compared with the existing models, this paper constructs a complete COVID-19 targeted drug discovery model and constructs a multi-dimensional comprehensive efficacy evaluation system integrating molecular drugs, cellular drugs, and animal drugs. Mechanisms of effective prescriptions such as protection of organs. Multi-dimensional analysis of antiviral active ingredients and immunomodulatory functional ingredients in TCM prescriptions, clarifying the material basis and mechanism of TCM prescriptions for the treatment of COVID-19, and comprehensively using modern biology, immunology, laboratory animals, and other multidisciplinary technologies. The active and functional components of TCM prescriptions were analyzed from molecular, cellular, and animal perspectives. From the perspective of systems biology, the molecular mechanism of the interaction between the active ingredients in TCM prescriptions and key antiviral proteins and host immune pathways was effectively analyzed and verified from the cellular and animal levels. Through the multi-omics combined detection of effective prescriptions and selected effective monomers, the core mechanisms of effective prescriptions to improve lung inflammation, regulate immunity and protect internal organs were elucidated. Based on multi-disciplinary research methods, we conduct drug evaluation and interpret the scientific connotation of effective targeted TCM in blocking mild and severe diseases, improving lung damage, and protecting the lungs. Solve the key issues of TCM in the treatment of COVID-19, such as how to play a multi-link, multi-target, and multi-level network mechanism around the interaction between the virus and the host, to reveal the mechanism of TCM to prevent and treat COVID-19, and discover drug targets to guide the research and development of new TCM, and establish a multi-dimensional technology system for the research and development of new anti-COVID-19 drugs. Complete the early pharmacological toxicity evaluation of the optimized new ingredients and develop new TCM-targeted prescriptions for the treatment of COVID-19 with clear ingredients and clear mechanisms of action. Therefore, compared with the current model, the current targeted drug exploration model mostly comes from viral proteins or computer simulations, lacking effective basic research, and the multi-dimensional drug efficacy evaluation system in this article is exploring the targets for the treatment of COVID-19 from multiple aspects It has certain advantages in terms of drugs.

However, targeted TCM also has certain limitations. There are still many uncertainties about COVID-19, and some of the 29 proteins expressed by SARS-CoV-2 have not yet been identified, so there may be certain limitations in the exploration of targeted TCM. In addition, the COVID-19 virus continues to evolve. Since the emergence of the SARS-CoV-2 virus, it has continued to evolve. WHO has so far designated five variants as SARS-CoV-2 Variants of Concern (VOC) – Alpha, Beta, Gamma, Delta, and Omicron – due to differences in transmission, disease severity, or immunity [104]. Therefore, the exploration of targeted Chinese medicine is also difficult and limited. At the same time, it takes a certain amount of time to establish a complete exploration model of COVID-19 targeted drugs and a multi-dimensional comprehensive efficacy evaluation system, and there are also certain requirements for the experimental process. Therefore, it will take longer and more research than the existing model.

4.2. Summary

Effective formulations are effective in the treatment of COVID-19. To deal with new infectious diseases and discover innovative and effective drugs against COVID-19, it is necessary to establish a pharmacodynamic evaluation system to discover effective molecules, ingredients, and formulations quickly and efficiently. Therefore, a comprehensive pharmacodynamic evaluation system is essential. This article established a complete COVID-19 targeted drug discovery model, and a multi-dimensional integrated effect evaluation system of molecules, cells, and animals was constructed. 19 Targeted TCM treatment provides new ideas and directions based on experiments and lays a good foundation for

future clinical work. Prepare for new infectious disease outbreaks while making emergency stockpiles.

Declaration

Author contribution statement

Yuting Sun, Xuedong An: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Liyun Duan, Yuehong Zhang, Cunqing Yang, De Jin, Yingying Duan, Rongrong Zhou, Yiru Zhao, Yuqing Zhang, Xiaomin Kang, Linlin Jiang: Analyzed and interpreted the data; Wrote the paper.

Fengmei Lian: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Declaration of interest's statement

The authors declare no competing interests.

Data availability statement

No data was used for the research described in the article.

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Additional information

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