Supplementary Table 1. List of TCM patent medicines used in the treatment of COVID-19

Pinyin	Simplified Chinese	Classified Chinese
An Gong Niu Huang Pill	安宫牛黄丸	安宮牛黃丸
Dang Gui Long Hui Pill	当归龙荟丸	當歸龍薈丸
Gu Biao Jie Du Ling	固表解毒灵	固表解毒靈
Huo Xiang Zheng Qi Shui	藿香正气水	藿香正氣水
Jin Hua Qing Gan Granule	金花清感颗粒	金花清感顆粒
Jin Yin Hua Tang	金银花汤剂	金銀花湯劑
Jing Yin Granule	荆银颗粒	荊銀顆粒
Kang Bing Du Granules	抗病毒颗粒	抗病毒顆粒
Kang Du Bu Fei Tang	抗毒补肺汤	抗毒補肺湯
Ke Qing Capsule	咳清胶囊	咳清膠囊
Ke Su Ting Syrup	咳速停糖浆	咳速停糖漿
Lian Hua Qing Wen Capsule	连花清瘟胶囊	連花清瘟膠囊
Ma Xin Gan Shi Tang	麻杏甘石汤	麻杏甘石湯
Qing Fei Pai Du Tang	清肺排毒汤	清肺排毒湯
Qing Yi-4	清疫 4 号	清疫 4 號
Re Du Ning Injection	热毒宁注射液	熱毒寧注射液
Sang Ju Yin	桑菊饮	桑菊飲
Shen Fu Injection	参附注射液	參附注射液
Shen Mai Injection	参麦注射液	參麥注射液
Shen Qi Fu Zheng Injection	参芪扶正注射液	參芪扶正注射液
Sheng Mai Injection	生脉注射液	生脈注射液
Shu Feng Jie Du Capsule	疏风解毒胶囊	疏風解毒膠囊
Shuang Huang Lian Oral Liquid	双黄连口服液	雙黃連口服液
Su He Xiang Pill	苏合香丸	蘇合香丸

Tan Re Qing Injection	痰热清注射液	痰熱清注射液
Xi Yan Ping Injection	喜炎平注射液	喜炎平注射液
Xin Guan-1 Formula	新冠一号方	新冠一號方
Xin Guan-2 Formula	新冠二号方	新冠二號方
Xing Nao Jing Injection	醒脑静注射液	醒腦靜注射液
Xue Bi Jing Injection	血必净注射液	血必淨注射液
Yu Ping Feng San	玉屏风散	玉屏風散

The clinical results of "Clear Lung Detox Soup" were published in the International Journal: The earlier the clinical outcome of using Clear Lung Detox Soup to treat new coronary pneumonia, the better

In the current external defense input, internal defense rebound of the epidemic prevention and control of a new stage, "clear lung detox soup" emergency project team carried out a clinical research results published in international journals. The study showed that "clear lung detox soup" is an effective treatment for new coronary pneumonia, and early intervention can lead to better clinical outcomes. This result provides solid evidence for the good efficacy of Chinese medicine in treating patients with new crown pneumonia.

The study, a large sample multicenter retrospective cohort study, included data on 782 new coronary pneumonia in 54 hospitals in nine provinces in China. All patients were treated with "clear lung detox soup" on the basis of the use of Western medicine in accordance with the New Coronary Pneumonia Treatment Programme (Trial 7th Edition). According to the time from the onset of the first clinical symptoms to the beginning of the use of "clear lung detox soup", patients were divided into ≤ 1 week group (≤ 7 days), 2 weeks group (> 7 days and ≤ 14 days), 3 weeks group (> 14 days and ≤ 21 days) and > 3 weeks group (> 21 days) to explore the relationship between the treatment time of "clear lung detox soup" early and late and clinical outcome improvement.

The results show that the early use of "clear lung detox soup" can significantly improve the time of clinical healing, clinical healing rate and nucleic acid to cloudy days, the duration of the disease and other indicators. Compared with the treatment of

"clear lung detox soup" after 3 weeks of symptoms for the first time, the clinical recovery time of patients treated with "clear lung detox soup" in early stages was significantly reduced by 2-3 times (≤ 1 week group vs> 3 weeks group was 3.81, 95% CI: 2.65-5.48; 2-week group vs >3-week group: 2.63, 95% CI: 1.86-3.73; 3-week group vs >3-week group: 1.92, 95% CI: 1.34-2.75); The medium time of viral nucleic acid transition decreased from 17 days to 13 days, 12 days and 12 days respectively (P s 0.0137); The intermediate time of the course of illness decreased from 34 days to 24 days, 21 days and 18 days (P< 0.0001); The medium length of hospitalization decreased from 18 days to 15 days, 15 days and 14 days (P< 0.0001).

The findings were published on the Lancet preprinted platform on July 31, 2020, and once published, received widespread attention and downloads, and were eventually published in the journal Pharmacological Research (IF:5.893) under the title "Association between early treatment and Qing Payfeiu decoction and favorable." clinical outcomes in patients with COVID-19: A retrospective multicenter cohort study》 。

Since the outbreak of New Coronary Pneumonia, the State Administration of Traditional Chinese Medicine has urgently initiated the screening and clinical observation of effective prescription drugs, guided by clinical emergency use, practicality and utility. On January 27th, Ge Hewen, a special researcher of the Chinese Academy of Traditional Chinese Medicine, used the "clear lung detox soup" for the clinical treatment of confirmed patients based on the core disease machine of new crown pneumonia, based on ancient classics, and achieved good results, reflecting the unity of integrity and innovation, disease identification and dialectics. On February 6th, the clinical observation results of the treatment of confirmed patients with "clear lung detox soup" were published to the whole society, as well as prescriptions and usages, which

released the signal that there was a cure, improved the accessibility of Chinese medicine, and greatly boosted the self-confidence of Chinese medicine practitioners. In the sixth, seventh and eighth editions of the national treatment program, "clear lung detox soup" is the only treatment of light, ordinary, heavy and critical heavy patients of the general prescription agent, in 28 provinces (regions, cities) in the country has been widely used, but also Hubei, Wuhan anti-epidemic main battlefield, as well as assistance to international anti-epidemic and treatment of imported cases of the largest use, the best effect of Chinese medicine prescription.

“清肺排毒汤”临床研究成果在国际期刊上发表：越早使用清肺排毒汤治疗新冠肺炎临床结局越好

来源： 时间：2020-12-15 08:45:23

在当下外防输入、内防反弹的疫情防控新阶段，“清肺排毒汤”应急项目组开展的一项临床研究成果在国际期刊上发表。该研究显示，“清肺排毒汤”是治疗新冠肺炎的有效措施，且早期干预能够获得更好的临床结局。此项成果为中医药救治新冠肺炎患者的良好疗效提供了坚实的证据。

此项研究为大样本多中心回顾性队列研究，共纳入中国 9 个省份 54 家医院 782 例新冠肺炎住院患者的数据。所有患者均在使用西药的基础上按《新型冠状病毒肺炎诊疗方案(试行第七版)》使用“清肺排毒汤”进行治疗。根据首次临床症状出现至开始使用“清肺排毒汤”的时间，将患者分为≤1 周组(≤7 天)、2 周组(>7 天和≤14 天)、3 周组(>14 天和≤21 天)和>3 周组(>21 天)，以探讨“清肺排毒汤”治疗时间早晚与临床结局改善的关系。

研究结果发现，在轻型、普通型患者和重症、危重型患者中，早期使用“清肺排毒汤”可显著改善临床痊愈时间、临床痊愈率及核酸转阴天

数、病程时间等指标。与首次出现症状 3 周后给予“清肺排毒汤”治疗相比，早期使用“清肺排毒汤”治疗的患者临床痊愈时间显著缩短 2-3 倍(≤1 周组 vs >3 周组=3.81，95% CI：2.65-5.48；2 周组 vs >3 周组=2.63，95% CI：1.86-3.73；3 周组 vs >3 周组=1.92，95% CI：1.34-2.75)；病毒核酸转阴中位时间分别从 17 天依次递减为 13 天、12 天、12 天(P = 0.0137)；病程中位时间从 34 天依次递减为 24 天、21 天和 18 天(P < 0.0001)；住院中位时间由 18 天依次递减为 15 天、15 天、14 天(P < 0.0001)。

研究结果于 2020 年 7 月 31 日在《柳叶刀》预印版平台发表，一经发表获得了广泛关注和下载量，并最终发表在《Pharmacological Research》(IF:5.893)杂志上，发表题目为《Association between early treatment with Qingfei Paidu decoction and favorable clinical outcomes in patients with COVID-19: A retrospective multicenter cohort study》。

新冠肺炎疫情发生以来，国家中医药管理局以临床急用、实用、效用为导向，紧急启动有效方药筛选和临床观察工作。1 月 27 日，将中国中医科学院特聘研究员葛又文针对新冠肺炎核心病机、基于古代经典名方，精心化裁的“清肺排毒汤”用于确诊患者临床救治，取得了良好疗效，体现了守正与创新的统一、辨病与辨证的统一。2 月 6 日，向全社会公布了“清肺排毒汤”救治确诊患者的临床观察结果，以及处方和用法，释放了有药可治的信号，提高了中医药的可及性，也大大提振了中医人自信心。在新冠肺炎第六版、第七版、第八版国家诊疗方案中，“清肺排毒汤”是唯一一个治疗轻型、普通型、重型和危重型患者的通用方剂，在全国 28 个省(区、市)得到广泛使用，也是湖北、武汉抗疫主战场，以及援助国际抗疫和治疗输入性病例使用量最大、效果最好的中药方剂。

http://www.satcm.gov.cn/hudongjiaoliu/guanfangweixin/2020-12-15/18987.html

Chinese medicine "clear lung detox soup" cured 104 cases of new crown pneumonia patients

Heilongjiang Province, the Chinese Medicine_Administration announced on the 19th, as One Of China'S Four "Clear Lung_DetoX_Soup" Clinical Application Pilot ProvinceS, As Of The 18th, Heilongjiang Province Has Been Cured And DischargeD From The New Coronary Pneumonia (COVID-19, Commonly known As WuhaN Pneumonia) PatientS Reached 104 CaseS, Of Which The Use Of Chinese Medicine To Participate In The Treatment Of Confirmed CaseS Of 95.26%, More Than The Chinese Average.

China News Agency reported that since the outbreak of Xinguan pneumonia,_Heilongjiang Province to promote Chinese medicine to participate in the prevention and treatment of New Coronary Pneumonia, the establishment of Chinese medicine treatment groups in various cities, the designated hospitals adhere to the combination of Chinese and

Western medicine treatment. The provincial Administration of Traditional Chinese Medicine sent five groups of more than 60 Chinese medicine experts to support the New Coronation Pneumonia Fixed-Point Hospital and Heilongjiang Provincial Intensive Care Center.

As of the 18th, the cumulative number of chinese medicine cases involved in the treatment of confirmed cases is 95.26 percent, more than the Chinese average, of which the use of Traditional Chinese medicine_soup accounted for 63.12 percent, but also the use of traditional Chinese medicine and Chinese medicine injections.

"Clear Lung Detox Soup" by Zhang Zhongjing of the Han Dynasty, "typhoid hybrid theory" in a number of treatments caused by cold evil of the classic prescription of fever, from the chinese medicine theory, the new crown pneumonia belongs to internal humidity, external cold induced, so the use of clear lung detox soup for its treatment.

Jiamus City, Heilongjiang Province, to the city's patients have been applied "clear lung detox soup", after a combination of Chinese and Western medicine treatment, 15 confirmed patients have been 11 recovered from the hospital. The city's famous Chinese medicine formulated anti-flu tea No. 1, has been used by 4,700 people, the effect is good, Chixi City, the use of Traditional Chinese medicine to treat new coronavirus infection, as of February 17, the city received Chinese medicine treatment 25 people, 100% efficiency, 2 people have been treated by professional Chinese medicine to recover from the hospital.

At present, the province has prepared three teams of Chinese medicine medical personnel, taking the lead in the preparation of three Chinese and Western medicine collaborative treatment wards, set up 1000 beds, now has the basic conditions for treating patients, ready to be put into use.

中醫藥「清肺排毒湯」 治癒 104 例新冠肺炎患者

黑龍江省中醫藥管理局 19 日發布消息，作為中國確定的 4 個「清肺排毒湯」臨床應用試點省份之一，截至 18 日，黑龍江省已治癒並出院的新型冠狀肺炎(COVID-19，俗稱武漢肺炎)患者達 104 例，其中使用中醫藥參與治療的確診病例為 95.26%，

超過中國平均水平。中藥示意圖。 圖 / 123RF

中新社報導，自新冠肺炎疫情發生後，黑龍江省推動中醫藥參與新冠肺炎防治，各地市成立中醫藥救治組，各定點醫院堅持中西醫結合治療。該省中醫藥管理局派出了 5 批 60 多人的中醫專家，支援新冠肺炎定點救治醫院和黑龍江省重症救治中心。

截至 18 日，中醫藥參與治療確診病例累計例數占比是 95.26%，超過中國平均水平，其中使用中藥湯劑佔比是 63.12%，也使用中成藥和中藥注射劑。

「清肺排毒湯」由漢代張仲景所著「傷寒雜病論」中多個治療由寒邪引起的外感熱病的經典方劑優化組合而成；從中醫理論來講，新冠肺炎屬於內濕為患、外寒誘發，所以使用清肺排毒湯對其治療。

黑龍江省佳木斯市給該市患者均應用了「清肺排毒湯」，經中西醫結合治療，15 名確診患者已有 11 名痊癒出院。該市名中醫配製出抗流感茶飲 1 號，已對 4700 人使用，效果良好；雞西市採用中藥救治新型冠狀病毒感染，截至 2 月 17 日，該市接受中藥治療 25 人，有效率 100%，已有 2 人經過專業的中醫治療痊癒出院。

目前，該省準備了 3 支中醫醫務人員隊伍，帶頭籌建 3 個中西醫協作救治病房，設 1000 張床位，現已具備收治病人的基本條件，隨時可以投入使用。

https://health.udn.com/health/story/120950/4360037

frontiers in Pharmacology

REVIEW
published: 27 January 2021
doi: 10.3389/fphar.2020.589714

Research Advance on Qingfei Paidu Decoction in Prescription Principle, Mechanism Analysis and Clinical Application

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Since the sudden epidemic of coronavirus disease 2019 (COVID-19), the State Administration of Traditional Chinese Medicine immediately organized experts to formulate and screen the effective prescriptions of traditional Chinese medicine according to the characteristics of the novel coronavirus infection. Qingfei Paidu decoction (QFPDD) has been proven to be effective in multi-provincial clinical trials, and has been selected as a general prescription for the treatment of COVID-19 in different stages that was later promoted to be used nationwide. This review highlights the latest advances of QFPDD, focusing on the TCM theory, mechanism analysis, clinical application of QFPDD and its future perspectives. Moreover, an in-depth discussion of some valuable issues and possible development for future research on QFPDD is also discussed, aiming to provide a novel guide to combat the global epidemic COVID-19.

Keywords: qingfei paidu decoction, novel coronavirus pneumonia, prescription principle, mechanism analysis, clinical application

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: *31 July 2020*
Accepted: *10 November 2020*
Published: *27 January 2021*

Citation:
Ren W, Ma Y, Wang R, Liang P, Sun Q, Pu Q, Dong L, Mazhar M, Luo G and Yang S (2021) Research Advance on Qingfei Paidu Decoction in Prescription Principle, Mechanism Analysis and Clinical Application. Front. Pharmacol. 11:589714. doi: 10.3389/fphar.2020.589714

1 INTRODUCTION

As of November 1, 2020, novel coronavirus pneumonia (COVID-19), has spread over 211 countries around the world including all the continents, except Antarctica with around 46.43 million cumulative confirmed cases and 1.2 million deaths due to its strong infectiousness. The prevalence of COVID-19 has surpassed that of SARS in 2003, and is recognized as a severe health menace worldwide.

Since December 1, 2019, COVID-19 was emerged in Wuhan, Hubei province, China. Subsequently, the epidemic broke out throughout the country with the floating population during the Spring Festival. The mode of transmission for COVID-19 was soon recognized to be the inhalation of droplets from sneezing and coughing or the physical contact with the mucous secretions from infected individuals. People were generally susceptible and contracting the COVID-19 infection at exponentially high rate. Due to the sudden rise in the number of COVID-19 cases, China immediately launched the nationwide strict epidemic prevention and control guidelines. According to the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases, the COVID-19 epidemic is listed in class-B infectious disease while it is managed in accordance with Class A infectious diseases (Xue et al., 2020). Until now, the number of cases infected by COVID-19 continues to grow around the globe, and it is predicted to be continued for longer period of time (Guan et al., 2020). Still until now, proper and effective targeted therapy, drugs

or vaccines, for COVID-19 epidemic control has not been identified. The accessibility of traditional drugs based on natural origin with effective therapeutic potential and the valuable historical treatment experience provide a more prominent therapeutic approach against COVID-19. Traditional Chinese medicine (TCM) has accumulated rich experience in the long-term practice of epidemic prevention and treatment, and it is characterized by broad-spectrum immunity, universal adaptability, foresight and so on. The unique advantages of TCM have attracted more and more attention to the epidemic prevention and treatment of COVID-19 (Yang et al., 2020a). Therefore, on January 27, 2019, the National Administration of Traditional Chinese Medicine launched the "Clinical screening for effective prescriptions of TCM for the prevention and treatment of pneumonia caused by novel coronavirus (2019-nCoV) infection" under the criteria "urgent, practical and effective"; nationwide. Qingfei Paidu Decoction (QFPDD), a multi-component herbal formula, was used clinically to treat 214 confirmed cases of COVID-19 with for three consecutive days as a course of treatment in four different pilot provinces in China from January 27, 2020 to February 5, 2020. The total effective rate was more than 90%, among them more than 60% of the cases showed significant improvement of symptoms and imaging manifestations and 30% of the patients showed stability of symptoms without aggravation or worsening (Yao et al., 2020). On February 18, 2020, the National Health Commission and National Administration of TCM jointly issued document No. 145 Diagnosis and Treatment Program of Novel Coronavirus Pneumonia (Trial Sixth edition). The document proposed to officially include TCM, QFPDD, in the clinical treatment of confirmed COVID-19 cases. QFPDD has been recommended as a general treatment prescription of TCM treatment for COVID-19 and has been promoted to the whole country for its remarkable clinical effect in the clinical prescription screening (Qin et al., 2020). Throughout the country, around 28 provinces, autonomous regions, and cities have been using this prescription, which is suitable for all periods and symptoms of COVID-19. Currently its use has been extended to treat suspected cases which has also been found effective and the feedback received is good.

QFPDD is formulated by the combination of syndrome differentiation and innovation based on the four classical prescriptions in *Treatise on Febrile Diseases* according to the pathogenic characteristics and development laws of COVID-19 (Jin, 2020). QFPDD has manifested its potential advantages and beneficial effects for the treatment of COVID-19. In this review, after summarizing the extant literature including CNKI, PubMed, Springer, Taylor & Francis, Google Scholar, and Baidu Scholar databases and other scientific resources e.g., Chinese Pharmacopoeia, 2020 edition, postgraduate research (PhD and MSc thesis, etc.), we have systematically summarized the TCM theory, modern mechanism analysis, clinical practice and application of QFPDD, hoping that it could offer some enlightenment for the further development and propel the research forward for efficiency, safety and controllable quality of QFPDD, so as to provide strong support for the global fight against the COVID-19 (**Supplementary Figure**).

1.1 TCM Theory and Prescription Principle of QFPDD

According to TCM theory, the experts have reached a consensus that COVID-19 belongs to a category of phytophthora blight (Li et al., 2020a), however, different experts have different understandings of COVID-19, including damp-toxin epidemic, cold-damp epidemic, and damp-heat epidemic. Wang and Miao et al. (2020) proposed COVID-19 as a damp-toxin epidemic caused by the damp toxin that belongs to yin, with the injury of *Yang* as the mainline (Miao et al., 2020; Wang et al., 2020c). Some believed that COVID-19 is a cold-damp epidemic caused by noxious dampness, and the basic pathogenesis is characterized by dampness, poison, blood stasis, and closure (Tong et al., 2020; Wang et al., 2020c; Xue et al., 2020). Luo and Zeng (2020) considered that COVID-19 is a damp-heat type caused by damp-heat epidemic toxin, and the main pathogenesis is the dampness, heat block of the Qi movement, endogenesis of phlegm and its transformation into fire and toxin, cremation of toxin and the combination of heat and blood stasis (Luo et al., 2020; Zeng and Sun, 2020).

Based on comprehensive analysis of COVID-19 clinical manifestations and syndrome types issued by the National Health Commission of PRC and various provinces in response to local conditions (**Table 1**), it is considered that COVID-19 is a damp-heat lung plague caused by damp-heat and epidemic toxin, and the pathogenesis and evolution process can be dry, and fire, and wind. At the very beginning, the pestilence attacks from the *Taiyang* meridian into the *Yangming* meridian quickly, or straight into the three *Yang* meridians, which is called *concurrent disease of three yang meridians*. But sometimes there is cold-dampness surrounding the exterior along with intense interior pathogenic fire. Or at the beginning, the exogenous pathogenic factors invade into three *Yin* meridians quickly, which may conduce to the syndrome of internal blockade and external collapse or syncope and collapse syndrome. The intermingled dampness and heat block the Qi movement, turbid phlegm hence appears inside and transforms into fire and toxin, intermingled heat and stasis is its main pathogenesis. Therefore, the treatment should be focused on dispelling dampness, heat and damp toxin, clearing dampness in triple warmer as well as strengthening vital Qi to eliminate pathogenic factors (Luo and Chen, 2020; Zhou, 2020). The most typical syndrome of COVID-19 is concurrent disease of three *Yang* meridians, which is often common in mild, moderate and part of severe cases. Hence, QFPDD is prescribed especially for this kind of syndrome.

QFPDD is composed of 21 TCMs, including *Herba Ephedrae* (*Ephedra sinica* Stapf; 9 g), *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.; 6 g; baked), *Semen Armeniacae Amarum* (*Prunus armeniaca* L.; 9 g), *Raw Gypsum* (15–30 g; first decocted), *Ramulus Cinnamomi* (*Cinnamomum cassia* (L.) J.Presl; 9 g), *Rhizoma Alismatis* (*Alisma plantago-aquatica* Linn.; 9 g), *Polyporus Umbellatus* (*Polyporus umbellaru* (Pers.) Fr.; 9 g), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala* Koidz.; 9 g), *Poria* (*Poria cocos* (Schw.) Wolf.;

TABLE 1 | TCM Syndrome Types of COVID-19 in national and various provinces' prevention and control programs.

Countries and regions	Mild	Moderate/Ordinary	Severe	Critical	Convalescence
National health commission	Cold-dampness retention lung syndrome; damp-heat retention lung syndrome	Pathogenic dampness retention lung syndrome; cold-dampness stagnating the lung	Syndrome of epidemic toxin obstructing lung; syndrome of flaring heat in qifen and yingfen	Syndrome of internal blockade and external collapse	Lung and spleen qi deficiency, deficiency of both qi and yin
Hubei province	Syndrome of heat-toxin invading lung	Pathogenic dampness retention lung syndrome	Syndrome of accumulated dampness-toxicity	Syndrome of blazing heat-toxin	NA
Heilongjiang province	Damp warm retention lung syndrome	Phlegm-heat retention lung syndrome	Syndrome of pathogenic toxin obstructing lung	Syndrome of pathogenic toxin clouding orifices	Pathogenic factors residue, deficiency of both qi and yin
Beijing province	Syndrome of epidemic toxin invading lungs	NA	Epidemic toxin retention lung syndrome	Syndrome of epidemic toxin obstructing lung	Deficiency of both qi and yin
Shanghai province	Noxious dampness retention lung syndrome	NA	Syndrome of heat-toxin obstructing lung	Syndrome of internal blockade and external collapse	Lung and spleen qi deficiency, deficiency of both qi and yin
Guangdong province	Pathogenic dampness stagnating the lung, cardinal disadvantageous; syndrome of pathogenic heat congesting lung, impairment of the ascending and descending function of the lung	NA	Pathogenic heat obstructing lung syndrome, obstruction of fu-qi; warm-heat obstructing lung syndrome	Syndrome of internal blockade and external collapse	Pathogenic factors residue, deficiency of both qi and yin, deficiency of both lung and spleen
Jiangxi province	Noxious dampness retention lung syndrome, cardinal disadvantageous	Heat-toxin with dampness syndrome, impairment of the ascending and descending function of the lung	Syndrome of heat-toxin obstructing lung, obstruction of fu-qi	Syndrome of internal blockade and external collapse	NA
Shanxi province	Syndrome of exterior tightened by cold-dampness, impairment of fluid due to heat retention; syndrome of heat-toxin invading lung; external-cold and internal-heat	NA	Heat-toxin retention lung syndrome	Syndrome of internal blockade and external collapse	Syndrome of lingering heat, deficiency of both qi and yin
Tianjin province	Syndrome of heat-toxin invading lung	Pathogenic dampness retention lung syndrome	Syndrome of accumulated dampness-toxicity	Syndrome of blazing heat-toxin	NA
Yunnan province	Dampness-heat retention lung syndrome	Pathogenic heat retention lung syndrome	Syndrome of pathogenic toxin obstructing lung	Syndrome of internal blockade and external collapse	NA
Sichuan province	Wind heat with dampness syndrome; wind chill with dampness syndrome	Pathogenic dampness retention lung syndrome; dampness-heat retention lung syndrome	Pathogenic heat retention lung syndrome; epidemic toxin obstructing lung syndrome	Syndrome of internal blockade and external collapse	Pathogenic factors residue, deficiency of both qi and yin
Gansu province	Syndrome of warm pathogen attacking lung	Warm-heat retention lung syndrome	Syndrome of warm toxin obstructing lung	Syndrome of internal blockade and external collapse	NA

15 g), *Radix Bupleuri* (*Bupleurum chinensis* DC.; 16 g), *Radix Scutellariae* (*Scutellaria baicalensis* Georgi; 6 g), *Rhizome Pinelliae Preparata* (*Pinellia ternata* (Thunb.) Breit.; 9 g; processed with ginger), *Rhizoma Zingiberis Recens* (*Zingiber officinale* Roscoe; 9 g), *Radix Asteris* (*Aster tataricus* Linn. f.; 9 g), *Flos Farfarae* (*Tussilago farfara* Linn.; 9 g), *Rhizoma Belamcandae* (*Iris domestica* (L.) Goldblatt & Mabb.; 9 g), *Herba Asari* (*Asarum sieboldii* Miq.; 6 g), *Rhizoma Dioscoreae* (*Dioscorea oppositifolia* L.; 12 g), *Fructus Aurantii Immaturus* (*Citrus sinensis* Osbeck; 6 g), *Pericarpium Citri Reticulatae* (*Citrus aurantium* L.; 6 g), and *Herba Pogostemonis* (*Pogostemon cablin (Blanco)* Benth.; 9 g). This prescription is mainly composed of Maxing Shigan decoction, Shegan Mahuang decoction, Xiaochaihu decoction and Wuling powder. In addition, it also incorporates Daqinglong decoction, Juzhijiang decoction, Fuling Xingren Gancao decoction, etc. QFPDD is a syncretic innovation of classical prescriptions from *Treatise on Febrile Diseases*, which act on different stages and viscera of water, dampness, phlegm, and fluid (Fan et al., 2020). This formula is suitable for the pathogenesis of COVID-19, affecting cold, dryness, damp toxin and dampness, and can effectively improve the symptoms. TCM theory and composition mechanism of QFPDD are summarized in **Figure 1**. The meridian tropisms of drugs in QFPDD are shown in **Figure 2**, where the top meridian tropism in QFPDD is lung meridian, indicating that drugs in QFPDD are mainly specific for lung diseases. The prescriptions of QFPDD are synergistic and complementary and the prescription principle of QFPDD is shown in **Figure 3**. Maxing Shigan decoction is to relieve exterior *Taiyang* syndrome, relieve superficies and ventilate lung Qi, clear heat and relieve panting; Shegan Mahuang decoction (*Fructus Jujubae* and *Fructus Schisandra chinensis* were taken out) is for lowering the adverse Qi and resolving fluid ventilate lung Qi, dispelling phlegm and relieving cough; Xiaochaihu decoction is for harmonizing half-superficies and half-interior *Shaoyang* syndrome, and large dose of raw gypsum is used to clear interior heat of the *Yangming* meridian, and Wuling powder is to warm the triple energizer and transform Qi and remove dampness by promoting diuresis; Juzhijiang decoction can activate Qi and dispel phlegm; *Herba Pogostemonis* can exorcise toxins and eliminate dampness; and *Rhizoma Dioscoreae* can strengthen the spleen and supplement the lung (Shen et al., 2020; Wang and Jin, 2020). The combination of *Ramulus Cinnamomi* and *Radix Glycyrrhizae* can nourish *Yang* and support healthy energy. QFPDD is not made up of drugs but multiple concordant prescriptions contributing to get twice the result with half the effort, so that the damp-heat and epidemic toxin can be quickly discharged (Wang et al., 2020a).

1.2 Mechanism Analysis of QFPDD

As described earlier, QFPDD contains a total of 21 TCMs, therefore, it is difficult to clearly explain the complex mechanisms of QFPDD in the treatment of COVID-19. Modern research on TCM holds that Chinese herbal compound formula plays an omnidirectional and overall regulatory role in the body due to the characteristics of multi-components, multi-targets and multi-path of the formula.

Recently, based on the reported components of QFPDD, several research groups have adopted the method of network pharmacology, molecular docking, and computer-aided drug design to provide data and clues for the multi-directional exploration of the material basis and pharmacodynamic mechanism of QFPDD in the treatment of COVID-19. Xu et al. (2020b) used the network pharmacology to screen significant effective compounds and key targets. Using TCMSP database, 148 related targets of 302 bioactive components in QFPDD were screened. Another database, GeneCards, using "COVID-19", "2019-nCoV" and "Novel Coronavirus Pneumonia" as keywords, was used to screen 362 COVID-19 related targets where a total of 23 intersection targets were obtained by Venn analysis. By using the CentiScaPe plug-in of Cytoscape software, the network topology diagram of the 10 significant effective compounds, i.e., quercetin, luteolin, naringenin, kaempferol, beta-sitosterol, stigmasterol, baicalein, isorhamnetin, nobiletin, and wogonin (**Table 2**); and five pivotal targets, i.e., PTGS2, NOS2, PPARG, MAPK14, and PTGS1 were analyzed (**Table 3**). The results of molecular docking of the above most significant compound, quercetin, and target, PTGS2, with the highest degree value showed that the binding and interaction ability between these molecules was strong. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the key targets were done using the Cluster Profiler package of R software, which showed that significant compounds such as quercetin, luteolin, naringin, kaempferol, and baicalein have expectorant, antitussive, antiviral and anti-inflammatory effects in various degrees. The key targets were mainly concentrated in 144 related signaling pathways including IL-17, tuberculosis, human cytomegalovirus infection, TNF, MAPK, Hepatitis B, etc. (**Table 4**). It contained 28 biological effects including cytokine receptor binding, MAP kinase activity and phosphatase binding to regulate and control metabolism, immune regulation, lung function, inflammation, and other physiological processes (Xu et al., 2020b). Xu et al. (2020a) showed that 217 related targets of 186 active components and 200 COVID-19 related targets were screened, and 51 common drug-disease targets were obtained by Venn analysis. Then, five significantly effective compounds i.e., quercetin, luteolin, kaempferol, naringin, and isorhamnetin were obtained by using the CentiScaPe plug-in of Cytoscape software to further construct the network topology diagram. The GO and KEGG pathway enrichment analysis indicated that the key targets were mainly concentrated in 30 related signal pathways such as IL-17, NF-κB, TNF, MAPK, Th17, etc. It involved several biological functions such as inflammation, immune regulation, neuroprotection, reduction of lung injury, and other physiological processes (Xu et al., 2020a).

Zhao et al. revealed that 464 compounds of QFPDD corresponded to 790 different putative targets, of which 232 targets were co-expressed with angiotensin-converting enzyme 2 (ACE2), the receptor of 2019-nCoV. Main signaling pathways regulated by key targets of QFPDD are shown in **Table 3**, where the main targets are concentrated on two types of disease pathways i.e., virus infection and lung injury. In addition, 48 important targets interacted densely

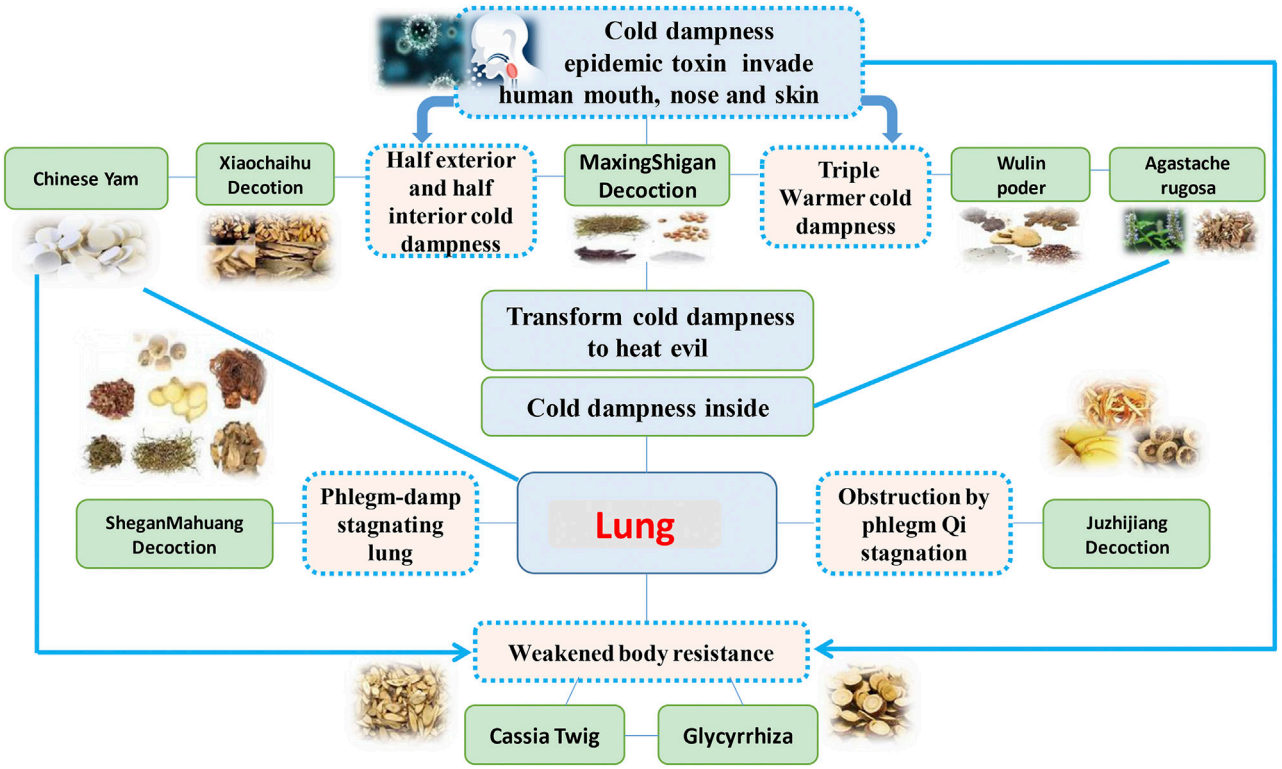

FIGURE 1 | TCM theory and composition mechanism of durgs in QFPDD.

with six proteins of HIV, indicating its potential antiviral effect. Key targets regulated a series of signaling pathways in biological processes such as endocrine system, immune system, translation, nervous system, and signal transduction (Zhao et al., 2020).

Wu et al. (2020b) showed that the QFPDD compound-pneumonia target network contained 292 compounds and 214 corresponding potential targets and the top five pivotal targets were AKT serine/threonine kinase 1 (AKT1), interleukin-6 (IL-6), mitogen-activated protein kinase 8 (MAPK8), mitogen-

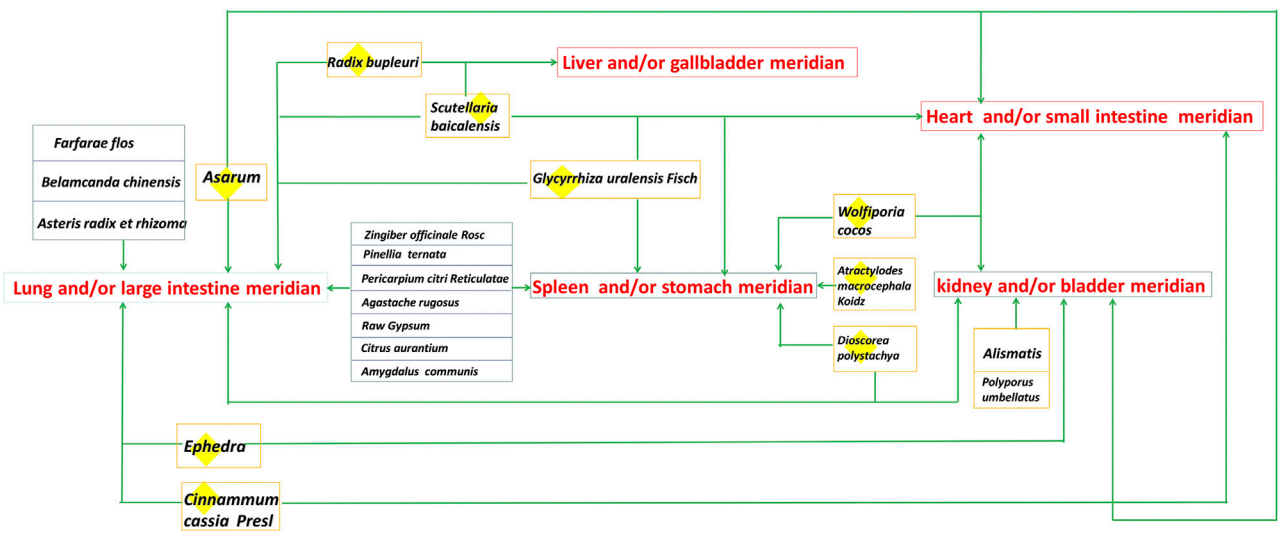

FIGURE 2 | Drug-meridian network of QFPDD.

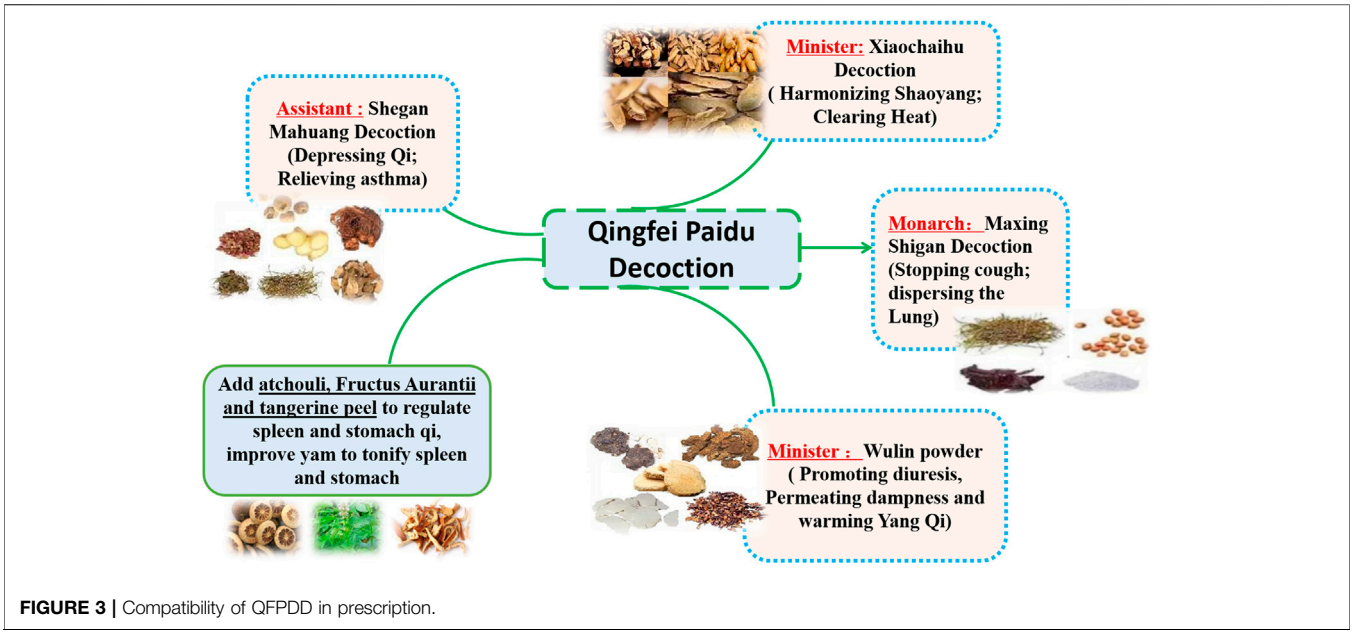

FIGURE 3 | Compatibility of QFPDD in prescription.

activated protein kinase 1 (MAPK1), and jun proto-oncogene (JUN). The GO and KEGG enrichment analysis and screening yielded 122 related signaling pathways, including non-small cell lung cancer, small cell lung cancer, hypoxia inducible factor-1, toll-like receptor signaling pathway, T cell receptor signaling pathway and other pathways related to pneumonia. Moreover, the same enrichment analysis also included TNF signaling pathway, P13k-Akt signaling pathway, MAPK signaling pathway, B cell receptor signaling pathway, apoptosis, and other pathways related to the reduction of lung injury (**Table 4**). The molecular docking results indicated that some core compounds such as ergosterol, shionone, tussilagone, etc. of the TCMs present in QFPDD had a certain degree of binding activity for 2019-nCoV 3C-like protease (3CLpro) and ACE2. It was worthwhile pointing out that ergosterol is the only one that can form a hydrogen bond with 3CLpro of 2019-nCoV (Wu et al., 2020b). In another study by Yan et al. (2020) QFPDD compound-2019-nCoV and COVID-19 target-biological function network was screened, it contained 163 active ingredients, 10 protein targets, and 42 biological functions such as renin-angiotensin regulation of blood volume and systemic arterial blood pressure to treat COVID-19. The results of preliminary molecular docking showed that the core ingredients had a good affinity with SARS-CoV-2 3CL hydrolase to form complexes with stable conformations and high binding energy, indicating that QFPDD might treat COVID-19 through RAS signaling pathway (Yan et al., 2020). Cytokine storm is considered one of the central causes of clinical sudden deterioration of COVID-19. It has been reported that QFPDD had an inhibitory effect on cytokine storm in the treatment of COVID-19 by acting on multiple targets and pathways with multiple components (Zhou et al., 2020). Duan et al. (2020) revealed that QFPDD had a potential common action mechanism in the treatment of SARS, MERS, and COVID-19. 337 corresponding targets of 246 components in QFPDD and 148 common disease-related targets for SARS, MERS, and COVID-19 were screened, and 44 common drug-disease targets were obtained by Venn analysis. The GO and KEGG pathway enrichment analysis of the key targets indicated that the key targets were mainly concentrated in 77 related signal pathways such as pertussis, tuberculosis, MAPK, FoxO, TNF, NOD-like receptor signaling pathways, and other pathways related to viral pneumonia. biological angiogenesis, immune

TABLE 2 | The key active compounds of QFPDD.

Compounds	References	Compounds	References
Quercetin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Beta-sitosterol	Xu et al. (2020b), Wu et al., 2020b
Luteolin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Wogonin	Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020b)
Kaempferol	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Wu et al., 2020b, Xu et al. (2020b)	Baicalein	Xu et al. (2020b)
Naringenin	Xu et al. (2020a), Zhou et al. (2020), Duan et al. (2020), Xu et al. (2020a), Xu et al. (2020b)	Nobiletin	Xu et al. (2020b)
Isorhamnetin	Xu et al. (2020a), Xu et al. (2020b)	Stigmasterol	Xu et al. (2020b)

TABLE 3 | The main key targets of QFPDD in the treatment of COVID-19.

Key targets	References	Key targets	References
Cell tumor antigen p53 (TP53)	Peng et al. (2020), Duan et al. (2020), Zhou et al. (2020)	Caspase 3 (CASP3)	Peng et al. (2020), Xu et al. (2020a), Yan et al. (2020), Duan et al. (2020), Zhou et al. (2020)
Protein kinase B1(Akt1)	Peng et al. (2020), Wu et al., 2020b	Janus kinase 2 (JAK2)	Peng et al. (2020)
Nuclear factor nuclear transcription factor-κB p105 subunit (NFKB1)	Peng et al. (2020)	Nuclear factor transcription factor-κB p100 subunit (NFKB2)	Peng et al. (2020)
Nuclear factor p65 subunit (RELA)	Peng et al. (2020)	Calmodulin 1 (CALM1)	Peng et al. (2020), Xu et al. (2020a)
Adenylate cyclase type 1 (ADCY1)	Peng et al. (2020)	Eukaryotic translation initiation factor 2, subunit 3 (EIF2S3)	Peng et al. (2020)
Adenylate cyclase type 2 (ADCY2)	Peng et al. (2020)	B-cell CLL/lymphoma 2 (BCL2)	Peng et al. (2020), Xu et al. (2020a), Zhou et al. (2020)
Heat shock protein α A1 (HSP90AA1)	Peng et al. (2020)	Protein kinase C-delta (PRKCD)	Peng et al. (2020)
Adenylate cyclase type 5 (ADCY5)	Peng et al. (2020)	Jun proto-oncogene (JUN)	Peng et al. (2020), Wu et al., 2020b
Recombinant human glucocorticoid receptor (NR3C1)	Peng et al. (2020)	Prostaglandin-endoperoxide synthase 2 (PTGS2)	Xu et al. (2020a), Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)
Mitogen-activated protein kinase 8 (MAPK8)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020), Wu et al., 2020b	Prostaglandin-endoperoxide synthase 1 (PTGS1)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)
Mitogen-activated protein kinase 3 (MAPK3)	Peng et al. (2020), Xu et al. (2020a), Zhou et al. (2020)	Dipeptidyl peptidase-4 (DPP4)	Xu et al. (2020a), Yan et al. (2020)
Human NK-κB inhibited protein α (NFKBIA)	Peng et al. (2020)	V-rel reticuloendotheliosis viral oncogene homolog A (RELA)	Xu et al. (2020a), Zhou et al. (2020)
Bcl2-associated X protein (BAX)	Xu et al. (2020a), Zhou et al. (2020)	V-fos FBJ murine osteosarcoma viral oncogene homolog (FOS)	Xu et al. (2020a), Zhou et al. (2020)
Apolipoprotein D (APOD)	Xu et al. (2020a)	Lymphocyte specific tyrosine kinase (LCK)	Peng et al. (2020)
Peroxisome proliferative activated receptor, gamma (PPARG)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)	Signal transducerand activator of transcription 1(STAT1)	Xu et al. (2020a), Zhou et al. (2020)
Nitric oxide synthase (NOS2)	Xu et al. (2020a), Xu et al. (2020b), Zhou et al. (2020)	Retinoblastoma 1 (RB1)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)
Mitogen-activated protein kinase 14 (MAPK14)	Xu et al. (2020a), Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)	Interleukin-6 (IL-6)	Xu et al. (2020a), Zhou et al. (2020), Wu et al., 2020b
Heme oxygenase (decycling) 1 (HMOX1))	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Apoptosis-related cysteine peptidase (CASP8)	Xu et al. (2020a), Zhou et al. (2020)
Intercellular adhesion molecule 1 (ICAM1)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)Z	Superoxide dismutase 1 (SOD1)	Xu et al. (2020a), Zhou et al. (2020)
Epidermal growth factor receptor (EGFR)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Protein kinase C alpha type (PRKCA)	Xu et al. (2020a), Zhou et al. (2020)
Bcl-2-like protein 1 (BCL2L1)	Xu et al. (2020a), Zhou et al. (2020)	Heat shock 70 kDa protein 5 (HSPA5)	Xu et al. (2020a), Zhou et al. (2020)
Mitogen-activated protein kinase 1 (MAPK1)	Xu et al. (2020a), Duan et al. (2020), Wu et al., 2020b	Interleukin-1β (IL-1β)	Xu et al. (2020a), Duan et al. (2020)
Chemokine (C-C motif) ligand 2 (CCL2)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Protein kinase C beta type (PRKCB)	Xu et al. (2020a), Zhou et al. (2020)
Serine protease inhibitor protein E1 (SERPINE1)	Xu et al. (2020a), Zhou et al. (2020)	Nitric oxide synthase 3 (NOS3)	Xu et al. (2020a), Zhou et al. (2020)
Interleukin-2 (IL-2)	Xu et al. (2020a), Zhou et al. (2020)	Heat shock 27 kDa protein 1 (HSPB1)	Xu et al. (2020a), Zhou et al. (2020)
Interleukin-1α (IL-lα)	Xu et al. (2020a), Zhou et al. (2020)	Poly ADP-ribose polymerase 1 (PARP1)	Xu et al. (2020a), Zhou et al. (2020)
Chemokine CXC motif ligand 2 (CXCL2)	Xu et al. (2020a), Zhou et al. (2020)	Chemokine CXC motif ligand 11 (CXCL11)	Xu et al. (2020a), Zhou et al. (2020)
C-reactive protein (CRP)	Xu et al. (2020a), Zhou et al. (2020)	Chemokine CXC motif ligand 10 (CXCL10)	Xu et al. (2020a), Zhou et al. (2020)
CD40 ligand (CD40LG)	Xu et al. (2020a), Zhou et al. (2020)	BCL2-antagonist of cell death (BAD)	Xu et al. (2020a), Zhou et al. (2020)
Interferon regulatory factor 1 (IRF1)	Xu et al. (2020a), Zhou et al. (2020)	Catalase (CAT)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)
Phospholipase A2 (PLA2G4A)	Xu et al. (2020a), Zhou et al. (2020)	cAMP responsive element binding protein 1 (CREB1)	Xu et al. (2020a), Zhou et al. (2020)
Cyclin D3 (CCND3)	Xu et al. (2020a)	Myeloid cell leukemia sequence 1 (MCLl)	Xu et al. (2020a)
Interleukin-4 (IL-4)	Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020)	Cyclin-dependent kinase 4 (CDK4)	Xu et al. (2020a), Zhou et al. (2020)
Angiotensin I-converting enzyme (ACE)	Yan et al. (2020)	Glucose-6-phosphate dehydrogenase (G6PD)	Xu et al. (2020a), Zhou et al. (2020)
Angiotensin I-converting enzyme 2 (ACE2)	Yan et al. (2020)	Furin (FURIN)	Yan et al. (2020)
Angiotensin II type 1 receptor (AT1R/ AGTR1)	Yan et al. (2020)	Caspase 6 (CASP6)	Yan et al. (2020)
Myeloid cell Leukemia sequence 1 (MCL1)	Yan et al. (2020), Zhou et al. (2020)	Polymerase (DNA directed), delta 1, catalytic subunit 125 kDa (POLD1)	Yan et al. (2020)
Tumor necrosis factor (TNF)	Yan et al. (2020), Zhou et al. (2020)	Interleukin- 10 (IL-10)	Duan et al. (2020), Zhou et al. (2020)
Interferon, gamma (IFNG)	Duan et al. (2020), Zhou et al. (2020)	Interleukin-8 (IL-8)	Duan et al. (2020), Zhou et al. (2020)
Transforming growth factor, beta 1 (TGFB1)	Zhou et al. (2020)		

TABLE 4 | Main enriched signaling pathways of QFPDD in the treatment of COVID-19.

Pathway name	References	Pathway name	References
Adherens junction	Zhao et al. (2020)	AGE-RAGE signaling pathway in diabetic complications	Xu et al. (2020b), Xu et al. (2020a)
Focal adhesion	Zhao et al. (2020)	C-type lectin receptor signaling pathway	Xu et al. (2020b), Xu et al. (2020a)
Osteoclast differentiation	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	HIF-1 signaling pathway	Xu et al. (2020b), Xu et al. (2020a), Wu et al., 2020b
Estrogen signaling pathway	Zhao et al. (2020)	Toxoplasmosis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
Thyroid hormone signaling pathway	Zhao et al. (2020), Wu et al., 2020b	*Yersinia* infection	Xu et al. (2020b)
Relaxin signaling pathway	Zhao et al. (2020)	Hepatitis B	Xu et al. (2020b), Xu et al. (2020a), Wu et al., 2020b
Prolactin signaling pathway	Zhao et al. (2020), Wu et al., 2020b	NOD-like receptor signaling pathway	Xu et al. (2020b), Wu et al., 2020b, Duan et al. (2020), Zhou et al. (2020)
Oxytocin signaling pathway	Zhao et al. (2020)	Kaposi sarcoma-associated herpesvirus infection	Xu et al. (2020b), Xu et al. (2020a)
Glucagon signaling pathway	Zhao et al. (2020)	Pertussis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
Th17 cell differentiation	Zhao et al. (2020), Xu et al. (2020b)	Leishmaniasis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)
B cell receptor signaling pathway	Zhao et al. (2020), Wu et al., 2020b	Endocrine resistance	Xu et al. (2020b)
T cell receptor signaling pathway	Zhao et al. (2020), Wu et al., 2020b	FoxO signaling pathway	Xu et al. (2020b), Wu et al., 2020b, Duan et al. (2020)
Neurotrophin signaling pathway	Zhao et al. (2020)	Prion diseases	Xu et al. (2020b)
Dopaminergic synapse	Zhao et al. (2020)	Pancreatic cancer	Wu et al., 2020b, Xu et al. (2020b)
ErbB signaling pathway	Zhao et al. (2020), Wu et al., 2020b	Hepatitis C	Wu et al., 2020b, Duan et al. (2020)
MAPK signaling pathway	Zhao et al. (2020), Duan et al. (2020), Zhou et al. (2020)	Ras signaling pathway	Wu et al., 2020b
PI3K-Akt signaling pathway	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Bladder cancer	Wu et al., 2020b
TNF signaling pathway	Zhao et al. (2020), Wu et al., 2020b, Xu et al. (2020b), Duan et al. (2020), Zhou et al. (2020)	Prostate cancer	Wu et al., 2020b
Wnt signaling pathway	Zhao et al. (2020)	Melanama	Wu et al., 2020b
VEGF signaling pathway	Zhao et al. (2020), Xu et al. (2020b), Wu et al., 2020b	Thyroid hormone signaling pathway	Wu et al., 2020b
Ribosome	Zhao et al. (2020)	Chronic myeloid leukemia	Wu et al., 2020b
IL-17 signaling pathway	Xu et al. (2020b), Xu et al. (2020a)	Glioma	Wu et al., 2020b
Chagas disease (American trypanosomiasis)	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)	Endometrial cancer	Wu et al., 2020b
Tuberculosis	Xu et al. (2020b), Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020)	Influenza A	Wu et al., 2020b, Xu et al. (2020b), Duan et al. (2020)
Human cytomegalovius infection	Xu et al. (2020b), Xu et al. (2020a)	Toll-like receptor signaling pathway	Wu et al., 2020b, Xu et al. (2020a), Duan et al. (2020), Zhou et al. (2020), Yang et al. (2020a)
Epithelial cell signaling in helicobacter pylori infection	Wu et al., 2020b	*Salmonella* infection	Wu et al., 2020b, Duan et al. (2020)
Melanoma	Wu et al., 2020b	Colorectal cancer	Wu et al., 2020b
RIG-I-like receptor signaling pathway	Wu et al., 2020b	Small cell lung cancer	Wu et al., 2020b
Herpes simplex infection	Wu et al., 2020b, Duan et al. (2020)	Non-alcoholic fatty liver disease	Wu et al., 2020b
Shigellosis	Wu et al., 2020b	HTLV-I infection	Wu et al., 2020b
Cytosolic DNA-sensing pathway	Wu et al., 2020b	Apoptosis	Xu et al. (2020a)
Acute myeloid leukemia	Wu et al., 2020b	Human immunodeficiency virus 1 infection	Xu et al. (2020a)
Measlea	Xu et al. (2020a)	Proteoglycans in cancer	Wu et al., 2020b
Non-small cell lung cancer	Wu et al., 2020b	Glutamatergic synapse	Jin et al. (2020)
Amphetamine addiction	Jin et al. (2020)	Long-term potentiation	Jin et al. (2020)
Long-term depressio	Jin et al. (2020)	Retrograde endocannabinoid signaling	Jin et al. (2020)
Cocaine addiction	Jin et al. (2020)	Nitrogen metabolism	Jin et al. (2020)
Nicotine addiction	Jin et al. (2020)	Neuroactive ligand-receptor interaction	Jin et al. (2020), Chen et al. (2020a)
Interleukin-4 and interleukin-13 signaling	Peng et al. (2020)	Interleukin-1 processing	Peng et al. (2020)
Adrenoceptors	Peng et al. (2020)	IκBα variant leads to EDA-ID	Peng et al. (2020)
CLEC7A/inflammasome pathway	Peng et al. (2020)	DEx/H-box helicases activate type I IFN and inflammatory cytokines production	Peng et al. (2020)
G alpha (s) signaling events	Peng et al. (2020)	G alpha(z) signaling events	Peng et al. (2020)

(Continued on following page)

TABLE 4 | (*Continued*) Main enriched signaling pathways of QFPDD in the treatment of COVID-19.

Pathway name	References	Pathway name	References
Tp53 regulates transcription of DNA repair	Peng et al. (2020)	RIP-mediated NF-κB activation via ZBP1	Peng et al. (2020)
Interleukin-21 signaling	Peng et al. (2020)	PI5P, PP2A and IER3 regulate PI3K/Akt signaling	Peng et al. (2020)
Interleukin-2 signaling	Peng et al. (2020)	Signaling by SCF-KIT	Peng et al. (2020)
Erythropoietin activatesPphosphoinositide-3-kinase (PI3K)	Peng et al. (2020)	Activation of the AP-1 family of transcription factors	Peng et al. (2020)
Interleukin-10 signaling	Peng et al. (2020)	Interleukin receptor SHC signaling	Peng et al. (2020)
Adenylate cyclase inhibitory pathway	Peng et al. (2020)	Calmodulin induced events	Peng et al. (2020)
Inflammatory bowel disease (IBD)	Duan et al. (2020)	Cytokine-cytokine receptor interaction	Duan et al. (2020), Zhou et al. (2020)
Rheumatoid arthritis	Duan et al. (2020)	Amebiasis	Duan et al. (2020)
African trypanosomiasis	Duan et al. (2020)	Malaria	Duan et al. (2020)
Dteroid biosynthesis	Chen et al. (2020a)	PPAR signaling pathway	Chen et al. (2020a)
Adipocytokine signaling pathway	Chen et al. (2020a)	Steroid hormone biosynthesis	Chen et al. (2020a)

response, nitric oxide synthesis and cell apoptosis might be the potential common mechanisms of QFPDD in the treatment of SARS, MERS, and COVID-19 (Duan et al., 2020).

In addition, Peng et al. (2020) constructed the interaction network of *Formula-Herb-Disease-Targets-Pathways* based on the three main clinical symptoms of COVID-19: pneumonia, fever, and cough. The research results indicated that key-targets such as cell tumor antigen p53 (tp53), protein kinase B1 (Akt1), nuclear factor nuclear transcription factor-κB (NK-κB) p105 subunit (NFKB1), nuclear factor p65 subunit (RELA), human NK-κB inhibited protein α (NFKBIA), etc. were mainly related to the regulation of apoptosis and immune response, inflammatory response, improving lung function, etc. The GO and KEGG pathway enrichment analysis indicated that the 103 key targets were mainly concentrated in the signal pathways such as interleukin signaling, adrenoceptors, seven members of the family of c-type lectin domains A (CLEC7A)/inflammasome pathway, phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) inflammatory signaling pathway, tp53 regulates transcription of DNA repair, etc. which might be the main pathways related to QFPDD's effect on the treatment of COVID-19 accompany with lung injury, fever, cough, and other symptoms (Peng et al., 2020).

Based on computer-aided drug design, Jin et al. (2020) systematically explored and analyzed the material basis and molecular mechanism of QFPDD in the three aspects of detoxification, anti-inflammatory storm, and diuresis-removing dampness. Molecular docking virtual screening was performed based on the 2,740 compounds in QFPDD and the targets including ACE2, interleukin-6 receptor (IL-6R), and aquaporins (APQ). The mechanism of action was predicted by reverse target prediction, GO and KEGG pathway enrichment analysis for *Atractylodes macrocephala, Polyporus umbellatus, Poria cocos, and Alisma plantago-aquatica*. Research showed that Xiaochaihu decoction ranked the first in the number of potentially active compounds to block the virus and suppress inflammatory storm among the five classic prescriptions of QFPDD. The top three most prominent drugs to block the key binding sites of the virus were *Radix Glycyrrhizae, Herba Ephedrae*, and *Citrus aurantium*, while the top three to suppress inflammatory storm were *Radix Glycyrrhizae, Radix Asteris, and Radix Bupleuri*. Quercetin and its derivatives, the potential dual-target active compounds, had a high binding ability to ACE2 and IL-6R targets. *Atractylodes macrocephala, Cinnamomum cassia, Poria cocos, Polyporus umbellatus, and Alisma plantago-aquatica* lacked compounds that blocked viruses and suppressed inflammatory storms, but dehydroeburicoic acid, scopoletin, alismoxide and alpha-D-galactose contained in the above drugs had the potential binding ability with AQP4. Each component of the sub-medical prescription is reasonably compatible and plays a role in prevention and treatment through multi-point cooperation and complementary advantages. The interaction between these targets can form a molecular network, and it is found that many active components of QFPDD play a role in virus invasion, virus replication, and multiple organ damage (Jin et al., 2020). Furthermore, Chen et al. (2020a) divided QFPDD into five functional units (four sub-medical prescriptions and the rest) in the light of the compatibility theory of TCM. Results showed that all the five functional units had a positive effect on COVID-19 independently, and it involved physiological processes such as inflammation, bacterial and viral responses, immune system, signaling transduction, etc (Chen et al., 2020a). Yang et al. (2020c) also reported the chemical composition and pharmacological mechanism of QFPDD which indicated the thrombin and Toll-like receptor (TLR) signaling pathway were suggested to be main pathways for Maxing Shigan decoction mediated anti-inflammatory effects (Yang et al., 2020c).

1.3 *In Vivo* Distribution and Metabolomics of QFPDD

Liu et al. (2020b) investigated the main chemical constituents in QFPDD and the tissues distribution of the main absorbed constituents in mice following oral administration of QFPDD. As shown in **Table 5**, a total of 39 compounds were identified from QFPDD using UHPLC-Q-Orbitrap HRMS. After administered QFPDD in mice (2.6 g/100 g, ig), 12, 9, 10, 8, 9,

TABLE 5 | Components of QFPDD distribution in the organs.

Name	CAS No	Distribution					
		Serum	Liver	Heart	Spleen	Lung	Kidney
Synephrine	94-07-5	-	-	-	-	-	-
Dihydroxyacetone	96-26-4	-	-	-	-	-	-
Gallic acid monohydrate	5,995-86-8	-	-	-	-	-	-
Neochlorogenic acid	906-33-2	-	-	-	-	-	-
(1R,2S)-2-(Methylamino)-1-phenylpropan-1-ol	299-42-3	+	+	+	+	+	+
Pseudoephedrine	90-82-4	+	+	+	+	+	+
Caffeic acid	331-39-5	-	-	-	-	-	-
Chlorogenic acid	327-97-9	-	-	-	-	-	-
Cryptochlorogenic acid	905-99-7	-	-	-	-	-	-
(R)-amygdalin	29,883-15-6	+	+	+	+	+	+
Benzeneacetonitrile, a-(b-D-glucopyranosyloxy)-, (aR)-	99-18-3	+	+	+	+	+	+
(-)-3,5-Dicaffeoyl quinic acid	89,919-62-0	-	-	-	-	-	-
Ferulic acid	1,135-24-6	-	-	-	-	-	-
Liquiritin	551-15-5	+	+	+	+	+	+
Isochlorogenic acid B	14,534-61-3	-	-	-	-	-	-
3,5-Dicaffeoylquinic acid	2,450-53-5	+	-	-	-	+	+
Hyperoside	482-36-0	+	+	+	-	+	+
Rutin	153-18-4	-	-	-	-	-	-
Resveratrol	501-36-0	-	-	-	-	-	-
Naringen	4,493-40-7	-	-	-	-	-	-
Hesperiden	520-26-3	+	+	+	+	+	+
Isochlorogenic acid C	57,378-72-0	-	-	-	-	-	-
Cinnamaldehyde	14,371-10-9	-				-	-
Baicalin	21,967-41-9	+	+	+	+	+	+
Quercetin	117-39-5	-	-	-	-	-	-
Luteolin	491-70-3	-	-	-	-	-	-
Kaempferol	520-18-3	-	-	-	-	-	-
Irisflorentin	41,743-73-1	+	+	+	+	+	+
Gingerol	23,513-14-6	-	-	-	-	-	-
2,5,7-trimethoxyphenanthren-3-ol	51,415-00-0	-	-	-	-	-	-
Asarinin	133-04-0	-	-	-	-	-	-
Glycyrrhizic acid	1,405-86-3	+	-	-	-	-	-
(8)-Gingerol	23,513-08-8	-	-	-	-	-	-
Atractylenolide I	73,069-13-3	-	-	-	-	-	-
Saikosaponin A	20,736-09-8	-	-	-	-	-	-
Tussilagone	104012-37-5	-	-	-	-	-	-
(10)-Gingerol	23,513-15-7	-	-	-	-	-	-
Alisol B,23-acetate	19,865-76-0	+	-	-	-	-	-
Pachymic acid	29,070-92-6	-	-	-	-	-	-

and 10 constituents were identified in serum, heart, lung, spleen, liver, and kidney, respectively. The results showed that these nine constituents (ephedrine, pseudoephedrine, amygdalin, prunasin, liquiritin, hyperoside, hesperidin, baicalin, and risflorentin) could be quickly absorbed into the circulation system and then widely distributed in various tissues. At 0.5 h, except baicalin, the exposure of the other eight target components reached a peak in serum and tissues. The exposure of baicalin was peaked at 2 or 4 h. At 0.5 h, the exposure of target components to lung tissue was ranked as follows: ephedrine (2,759.11 ± 784.39 ng/g), prunasin (1819.7 ± 427.28 ng/g), pseudoephedrine (880.6 ± 287.97 ng/g), amygdalin (304.43 ± 234.7 ng/g), hesperidin (78.33 ± 38.38 ng/g), risflorentin (8.62 ± 4.66 ng/g), baicalin (8.53 ± 1.91 ng/g), hyperoside (7.72 ± 1.63 ng/g), liquiritin (7.68 ± 5.19 ng/g). At 2 h, ephedrine (776.61 ± 148.4 ng/g), prunasin (173.77 ± 58.21 ng/g), pseudoephedrine (84.68 ± 59.04 ng/g), baicalin (49.33 ± 17.06 ng/g), amygdalin (1.26 ± 0.26 ng/g) (Liu et al., 2020b). Furthermore, Wu et al. (2020a) indicated that treatment with QFPDD (1.5, 6 g/kg/day, p.o.) for continued 5 days, could significantly regulate the host metabolism and gut microbiota composition in rats such as enriched romboutsia, turicibacter, and *clostridium*_sensu_stricto_1, and decreased norank_f_Lachnospiraceae. The results from GC-MS and LC-MS/MS identified a total of 23 and 43 differential metabolites respectively that were altered by QFPDD. The metabolic pathways of these differential metabolites included glycerophospholipid metabolism, linoleic acid metabolism, TCA cycle, and pyruvate metabolism (Wu et al., 2020a).

1.4 Clinical Application and Practice of QFPDD

QFPDD is taken as water decoction, once a day, administrated in the morning and at night separately, 40 min after meals and total of three doses as a course of treatment (Jiang and Chen, 2020). If possible, half a bowl of rice water can be taken after taking the decoction every time, and those suffering from body fluid deficiency can take one bowl of rice water (Tian et al., 2020).

Diagnosis and Treatment Program of COVID-19 (Seventh edition) issued by National Health Commission of the PCR has clearly stated that TCM treatment requires syndrome differentiation and treatment based on the local climate characteristics and different physical constitution. QFPDD, as a general prescription, could not take into account individual differences and may bring some related adverse reactions. Common adverse reactions of QFPDD include nausea and vomiting, dizziness, dermatitis, etc. Wang et al. (2020b) collected information about the entire diagnosis and treatment of 98 confirmed cases of COVID-9 treated with QFPDD in Sichuan province, and found that during the course of QFPDD treatment, four patients had nausea and vomiting, two patients had dizziness, one patient had a rash, and the incidence of adverse reactions was 7.14% (Wang et al., 2020b). In addition, Hu et al. revealed the observation on clinical effect of Qingfei Paidu granules in the treatment of 76 confirmed cases of COVID-9 in Hubei province, and found that during the course of Qingfei Paidu granules treatment, two patients had mild diarrhea, one patient had nausea and vomiting, one patient suffered from pruritus, and the incidence of adverse reactions was 5.26%, but above adverse reaction symptoms were mild and disappeared without special treatment (Hu et al., 2020). As shown in **Table 6**, some clinical observation of Qingfei Paidu prescription with different dosage forms in the treatment of COVID-19 indicated that QFPDD could effectively improve the symptoms and the effective rate is above 80%. The specific clinical indicators of TCM syndromes and main laboratory indices and safety observation which reflect the efficacy of QFPDD are shown in **Table 7** and **Table 8**, respectively. For each course of treatment, clinicians should objectively evaluate the efficacy and actual adverse reactions of QFPDD to adjust the prescription appropriately.

If the patient does not have a fever, the dosage of raw gypsum should be reduced, otherwise, the dosage of raw gypsum should be increased. If the symptoms are improved but not cured, the second course should be added. If the patient has other basic diseases, the second course of the prescription shall be modified according to the actual situation. (You et al., 2020). If the symptoms disappear, the patients can stop taking the medicine in the second course of treatment. For patients with obvious deficiency of spleen *Yang*, 15 g of raw gypsum can be used in the prescription; for patients with the deficiency of stomach *Yin*, the method of nourishing *Yin* and eliminating dampness can be followed for empirical treatment and for those with excessive sweating, high blood pressure, palpitation, and insomnia, the dosage of the prescription can be appropriately reduced, or the dosage of yam can be increased. In the case of hepatic insufficiency, clinicians should analyze the causes of hepatic insufficiency, stop taking drugs if necessary, or add liver protection therapy (Dong et al., 2020; Lai et al., 2020). As for the dosage of *Herba Asari*, QFPDD is used up to 6 g, although it does not follow "the dosage of *Herba Asari* is not more than 5 g", it is still in the range of commonly used clinical dosage and its fluctuation, which is more suitable for the patients with cold-dampness-yang injury and severe deficient cold. For those with severe heat and dampness, the dosage of *Herba Asari* should be

TABLE 6 | Observation on clinical effect of Qingfei Paidu prescription with different dosage forms in the treatment of COVID-19.

No	The number of cases	Pharmaceutical dosage form	Course of treatment	Cure rate (%)	Total effective rate (%)	Province	References
1	76 cases	Granules	5 days as a course of treatment, three courses of treatment	65.79%	88.16%	Hubei province	Hu et al. (2020)
2	98 cases	Decoction	3 days as a course of treatment, three courses of treatment	41.13%	92.09%	Sichuan province	Wang et al., 2020b
3	30 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	83.335	Hubei province	Li et al., 2020b
4	151 cases	Mixture	3 days as a course of treatment, three courses of treatment	43.70%	90.07%	Sichuan province	Lai et al. (2020)
5	108 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	91.67%	Hubei province	Meng et al. (2020)
6	214 cases	Decoction	3 days as a course of treatment, three courses of treatment	NA	90%	Shanxi, Hebei, Shaanxi, Hellongjiang province	General Office of National Health Commission, State Administration of Traditional Chinese Medicine (2020)

TABLE 7 | Clinical symptom rating scale of TCM syndromes of COVID-19.

Primary symptoms	Normal (0 point)	Slight (2 points)	Medium (4 points)	Severe (6 points)
Fever	≤37.2°C	37.2°C–38.2°C	38.3°C–39.0°C	>39.0°C
Cough	None	Occasionally, with a single cough	Often, but does not affect work and rest	Cough frequently with more than one cough, cause vomiting, affects work and rest
Asthma	The respiration is stable and the frequency is within the normal range of the corresponding age	Exceeding the upper limit of the normal value of the corresponding age (≤10 times/min), there is no flaring of nares and three concave sign	Exceeding the upper limit of the normal value of the corresponding age (11–20times/min), and/or intermittent wheezing, flapping of nasal wings, three concave sign	Exceeding the upper limit of the normal value of the corresponding age (≥21times/min), and/or continuous wheezing, flaring of nares, three concave sign
Expectoration	None	There is an occasional sound of phlegm in the throat and a small amount of sputum	The phlegm sound in the throat is hissing and the phlegm is yellow	There is a roar of phlegm sound in the throat and a large amount of yellow-phlegm
Nasal obstruction	None	Occasionally. It doesn't affect breathing through the nose	Patients often have the nasal obstruction during the day	Obvious nasal obstruction patients have to breathe through the mouths
Nasal discharge	None	Occasionally	Patients have runny nose in the morning and at night	Continuously
Dry mouth	None	Occasionally	Sometimes	Continuously
Pharyngalgia	None	Slightly	Dry pain, pain when swallowing	Burning pain, sharp pain when swallowing
Hypodynamia	Normal	Slightly	Obvious	General weakness
Anorexia	Normal	Poor appetite	Loss of appetite	The appetite is extremely poor, or the patients refuse to eat
Diarrhea	None	Less than 3 times a day Loose stool	Three to six times a day Loose stool	More than 7 times a day The stool is watery
Secondary symptoms	**Normal (0 point)**	**Slight (1 point)**	**Moderate(2 points)**	**Severe (4 points)**
Complexion	Normal	Flushing of face and lusterless complexion	Flushing of face and dim complexion	Pallor and dim complexion
Palpitation	None	Mildly	Sometimes	Continuously
Abdominal distension	None	Occasional abdominal distension or postprandial abdominal distension	Abdominal distension is severe, up to 6 hours a day	Abdominal distension all day long
Aversion to cold	None	Slightly	Moderately	Shivering
Cyanosis	None	Slight cyanosis [$P(O_2)$50 mmHg–80 mmHg,SaO_2 80%–90%]	Moderate cyanosis [$P(O_2)$30 mmHg–50 mmHg,SaO_2 60%–80%]	Severe cyanosis [$P(O_2)$<30 mmHg,SaO_2 <60%]
Hyperhidrosis	None	Usually the skin is slightly moist or occasionally hot and sweating	Usually the skin is moist, sweating if you move a little; hectic fever on the chest and back, sweating repeatedly	Sweat usually and sweat like washing with moving
Short breath	None	Slightly	Shortness of breath increases after exercise	Obviously affecting work and daily life
Insomnia	Normal	Difficulty falling asleep	Difficulty falling asleep, sleep lightly	Hard to sleep
Urination	Normal	Slightly yellow	Dark yellow	Dark urine
Tongue manifestation	**Normal (0 point)**		**Abnormal (2 points)**	
Tongue property	Light red tongue		Red or dark-red tongue, or with ecchymosis, or prickly tongue	
Coated tongue	The tongue coating is thin and white		Tongue coating is yellow, thick, greasy, etc.	
Pulse	**Normal (0 point)**		**Abnormal (2 points)**	
Pulse	Normal pulse		Irregular-rapid pulse, irregularly intermittent pulse, regularly intermittent pulse, etc.	

TABLE 8 | The main laboratory indices and safety observation in the treatment of COVID-19.

Detection of laboratory indices	References	Detection of laboratory indices	References	Safety observation	References
White blood cell count (WBC)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Lactate dehydrogenase (LDH)	Wang et al., 2020b	Throat swab nuclei cacid detection	Hu et al. (2020), Wang et al., 2020b
Lymphocyte percentage (LYMPH%)	Hu et al. (2020), Wang et al., (2020b), Meng et al. (2020)	Creatine kinase isozyme (CK-MB)	Wang et al., 2020b	Chest computed tomography	Hu et al. (2020), Wang et al., 2020b
Neutrophil percentage (NEUT%)	Hu et al. (2020), Wang et al., (2020b), Meng et al. (2020)	Creatine kinase (CK)	Wang et al., 2020b	Blood biochemistry	Hu et al. (2020), Wang et al., 2020b
Aspartate aminotransferase (AST)	Wang et al., 2020b, Meng et al. (2020)	C-reactive protein (CRP)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Electrocardiogram	Hu et al. (2020), Wang et al., 2020b
Erythrocyte sedimentation rate (ESR)	Hu et al. (2020), Wang et al., 2020b	Procalcitonin (PCT)	Hu et al. (2020), Wang et al., 2020b	Observation of adverse reactions	Hu et al. (2020), Wang et al., 2020b
Albumin (ALB)	Hu et al. (2020), Meng et al. (2020)	D-dimer (D-dimer)	Hu et al. (2020), Wang et al., 2020b		
Urea (UREA)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)	Alanine aminotransferase (ALT)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)		
Creatinine (CREA)	Hu et al. (2020), Wang et al., 2020b, Meng et al. (2020)				

reduced as appropriate (Liu et al., 2020a). Some scholars also have recommended the modified QFPDD combined with western medicine such as alpha-interferon, oseltamivir, chloroquine phosphate, arbidol, ribavirin in the treatment of COVID-19, and found that it was more effective than the treatment of western medicine alone, which could significantly shorten the patient's hospitalization time, the time of clinical symptom improvement and the time of lung CT improvement (Fang et al., 2020; Li et al., 2020b; Yang et al., 2020b).

2 CONCLUSION AND PERSPECTIVES

COVID-19 is a new type of infectious disease. Western medicine mainly focuses on symptomatic relief. TCM has been applied for treating epidemics for thousands of years, and many clinicians have conducted in-depth research on COVID-19 etiology, pathogenesis, and syndrome differentiation. Since TCM played a huge role in the treatment of SARS in China in 2003, the National Health Commission and the National Administration of TCM jointly issued the "New Coronavirus Infection Pneumonia Diagnosis and Treatment Program (Fourth, Fifth, Sixth, Seventh and Trial Eighth edition)", which advocated the integration of Chinese and Western medicine, strived to shorten the course of the disease, improve clinical efficacy and reduce the incidence and mortality of critically ill patients (Lu and Lu, 2020; Xie, 2020).

In the process of the treatment of COVID-19, under the guidance of TCM theory, based on clinical practice and patient-oriented principle combined with data mining and basic research of modern biology and pharmacology, China established treatment methods for different stages and syndromes in different regions by systematically sorting out several classic and effective prescriptions and quickly put them into the clinical application (Zhang et al., 2020). Given the current epidemic situation of COVID-19, early intervention of TCM has played an important role in this epidemic control. Chinese and western advantages complement each other, which has a definite curative effect in reducing fever and other symptoms, controlling disease progression and reducing complications. QFPDD was selected and recommended by the National Administration of TCM as a general prescription for treating different stages of COVID-19. QFPDD is combined with multiple prescriptions and has the properties and flavors of pungent-warm and pungent-cool, aiming at the pathogenesis of COVID-19, including cold, dampness, heat, toxin, and deficiency (Chen et al., 2020b). QFPDD has the functions of dispelling cold and dampness, eliminating heat and turbidity, promoting and nourishing lung and spleen, detoxifying and removing pathogenic factors, etc. Modern pharmacologic studies have also confirmed the anti-inflammatory, antiviral and immunological functions of QFPDD which is attributed to the multi-component, multi-target, and multi-pathway characteristics of TCM. QFPDD is also a widely accepted prescription for treating COVID-19 based on its successful and effective clinical observations. The successful use of QFPDD in this novel viral pneumonia epidemic has confirmed the advantages of TCM in treating emergencies. However, at present, the mechanism of QFPDD is still unclear. It is necessary to further

comprehensively evaluate the efficacy and safety of QFPDD and clearly explain the complex mechanisms of QFPDD in the treatment of COVID-19 through systematic reviews and meta-analysis (Gao et al., 2020). Currently, there is a lack of extended research with sufficient breadth and depth and the current research has just focused on QFPDD TCM theory, clinical experience, network pharmacology, etc., with only a small number of clinical research samples. In the follow-up research, it is not only essential to carry out more comprehensive chemical composition characterization, pharmacokinetic and pharmacodynamic studies *in vitro* and *in vivo*, but also extended clinical data should be evaluated to elucidate the material basis and systematically explain the effectiveness of QFPDD against COVID-19, and further provide a theoretical basis for the clinical scientific and rational application of QFPDD in the prevention and clinical treatment of COVID-19.

AUTHOR CONTRIBUTIONS

RW, YM, RQW, LP, GL, and SJY conceived and designed the review; QS, QGP, LD and MM reviewed the literature; RW and YM wrote the manuscript.

FUNDING

This work was supported by National Administration of Traditional Chinese Medicine (Grant NO. 2020ZYLCYJ02-3); Sichuan Administration of traditional Chinese Medicine ((Grant NO. 2020yj018) and (Grant NO. 2020yj024)); the fellowship of China Postdoctoral Science Foundation (Grant NO. 2020M683365); the National Traditional Chinese Medicine Clinical Research Base (Grant NO. (2020)33). The authors are sincerely thankful to all individuals who were involved in this study.

ACKNOWLEDGMENTS

The authors are thankful to Jiali Liu for assistance with the literature search. We also thank Ting Wang for valuable advice.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.589714/full#supplementary-material.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DOI:10.11656/j.issn.1672-1519.2020.09.09

“清肺排毒颗粒”治疗新型冠状病毒肺炎的临床疗效初探

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摘要:[目的] 观察用清肺排毒颗粒治疗新型冠状病毒肺炎的初步临床疗效。**[方法]** 将确诊的76例新型冠状病毒肺炎患者纳入观察,使用清肺排毒颗粒进行治疗,5 d为1个疗程,治疗3个疗程后,比较患者服药前和服药后的中医证候积分的变化和实验室相关指标的改善情况,中医证候依据症状的轻重程度,采用评分的方法判定。**[结果]** 与治疗前相比,运用清肺排毒颗粒治疗5、10、15 d后,其证候总积分均明显下降($P<0.01$);主症、次症总积分均较治疗前明显下降,均具有统计学意义($P<0.01$)。实验室检测显示,与治疗前相比,淋巴细胞百分率(LYMPH%)上升,丙氨酸氨基转移酶(ALT)、D-二聚体(D-Dimer)、C反应蛋白(CRP)和血沉(ESR)下降,逐渐恢复正常,差异具有统计学意义($P<0.01$),核酸转阴及肺部计算机断层扫描(CT)好转率为92.10%。治疗5 d后,基本痊愈18例,显效26例,总有效率为57.89%;治疗10 d后,基本痊愈32例,显效27例,总有效率为77.63%;治疗15 d后,基本痊愈50例,显效17例,总有效率为88.16%。**[结论]** 清肺排毒颗粒能有效控制患者的临床症状以及改善实验室异常检测指标,能很好地用于新型冠状病毒肺炎的治疗,其临床效果显著,不良反应少,值得推广使用。

关键词:新型冠状病毒肺炎;清肺排毒颗粒;临床研究
中图分类号:R511 **文献标志码:**A **文章编号:**1672-1519(2020)09-0999-06

2019年12月底在湖北省武汉市陆续出现多起不明原因的肺炎病例[1],随后在全国蔓延。国家卫生健康委员会发布通知,将这种肺炎暂命名为“新型冠状病毒肺炎”,简称“新冠肺炎”[2]。新冠肺炎潜伏期长、传染性强、各类人群普遍易感,对人民健康、经济发展和社会稳定产生巨大的影响。对于新冠肺炎的治疗,目前没有特效药物。怎样找到快速有效的药物以及治疗方案,是医学界当前亟需解决的重大难题。中医药对病毒感染性肺炎具有良好疗效,曾在严重急性呼吸综合征(SARS)治疗中显示了一定的效果。同时,国家卫生健康委员会颁布的《新型冠状病毒感染的肺炎诊疗方案》,均明确提出中医药的治疗策略[3]。2020年2月8日,根据近期运用中西医结合治疗新冠肺炎取得良好效果的情况,国家卫生健康委员会与国家中医药管理局发布通知,提出各地区可将复方中药清肺排毒汤(QFPDT)用于新冠肺炎的防治[4]。因此,笔者所在单位和团队采用中西医结合治疗新冠肺炎,将清肺排毒汤广泛用于临床治疗中,并进行临床观察和数据分析,研究清肺排毒汤治疗新冠肺炎的初步临床疗效以及安全性,取得了一定效果,报道如下。

1 资料与方法

1.1 一般资料 研究对象是2020年1—3月期间在湖北汉川市人民医院、武汉市第一医院、湖北六七二中西医结合骨科医院住院的新冠肺炎确诊患者。西医诊断标准以及中医临床证候分型参考《新型冠状病毒感染的肺炎诊疗方案(试行第六版)》制定[4]。共收集符合入组条件的患者76例,其中男40例,女36例。按照轻型、普通型、重型及危重型分组治疗,其中轻型18例,普通型48例,重型及危重型10例,分别占23.7%、63.1%、13.2%。76例患者的平均年龄为(40.56±15.01)岁,男性患者的平均年龄为(39.95±16.52)岁,女性患者的平均年龄为(14.21±15.61)岁。

1.2 纳入标准 1)参照国家卫生健康委员会颁布的第七版《新型冠状病毒肺炎诊疗方案》中诊断标准执行,将确诊病例纳入。2)年龄为14~82岁,男女不限。3)1个月内未服用免疫抑制药物者。4)患者被告知并同意。

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1.3 排除标准 1)14岁以下或82岁以上。2)合并其他严重疾病不耐受口服中药者。3)已知对中药或者成分过敏者,或过敏体质患者。4)免疫缺陷患者、1个月内服用免疫抑制药物者。5)孕妇或哺乳期妇女。6)临床医生认为不适合参与研究者。

1.4 脱落标准 1)证候明显改变患者。2)依从性差、自行退出者。3)资料不全和失访患者。

1.5 治疗方法 对确诊为新冠肺炎的患者使用清肺排毒汤治疗。清肺排毒汤为颗粒剂配方药,为培力健康产品有限公司提供的农本方清肺排毒汤配方颗粒(简称清肺排毒颗粒,QFPDKL),调配依据:国中医办医政函[2020]20号,功能主治:清肺排毒、止咳平喘、健脾祛湿、和解表里。适用于轻型、普通型及重型新冠肺炎患者,在危重型患者救治中可结合患者实际情况合理使用。用法用量:开水冲服,每次21 g,每日2次(饭后服用)。具体成分为:麻黄9 g,苦杏仁9 g,生石膏30 g,炙甘草6 g,柴胡16 g,姜半夏9 g,黄芩6 g,生姜9 g,猪苓9 g,桂枝9 g,茯苓15 g,炒白术9 g,泽泻9 g,紫菀9 g,射干9 g,款冬花9 g,细辛6 g,枳实6 g,山药12 g,广藿香9 g,陈皮6 g,等量折算为配方颗粒。5 d为1个疗程,共进行3个疗程的治疗。

1.6 疗效判断与观察指标

1.6.1 观察指标 比较服药前后中医证候的变化,并根据无、轻、中,重症状的程度给予相应评分。参考国家药品监督管理局发布的《证候类中药新药临床研究技术指导原则》[5],制定相应的《中医证候评分量表》,统计治疗前后的证候总积分,进行比较。

1.6.2 临床生化指标的检测 治疗前后分别观察患者的实验室指标。包括血液分析:白细胞计数(WBC)、淋巴细胞百分率(LYMPH%)、中性粒细胞百分率(NEUT%);血沉(ESR)、C反应蛋白(CRP)、降钙素原(PCT)、D-二聚体(D-Dimer);肝肾功能:丙氨酸氨基转移酶(ALT)、白蛋白(ALB)、尿素(UREA)、肌酐(CREA)等。

1.6.3 中医证候评分量表 制定新冠肺炎中医证候评分量表。见表1、表2。

1.6.4 临床疗效评估 评价标准参考《中医病证诊断疗效标准》[6]执行。疗效症状积分下降指数=[(治疗前评分-治疗后评分)/治疗前评分]×100%。治愈:疗效指数≥90%。显效:疗效指数<90%且≥60%。进步:疗效指数<60%且≥30%。无效:疗效指数<30%,总有效率=治愈率+显效率。

1.7 安全性观察 安全性观察:检测治疗前后患者呼吸道咽拭子核酸、胸部计算机断层扫描(CT)、血生化、心电图等,查看核酸转阴情况和肺部CT的吸收情况。同时观察患者治疗期间的不良反应。

1.8 统计学方法 采用SPSS 20.0软件进行统计学分析,以均数±标准差($\bar{x}\pm s$)表示计量资料,使用配对t检验进行组内前后的比较,使用重复测量方差分析进行重复测量资料的比较。以$P<0.05$表示有统计学差异。

2 结果

2.1 治疗前后中医证候总积分的变化比较 患者服用清肺排毒颗粒治疗后,与治疗前相比,治疗后5、10、15 d后,其证候总积分均显著下降,差异有统

表1 新冠肺炎中医证候主症评分量表

Tab.1 Primary symptom rating scale of TCM syndromes of COVID-19

主症	正常(0分)	轻度(2分)	中度(4分)	重度(6分)
发热	≤37.2 ℃	37.3~38.2 ℃	38.3~39 ℃	>39 ℃
咳嗽	无	偶作,每咳单声	阵作,每咳数声	咳嗽频繁,影响作息
咳痰	无	喉中时有痰声,少痰	喉中痰嘶,痰黄	喉中痰吼,痰黄量多
气喘	呼吸平稳	活动量大喘气	稍动或翻身喘气	平卧喘气,需吸氧
咽痛	无	微痛	干痛,吞咽时痛	灼痛,吞咽剧痛
口干	无	偶有口干咽燥	时有口干咽燥	持续口干咽燥
乏力	正常	轻微乏力	乏力明显	全身无力,不能起床
腹泻	无	大便次数小于3次/d	大便3~6次/d	大便大于等于7次/d
厌食	如常	口淡无味	口淡无味、食欲减少	纳呆
舌脉	正常0分		异常2分	
舌质	舌质淡红		舌质红,或有芒刺,暗红,有瘀斑	
舌苔	舌苔薄,白		舌苔黄、厚、腻等	
脉象	平脉		浮、沉、弦、促等异常脉象	

表 2 新冠肺炎中医证候次症评分量表

Tab.2 Secondary symptom rating scale of TCM syndromes of COVID-19

次症	正常(0分)	轻度(1分)	中度(2分)	重度(4分)
恶寒	无	有,无须加衣被	有,须加衣被	有,寒战
鼻塞	无	偶鼻塞,不影响呼吸	常有鼻塞不适感	鼻塞明显,需用口呼吸
面色	正常	潮红,少华	潮红,无华	灰白,无华
多汗	无	平素皮肤微潮	稍动则汗出	动则汗出如水洗状
心悸	无	轻微	心悸,时作时止	持续心悸
气短	无	感气短	气短活动加剧	明显气短,影响工作
腹胀	无	食后腹胀	腹胀较重,持续时间长	整日腹胀或腹胀如鼓
失眠	正常	入睡困难	入睡困难,易醒	难以入睡
小便	正常	稍黄	深黄	黄赤

计学意义(P<0.01);治疗 10 d 后与治疗 5 d 后比较,证候总积分均显著下降,差异有统计学意义(P<0.01);治疗 15 d 后与治 10 d 后比较,证候总积分均显著下降,差异有统计学意义(P<0.01)。见表 3、图 1。

表 3 清肺排毒颗粒治疗前后中医证候总积分比较($\bar{x}\pm s$)

Tab.3 Comparison of total scores of TCM syndromes before and after treatment of QFPDKL($\bar{x}\pm s$) 分

时间节点	例数	总积分
治疗前	76	3 192± 7
治疗 5 d 后	76	1 315±11*
治疗 10 d 后	76	702± 5*#
治疗 15 d 后	76	372± 4*#△

注:与治疗前比较,*P<0.01;与治疗 5 d 后比较,#P<0.01;与治疗 10 d 后比较,△P<0.01。

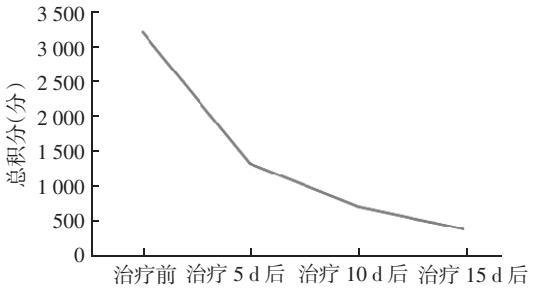

图 1 清肺排毒颗粒治疗前后证候总积分变化趋势

Fig.1 Change trend of syndrome total scores before and after treatment of QFPDKL

2.2 各症状治疗前后平均积分的变化 通过对 76 例患者的观察,可以看出治疗后患者症状的积分随着治疗时间的延长呈下降趋势。从治疗前的证候总积分可以发现,发热、咳嗽、喘气、厌食以及乏力为主要的临床主症状,患者使用清肺排毒颗粒治疗 5、10、15 d 后,与治疗前比较,其主症积分均明显下降,具有统计学意义(P<0.01)。面色、腹胀、失眠、多汗以及小便情况为次要的临床症状,患者使用清肺排毒颗粒治疗 5、10、15 d 后,其次症积分比治疗前均显著下降,均具有统计学意义(P<0.01)。见表 4、表 5。

表 4 清肺排毒颗粒治疗前后主要主症总积分变化($\bar{x}\pm s$)

Tab.4 Change of Primary symptom total scores before and after treatment of QFPDKL($\bar{x}\pm s$) 分

时间节点	例数	发热	咳嗽	气喘	乏力	厌食
治疗前	76	240±6	238±5	180±4	203±5	173±4
治疗后 5 d	76	40±2*	117±4*	109±3*	171±4*	147±3*
治疗后 10 d	76	10±1*	51±2*	23±1*	56±2*	54±2*
治疗后 15 d	76	5±1*	23±1*	5±1*	32±2*	28±1*

注:与治疗前比较,*P<0.01。

表 5 清肺排毒颗粒治疗前后主要次症总积分变化($\bar{x}\pm s$)

Tab.5 Change of secondary symptom total scores before and after treatment of QFPDKL ($\bar{x}\pm s$) 分

时间节点	例数	面色	腹胀	失眠	多汗	小便
治疗前	76	167±4	118±3	81±2	124±2	86±2
治疗后 5 d	76	152±3*	84±2*	58±2*	115±3*	55±2*
治疗后 10 d	76	53±2*	52±2*	24±1*	48±2*	15±1*
治疗后 15 d	76	15±1*	20±1*	18±1*	32±2*	8±1*

注:与治疗前比较,*P<0.01。

2.3 治疗前后实验室检查结果的变化 从 76 例患者治疗前的实验室检查结果来看,新冠肺炎患者的 WBC、NEUT%、PCT 和 CREA 均在正常范围;LYMPH%明显减少;ALT、D-Dimer、CRP 和 ESR 明显升高;治疗 5、10、15 d 后,与治疗前相比,LYMPH%上升,逐渐恢复正常,差异具有统计学意义(P<0.01);ALT、D-Dimer、CRP 和 ESR 下降,逐渐

表6 清肺排毒颗粒治疗前后实验室检查指标结果($\bar{x}\pm s$)

Tab.6 Results of laboratory examination indexes before and after treatment of QFPDKL($\bar{x}\pm s$)

时间节点	例数	WBC（×10⁹/L）	NEUT（%）	LYMPH（%）	CRP（mg/L）	ESR（mm/H）	PCT（ng/mL）	D-Dimer（mg/L）	ALT（U/L）	CREA（mmol/L）
治疗前	76	4.72±1.03	47.59±5.36	16.68±3.40	30.47±4.39	21.21±4.40	0.39±0.18	1.51±0.11	45.2±4.14	78.58±6.17
治疗5 d后	76	4.90±1.81	50.54±7.61	20.75±2.79*	15.32±3.74*	14.42±3.61*	0.38±0.21	1.05±0.07*	31.33±5.42*	73.44±6.26
治疗10 d后	76	5.10±1.31	51.32±6.75	22.77±3.74*#	7.03±2.01*#	11.87±3.27*#	0.23±0.09	0.53±0.05*	25.72±4.81*#	72.32±5.85
治疗15 d后	76	6.57±1.42	55.48±5.74	26.32±2.81*△	3.51±1.05*△	8.45±2.73*△	0.19±0.03	0.27±0.03*△	18.87±4.15*△	70.47±5.39

注：与治疗前比较，*P<0.05；与治疗5 d后比较，#P<0.05；与治疗10 d后比较，△P<0.05。

恢复正常，差异具有统计学意义（P<0.01）。见表6。

2.4 治疗5、10、15 d后的疗效评价 运用清肺排毒颗粒治疗后，患者的证候总积分与治疗前相比均明显下降，临床症状较治疗前显著改善甚至消失。治疗5 d后，基本痊愈18例，显效26例，进步26例，无效6例，总有效率为57.89%；治疗10 d后，基本痊愈32例，显效27例，进步13例，无效4例，总有效率为77.63%；治疗15 d后，基本痊愈50例，显效17例，进步7例，无效2例，总有效率为88.16%。

2.5 肺部CT及核酸转阴情况 76例患者经过3个疗程治疗后统计，其中70例核酸转阴，6例继续治疗，76例肺部CT较治疗前吸收好转，核酸转阴及CT好转率为92.10%。

2.6 不良反应情况 在使用清肺排毒颗粒治疗的过程中，76例患者中2例出现轻度腹泻，1例出现恶心呕吐症状，1例出现皮肤瘙痒，不良反应发生率为5.3%。不良反应症状较轻微，无特殊治疗即自行缓解消失，未影响疗程治疗。

3 讨论

新冠肺炎具有致病暴戾、起病急骤、家庭聚集性、传变迅速、致死率高、易耗气伤阴、易闭神机等特点[7]。《素问·刺法论》指出："五疫之至，皆相染易，无问大小，病状相似。"《温疫论》亦曰："温疫之为病，非风非寒，非暑非湿，乃天地间别有一种异气所感。"根据其临床症状和发病特点，本病属中医学"疫病"的范畴。根据气候特征以及地理特点等因素综合判断，此次疫毒多以湿邪为患，可称为"湿毒疫"[8]，也有专家指出此次新冠肺炎疫情发生时，气候以寒冷湿为主，建议称为"寒疫"[9]。多数学者认为新冠肺炎的核心病机为"湿毒"[10]。以肺脏为主要病位，湿邪为重要病机，脾胃的盛衰影响疾病的进退，正虚邪实决定疫病的发展[11]。通过分析部分新冠肺炎病例的临床表现后，发现不少患者出现明显的干咳、少痰甚至无痰等燥邪犯肺的情况[12]，考虑与疫毒猛烈，发展迅速，易耗伤阴液相关，有学者提出新冠肺炎"燥湿相关"的理论病机。综合考虑，对于此次新冠疫情，"湿、毒、瘀、燥、虚"是其主要病机[8]。湿性重浊黏滞、缠绵难解，气机阻滞，反复发作，变化多端，可寒可热，可燥可瘀，甚至危重时出现热深厥深，气不达外，导致内闭外脱，危及生命[7]，这同时给治疗新冠肺炎带来了很大难度。中医临床经验发现，尽管新冠肺炎的传变迅速，具有太多不确定性，但若采用方证相应的思想，随证治之，就能精准把握新冠肺炎表现的不同证候，及时使用有效方剂进行干预[13]。

通过不断临床实践，临床发现部分方药取得了不错的临床疗效。其中在第六版新冠肺炎诊疗方案[4]的中医治疗方面，推荐治疗各期加入了中医通用方剂"清肺排毒汤"。"清肺排毒汤"巧妙化裁《伤寒论》中的多首经方，符合新冠肺炎的病机，且方证相应，紧随疫病的发展变化，针对性强。其包含了5首经典方剂，由宣肺的麻杏石甘汤，调中的小柴胡汤，利湿的五苓散，平喘的射干麻黄汤、顾护脾胃的橘枳姜汤等加减优化组合而成，其主要功能是疏通三焦、清肺排毒、平喘止咳。组方配合严谨，贴合病机，有的放矢，驱邪扶正。针对湿邪为患，方中五苓散利水除湿，配伍麻黄宣肺利尿，增强功效，同时五苓散调节防止麻黄桂枝发汗太过，针对新冠肺炎表现肺闭不宣时，合射干麻黄汤利咽祛痰，宣通气机，最后用小柴胡汤合橘枳姜汤通利三焦，固护脾胃，并防止疫毒入里，截断病情深入发展[14]。加藿香为芳香化湿，用石膏防郁而化热。网络药理学研究提示[15]，清肺排毒汤的48个重要靶标与人类免疫缺陷病毒（HIV）的6个蛋白具有相互作用，显示了它潜在的抗病毒作用。

武汉地处华中，气候潮湿，时值冬春，疫疠之气具有寒湿特点。笔者临床观察到，新冠肺炎患者早期咳嗽、发热，伴有恶寒，舌质淡、暗，苔白腻，确为

属寒湿所主。这也符合王永炎、仝小林院士“寒湿疫”的判断[9,16]。清肺排毒汤的组方中有清有宣,有利有疏,有攻有补,处方全面,同时也考虑到后期寒湿化热化燥的情况。运用此方可明显减轻新冠肺炎患者的主要临床症状。本研究纳入的76例患者,使用清肺排毒颗粒治疗后,其各个症状均得到明显改善,其中在治疗5 d后,总有效率是57.89%,治疗10 d后总有效率为72.63%,治疗15 d后总有效率达88.16%。其中70例核酸检测两次转阴出院继续中药治疗,76例肺CT显示明显吸收恢复。治疗后中医证候总积分逐步下降,从统计结果分析来看,针对新冠肺炎的主要临床症状如咳嗽、发热、喘气、厌食以及乏力,临床次要症状如面色、腹胀、失眠、多汗及小便等症状,使用清肺排毒颗粒干预后,都具有明显的疗效。随着治疗时间的延长,总有效率越来越高,清肺排毒颗粒对普通型、轻型和重型均有明显疗效。在观察亦发现76例患者中,没有1例转重型或者危重型,使用至今无病例死亡。正如张伯礼院士[17]初步总结本次新冠肺炎的治疗优势时指出,中西医结合治疗新冠肺炎疗效更好,使用中药后,退热更快,止咳平喘效果更好,改善食欲乏力等症状优势明显;轻症患者恢复更快,重症患者转危重少,病死率低。

因病患众多,筛选中医药有效经方复方非常必要,及时选择有效的传染病通用方剂,将使更多的患者尽快使用中药,及早预防与治疗,从而可以极大的提高治愈率,降低其病死率,并减少后遗症。清肺排毒颗粒针对新冠肺炎的防治具有可靠的临床疗效,能最大限度地提高治愈率和降低病死率,提高了一线防控和临床治疗的有效性。这也增加了运用中医药战胜新冠肺炎疫情的信心与决心。同时,笔者团队也观察到,对于新冠肺炎恢复期的患者,还存在一些症状,如乏力、干咳、食欲不振等。可能原因为病毒引起的机体损伤,容易造成患者免疫力低下,抗御外邪能力减弱,修复需要一定的过程。疾病康复阶段,中医药仍然可以继续发挥其优势,进一步改善症状,恢复肺功能,提高生活质量。笔者期待更多的临床资料和实践数据,为进一步探索中医药治疗新冠肺炎的最佳途径和方法提供更为实用的依据。

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(收稿日期:2020-06-11)
(本文编辑:马 英,徐一兰)

Preliminary study on clinical efficacy of "Qingfei Paidu Granule" in treating coronavirus disease 2019

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Abstract:[Objective] To observe the preliminary clinical study on coronavirus disease 2019 (COVID-19) treated by Qingfei Paidu Granule (QFPDKL). **[Methods]** The 76 patients with COVID-19 were included in the observation and treated with QFPDKL,with 5 days as a course of treatment. After 3 courses of treatment,the changes of Traditional Chinese Medicine (TCM) syndrome scores and the improvement of laboratory related indicators were compared before and after treatment. TCM syndromes were judged by scoring method according to the severity of symptoms. **[Results]** Compared with before treatment,the total score of syndromes after treatment of QFPDKL for 5,10 and 15 days decreased significantly ($P<0.01$). The total scores of primary and secondary symptoms were significantly lower than those before treatment ($P<0.01$). Laboratory tests showed that compared with before treatment,lymphocyte percentage (LVMPH %) increased,alanine aminotransferase (ALT),D-Dimer,C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) decreased,and gradually returned to normal,the difference was statistically significant ($P<0.01$). The negative rate of nucleic acid and the improvement rate of lung computed tomography (CT) were 92.10%. After 5 days of treatment,18 cases were basically cured,26 cases were markedly effective,and the total effective rate was 57.89%. After 10 days of treatment,32 cases were basically cured,27 cases were markedly effective,and the total effective rate was 77.63%. After 15 days of treatment,50 cases were basically cured,17 cases were markedly effective and the total effective rate was 88.16%. **[Conclusion]** QFPDKL can effectively control patients' clinical symptoms and improve laboratory abnormal detection indicators,and can be well used in the treatment of COVID-19. Its clinical effect is remarkable,with few adverse reactions,and it is worth popularizing and using.

Keywords: coronavirus disease 2019;Qingfei Paidu Granule;clinical research

Phytomedicine 85 (2021) 153531

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Original Article

Association between use of Qingfei Paidu Tang and mortality in hospitalized patients with COVID-19: A national retrospective registry study

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ARTICLE INFO

Keywords:
Qingfei Paidu Tang
Mortality
COVID-19

ABSTRACT

Background: Qingfei Paidu Tang (QPT), a formula of traditional Chinese medicine, which was suggested to be able to ease symptoms in patients with Coronavirus Disease 2019 (COVID-19), has been recommended by clinical guidelines and widely used to treat COVID-19 in China. However, whether it decreases mortality remains unknown.

Purpose: We aimed to explore the association between QPT use and in-hospital mortality among patients hospitalized for COVID-19.

Study design: A retrospective study based on a real-world database was conducted.

Methods: We identified patients consecutively hospitalized with COVID-19 in 15 hospitals from a national retrospective registry in China, from January through May 2020. Data on patients' characteristics, treatments, and outcomes were extracted from the electronic medical records. The association of QPT use with COVID-19 related mortality was evaluated using Cox proportional hazards models based on propensity score analysis.

Results: Of the 8939 patients included, 28.7% received QPT. The COVID-19 related mortality was 1.2% (95% confidence interval [CI] 0.8% to 1.7%) among the patients receiving QPT and 4.8% (95% CI 4.3% to 5.3%) among those not receiving QPT. After adjustment for patient characteristics and concomitant treatments, QPT use was associated with a relative reduction of 50% in-hospital COVID-19 related mortality (hazard ratio, 0.50; 95% CI, 0.37 to 0.66 $p < 0.001$). This association was consistent across subgroups by sex and age. Meanwhile, the incidences of acute liver injury (8.9% [95% CI, 7.8% to 10.1%] vs. 9.9% [95% CI, 9.2% to 10.7%]; odds ratio, 0.96 [95% CI, 0.81% to 1.14%], $p = 0.658$) and acute kidney injury (1.6% [95% CI, 1.2% to 2.2%] vs. 3.0% [95% CI, 2.6% to 3.5%]; odds ratio, 0.85 [95% CI, 0.62 to 1.17], $p = 0.318$) were comparable between patients receiving QPT and those not receiving QPT. The major study limitations included that the study was an observational study based on real-world data rather than a randomized control trial, and the quality of data could be affected by the accuracy and completeness of medical records.

Conclusions: QPT was associated with a substantially lower risk of in-hospital mortality, without extra risk of acute liver injury or acute kidney injury among patients hospitalized with COVID-19.

Abbreviations: CI, confidence interval; COVID-19, Coronavirus Disease 2019; HR, hazard ratios; IPTW, inverse probability treatment weighting; IQR, interquartile range; OR, odds ratios; QPT, Qingfei Paidu Tang; SMD, standard mean difference.

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https://doi.org/10.1016/j.phymed.2021.153531
Received 24 November 2020; Received in revised form 11 February 2021; Accepted 23 February 2021
Available online 28 February 2021

Introduction

Coronavirus Disease 2019 (COVID-19), caused by a novel severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has posed a huge threat to global health as the largest pandemic in a century. Nearly 50 million people worldwide have been infected, of whom over 1.2 million died by mid-November2020 (World Health Organization, 2020). The pandemic is still evolving, effective treatments against COVID-19 are therefore urgently needed to reduce the mortality of COVID-19.

Qingfei Paidu Tang (QPT), a traditional Chinese medicine, was formulated on the basis of one of the classics of traditional Chinese medicine, Treatise on Febrile and Miscellaneous Diseases (*Shang Han Zabing Lun*) (National Administration of Traditional Chinese Medicine, 2020). It is a compound prescription containing four traditional Chinese medicine prescriptions, each of which has been widely applied as therapy of common cold, fever, influenza, and other virus infections (Shi. et al.,2019; Zheng et al., 2014; Hsieh et al., 2012; Lin et al., 2020; Yang et al., 2020). Basic research also found that QPT possessed properties such as antivirus (Zhao et al., 2020; Chen et al., 2020), anti-inflammation (Zhao et al., 2020; Chen et al., 2020; Yang et al., 2020; Xu et al., 2020a; Xu et al.,2020b; Wu et al., 2020), and immune regulation (Zhao et al., 2020; Xu et al., 2020a, 2020b; Wu et al., 2020), which might be beneficial for patients with COVID-19. Moreover, several small observational studies in China have suggested its potential effectiveness in relieving symptom (i.e., fever and cough) and preventing disease progression in patients with COVID-19 (Wang et al., 2020; Zhang et al., 2020; Li et al., 2020; Xin et al., 2020).Therefore, QPT has been recommended in the Chinese guidelines for the treatment of Coronavirus Disease 2019 (COVID-19) since early February 2020 and widely used in China (National Health Commission of the People's Republic of China, 2020). However, it is unknown whether it could reduce the mortality of COVID-19.

Accordingly, using the data from a national retrospective registry, we sought to evaluate the effectiveness and safety of QPT in COVID-19. Specifically, we hypothesized that QPT use would be associated with a lower risk of in-hospital mortality in patients with COVID-19, and tested it using propensity score analysis. We also assessed whether there was an association of QPT with the incidence of acute liver injury and acute renal injury during hospitalization.

Methods

Data sources

In a government-mandated national registry, hospitalizations for COVID-19 in all the designated hospitals across China were registered retrospectively. Information relating to patient characteristics, treatments, and outcomes, in the electronic medical records (EMR), were required to be submitted to a system deployed by the National Health Commission of China, in forms of either structured database for the front page, or unstructured text for the progress notes, lab test results, and physician's orders. By the date of May 6th 2020, over 40 thousand COVID-19 cases from more than five hundred hospitals have been included.

Ethical approval

The Ethics Committee at the National Center for Cardiovascular Diseases (NCCD)/Fuwai Hospital ethics committee approved this study and the Ethics Committee at the First Affiliated Hospital, Sun Yat-sen University approved the current analysis. Informed consent of individual patients was waived.

Study cohort

Among all the designated hospitals providing inpatient care for COVID-19 in the national registry, we excluded hospitals that were ineligible for data extraction or analysis for the following two reasons. First, the number of patients hospitalized with COVID-19 was less than 100. Second, the number of patients receiving QPT in the hospitals was less than 50. In the end, 15 hospitals were included in our study, all of which were located in Hubei province.

Among the eligible hospitals, we included all patients aged $\geq$18 years discharged between January and May 2020 with a confirmed diagnosis of COVID-19. We identified these patients, according to the International Classification of Diseases, Clinical Modification codes revision 10 (U07.100, U07.100.00x, U07.100.00 $\times$ 001, U07.100.00 $\times$ 002, U07.100.00 $\times$ 003), when available, or through principal diagnosis terms noted at discharge. We excluded patients who were transferred out, since the records of their hospitalizations were truncated. Patients who died or were discharged within 24 h of admission were also excluded from the analysis, because the testing and treatments for them were likely to be influenced due to the short length of hospital stay.

Data extraction

For each patient, the demographic characteristics (age and sex), prior medical histories/comorbidities (hypertension, diabetes, coronary heart disease, stroke, chronic kidney diseases, chronic obstructive pulmonary disease, and cancer), clinical status at admission (critical or not), and in-hospital death were obtained from the front-page database or progress notes. The vital signs (heart rate, blood pressure, and respiratory rate) at admission were extracted from the progress notes. The in-hospital medications (QPT, Arbidol, Ribavirin, Oseltamivir, Ganciclovir, Lopinavir, Lianhuaqingwen, Xuebijing, Diammonium Glycyrrhizinate, Methylprednisolone, Dexamethasone, and Interferon) were extracted from the physician orders, progress notes, and nurse records. The in-hospital acute liver injury and acute kidney injury were identified based on the front-page database, progress notes, and lab test results.

We searched predefined keywords in unstructured text of the submitted medical records using Python software (version 3.6) and MYSQL software (version 8.0), in order to extract the data. Particularly, research clinicians randomly selected and reviewed 5% of the medical records in the hospitals with QPT use rate under 20%, to ensure the exhaustion of synonyms of this medication and completeness of data extraction. Furthermore, to ensure data accuracy, research clinicians adjudicated the prior medical history/comorbidities based on the progress note.

Treatment and outcome measures

As the treatment of interest in our analysis, QPT use was defined as receiving this medication for no less than three days during the hospitalization, according to the Chinese diagnosis and treatment protocol for COVID-19 (Trial Version 6) (i.e., one formula a day, three formulas were defined as a course of treatment) (National Health Commission of the People's Republic of China, 2020). Correspondently, the study cohort was categorized into two treatment groups – patients receiving QPT and those not receiving QPT. Meanwhile, we also explored the effectiveness and safety of QPT between patients who ever received QPT during hospitalization and those who did not.

The outcome measure of effectiveness was in-hospital COVID-19 related mortality. The outcome measure of safety included acute liver injury and acute kidney injury during hospitalization. Acute liver injury was defined as documented acute liver injury, acute liver renal insufficiency, acute liver failure, hepatic encephalopathy, or hepatic coma, then adjudicated based on the elevation in aspartate aminotransferase, alanine aminotransferase, or total bilirubin. Acute renal injury was defined as documented acute renal failure, acute renal injury, or acute

renal insufficiency, then adjudicated based on the elevation in serum creatinine.

Statistical analysis

We described participant characteristics, treatments, and outcomes, with frequencies and percentages for categorical variables, while means ± standard deviations or median with interquartile range (IQR) for continuous variables. The difference between groups was estimated by standard mean difference (SMD), and absolute values less than 0.1 were considered small differences (Austin and Stuart, 2015).

We conducted a statistical power analysis in advance, based on the projected sample size of this retrospective registry. Assuming the in-hospital mortality rate was 4% in patients not receiving QPT, a total sample size of 9000 can achieve a statistical power of 80% at a 2-sided 0.05 significance level to detect a hazard ratio of 0.7 or below, for the treatment with a 30% or greater prevalence.

We used inverse probability treatment weighting (IPTW) based on the probability of receiving treatment to make the characteristics between the two treatment groups comparable. The probability of receiving QPT was estimated by multilevel logistic regression that adjusted for baseline characteristics including demographics, comorbidities, and prior histories extracted in previous referred (Table S1), with hospital as a random effect.

To assess the effectiveness of QPT, we obtained hazard ratios (HR) between treatment groups with developing frailty proportional hazards models for in-hospital death, accounted hospital as a random effect, adjusted for other in-hospital medications, and weighted with inverse probability of QPT use. We then plotted Kaplan-Meier curve in patients receiving and those not receiving QPT. To assess the safety of QPT, we obtained odds ratios (OR) with the multilevel logistic regression for acute liver injury and acute renal injury, which handled random effect, adjustment, and weight, using the similar approaches described earlier. We also added interaction items to explore the heterogeneity of effectiveness across subgroups by age (<60, 60–69, or ≥70 years), sex (male or female), and prior medical history/comorbidities (with any or without). In each subgroup, we recalculated inverse probability and reweighting separately, as aforementioned.

We conducted two sensitivity analysis. First, we matched propensity score between patients receiving and not receiving QPT using the nearest-neighbor method, to create two groups with similar characteristics and sample size. Second, we added the propensity score as a covariate in the frailty model without weighting, to account for the difference between treatment groups.

In the submitted medical records, small proportions of blood pressure (1.7%), heart rate (0.1%), and respiratory rate (0.2%) were missing. Assuming that these data were missing at random, we applied a multiple imputation method based on Markov Chain Monte Carlo by PROC MI procedure in SAS to impute missing values (Sterne et al., 2009).

Two-tailed P values were reported with $p < 0.05$ considered to indicate statistical significance. All statistical analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC).

Results

Study participants

There were 9115 patients with COVID-19 admitted to the 15 designated hospitals in this study, with the numbers of cases in each included hospital ranging from 140 to 1856. After excluding 96 patients with age <18 years, 66 patients transferred out, and 14 patients with the length of stay less than 24 h, 8939 eligible cases were included in the analysis (Fig. 1). Of them, the average age was 55.9 ± 15.6 years, and 53.4% (4771) were women. 4.4% (390) of patients were at critical state at admission, while 33.7% (3016) had hypertension, and 15.2% (1357) had diabetes.

Of these patients, 2833 (31.7%) ever received QPT during hospitalization, with a median treatment duration of 6 (4 to 9) days. Half of the

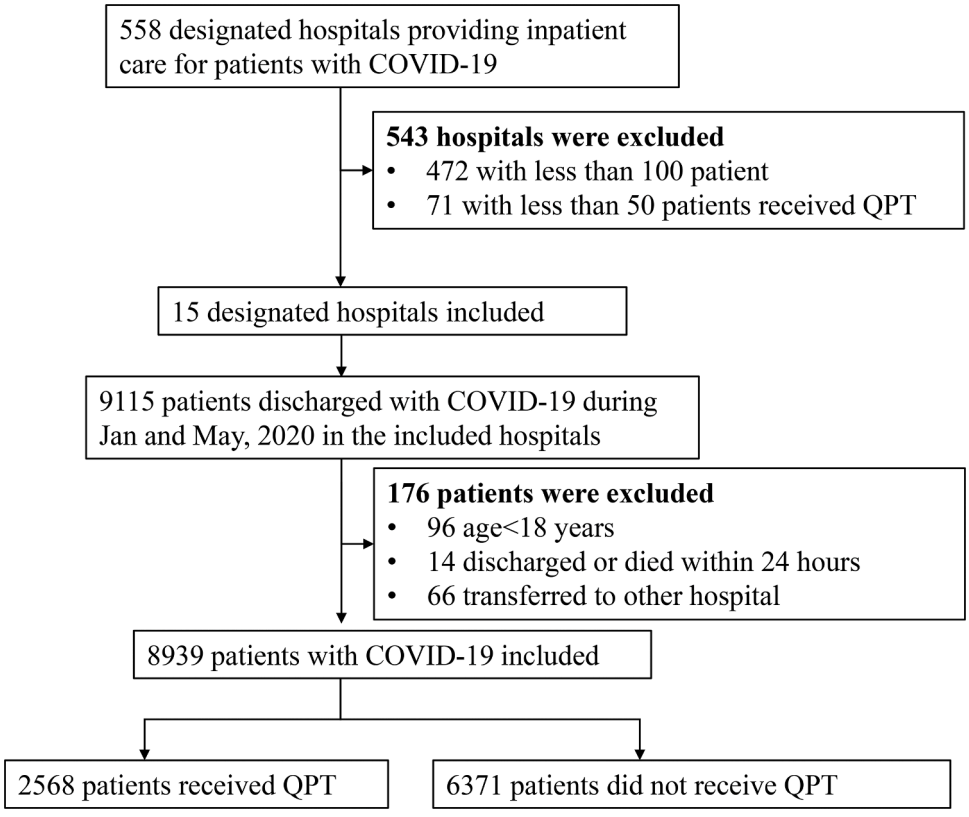

Fig. 1. Flowchart of the study cohort COVID-19, coronavirus disease 2019; QPT, Qingfei Paidu Tang.

QPT users received the first formula within 5 days after hospitalization. The timing of QPT use after hospitalization was shown in Figure S1.

In the study cohort, 2568 patients (28.7%) received QPT for no less than 3 days and 6371 (71.3%) did not. The patient characteristics of the two treatment groups were provided in Table 1. Unweighted comparisons showed that patients who received QPT were younger (SMD>0.1). After adjustment for inverse probability of treatment weighting, all covariates were well balanced (i.e., standardized mean differences were <0.1). The distributions of inverse probability score weights of the two treatment groups were shown separately in Figure S2.

Outcomes

During hospitalization with a median length of stay of 15 (9 to 21) days, 332 (3.7%) patients died from COVID-19. The mortality was 1.2% (95% confidence interval [CI], 0.8% to 1.7%) among patients who received QPT and 4.8% (95% CI, 4.3% to 5.3%) among patients who did not (Fig. 2). In the unadjusted analysis, patients who received QPT were less likely to die than patients who did not receive QPT (hazard ratio, 0.17; 95% CI, 0.11 to 0.25, $p < 0.001$). In the Cox model with inverse propensity score weighting, all covariates in the Cox model were shown in Table S2. QPT use was associated with a lower mortality risk (adjusted hazard ratio, 0.50; 95% CI, 0.37 to 0.66, $p < 0.001$).

In terms of sex and age, no significant differences were observed among their subgroups in the associations between QPT treatment and in-hospital mortality (all P for interaction > 0.05). Although significant heterogeneity in the associations between QPT treatment and in-hospital COVID-19 related mortality was detected between subgroups by prior medical history/comorbidities status (P for interaction = 0.020), the significantly lower mortality risk for patient receiving QPT was observed in both subgroups (Fig. 3).

Regarding the safety of QPT, patients who received QPT had comparable incidences of acute hepatic injury (crude rate, 8.9% [95% CI, 7.8% to 10.1%] vs 9.9% [95% CI, 9.2% to 10.7%]; adjusted OR, 0.96 [95% CI, 0.81 to 1.14], $p = 0.658$) and acute kidney injury (crude rate, 1.6% [95% CI, 1.2% to 2.2%] vs. 3.0% [95% CI, 2.6% to 3.5%]; adjusted OR, 0.85 [95% CI, 0.62 to 1.17], $p = 0.318$), in comparison with those who did not.

Furthermore, we also conducted an analysis of the effectiveness and safety of QPT between patients who ever received QPT during hospitalization and those who did not, and found similar results with those mentioned above (Table S3–4).

Sensitivity analyses

In addition to the IPTW analysis, we matched 3492 patients based on their propensity score (1746 patients receiving QPT and 1746 patients not receiving QPT). The two groups were well-balanced in characteristics and concomitant treatments (Table S5, Figure S3). The risk of mortality in patients who received QPT was significantly lower than in those who did not receive QPT (1.1% [95% CI, 0.7% to 1.7%] vs 2.7% [95% CI, 2.0% to 3.6%], HR, 0.40; 95%CI, 0.22 to 0.71; $p = 0.002$) (Table 2 and Figure S4). In the meantime, patients receiving QPT had a comparable incidence of acute kidney injury (1.1% [95% CI, 0.7% to 1.8%] vs. 1.9% [95% CI, 1.3% to 2.6%]; OR, 0.74 [95% CI, 0.40 to 1.35], $p = 0.327$) compared with the patients who did not, but a lower risk of acute liver injury (5.4% [95% CI, 4.4% to 6.5%]vs. 8.1% [95% CI, 6.9% to 9.5%]; OR, 0.72 [95% CI, 0.54 to 0.96], $p = 0.025$).

We also included the propensity score as an additional covariate in the models, in which patients who received QPT had a significantly lower risk of mortality than those who did not receive QPT (adjusted HR, 0.23 95% CI, 0.15 to 0.35; $p < 0.001$). Meanwhile, patients receiving QPT had comparable incidence of acute liver injury (OR, 0.93 [95% CI, 0.76 to 1.14], $p = 0.497$) and acute kidney injury (OR, 0.74 [95% CI, 0.50 to 1.10], $p = 0.133$) compared with the patients not receiving QPT.

Table 1
Baseline characteristics of patients by Qingfei Paidu Tang use.

	No QPT	QPT	SMD before IPTW	SMD after IPTW
	$N = 6371$	$N = 2568$		
Demographic				
Women	3401 (53.4)	1370 (53.3)	−0.0007	0.0115
Age, years			0.1081	0.0263
< 60	3626 (56.9)	1594 (62.1)		
60–70	1511 (23.7)	555 (21.6)		
> 70	1234 (19.4)	419 (16.3)		
Prior history/ Comorbidities				
Hypertension	2191 (34.4%)	825 (32.1%)	−0.0481	0.0175
Diabetes	1014 (15.9%)	343 (13.4%)	−0.0724	0.0130
Coronary heart disease	475 (7.5%)	211 (8.2%)	0.0283	0.0284
Stroke	469 (7.4%)	140 (5.5%)	−0.0781	0.0602
Chronic kidney disease	159 (2.5%)	57 (2.2%)	−0.0182	0.0265
COPD	116 (1.8%)	41 (1.6%)	−0.0173	0.0312
Cancer	201 (3.2%)	84 (3.3%)	0.0066	0.0190
Clinical characteristics at admission				
SBP, median (IQR), mmHg	130(120, 140)	128(120, 140)	−0.0308	0.0466
DBP, median (IQR), mmHg	80(74, 89)	80(74, 88)	0.0256	0.0316
HR, median (IQR), breaths per min	84(78, 95)	84(78, 96)	0.0077	0.0340
RR >24 breaths per min	592 (9.3%)	195 (7.6%)	−0.0611	0.0713
Critical state at admission	274 (4.3%)	116 (4.5%)	0.0105	−0.0019
Medication				
Antiviral				
Arbidol	3447 (54.1%)	1969 (76.7%)	0.4884	0.2971
Ribavirin	1150 (18.1%)	585 (22.8%)	0.1175	−0.0707
Oseltamivir	1347 (21.1%)	666 (25.9%)	0.1131	−0.0627
Ganciclovir	323 (5.1%)	183 (7.1%)	0.0860	−0.1166
Lopinavir/Ritonavir	777 (12.2%)	371 (14.4%)	0.0663	−0.0494
Traditional Chinese medicine				
Lianhua Qingwen	3172 (49.8%)	1563 (60.9%)	0.2242	0.1008
Xuebijing	624 (9.8%)	503 (19.6%)	0.2793	0.0284
Diammonium glycyrrhetate	996 (15.6%)	315 (12.3%)	−0.0973	0.0253
Corticosteroids				
Methylprednisolone	1251 (19.6%)	490 (19.1%)	−0.0140	−0.1184
Dexamethasone	334 (5.2%)	133 (5.2%)	−0.0029	−0.0077
Immunomodulator				
Interferon-alpha	2242 (35.2%)	857 (33.4%)	−0.0383	−0.1823

Abbreviations: QPT, Qingfei Paidu Tang; IPTW, inverse probability of treatment weighting; SMD, standardized mean difference; IQR, inter-quartile range; HR, heart rate; RR, respiratory rate; COPD: chronic obstructive pulmonary disease.

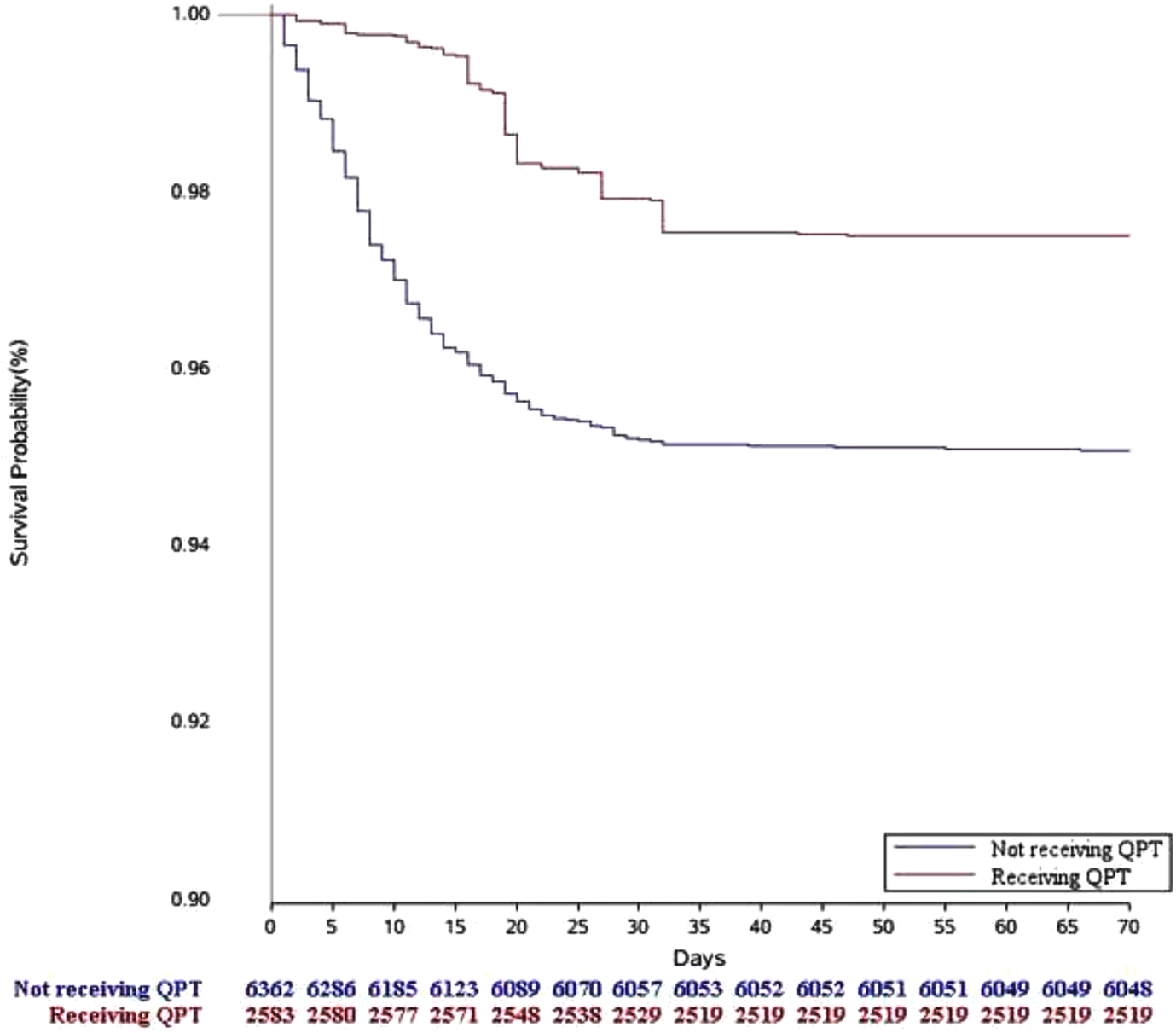

Fig. 2. Kaplan–Meier survival curves for in-hospital mortality in inverse probability treatment weighting analysis QPT, Qingfei Paidu Tang.

Discussion

In this analysis based on a national registry of hospitalized patients with COVID-19, we first demonstrated that QPT use was associated with halving the risk of in-hospital mortality, without any significant increase in the risk of adverse effects, such as acute liver injury or acute kidney injury. Our findings have provided new evidence and insights regarding the treatment of COVID-19.

Our study has extended the literature on the effectiveness of QPT for patients with COVID-19. First, this is the first study assessing the association between the QPT use and in-hospital mortality that is considered the most important and objective outcome metrics, rather than surrogate indicators widely used before. Second, in comparison with prior studies in China about QPT for COVID-19 treatment (Wang et al., 2020; Zhang et al., 2020; Li et al., 2020; Xin et al., 2020), our study has involved an over ninety-time larger sample size that ensured sufficient statistical power even for subgroup analysis. Third, using various propensity score approaches, we established control groups to enable appropriate comparisons in both effectiveness and safety of QPT. Fourth, this national registry included consecutive patients from multiple Chinese hospitals, which represented the use and effectiveness of QPT in real-world practice.

The effects of QPT on decreasing mortality of COVID-19 observed in our study are supported by the mechanisms shown in prior experimental studies, which included antivirus (Zhao et al., 2020; Chen et al., 2020), anti-inflammation (Zhao et al., 2020; Chen et al., 2020; Yang et al., 2020; Xu et al., 2020a, 2020b; Wu et al., 2020), immune regulation (Zhao et al., 2020; Xu et al., 2020a, 2020b; Wu et al., 2020), regulating metabolism (Chen et al., 2020; Xu et al., 2020a), anti-platelet aggregation (Yang et al., 2020), and organ protection (Xu et al., 2020a; Wu et al., 2020). QPT was composed of four traditional Chinese medicine prescriptions, which were shown to be separately effective in antivirus (Shi et al., 2019; Hsieh et al., 2012), anti-inflammatory (Yang et al., 2015), or immuno-modulating (Lin et al., 2020). QPT has multiple components acting on the multiple pathways. Some studies employed molecular network and network pharmacology to analyze the ingredients of QPT, and found that the key active ingredients, including quercetin, luteolin, kaempferol, naringenin, and isorhamnetin, could alleviate excessive

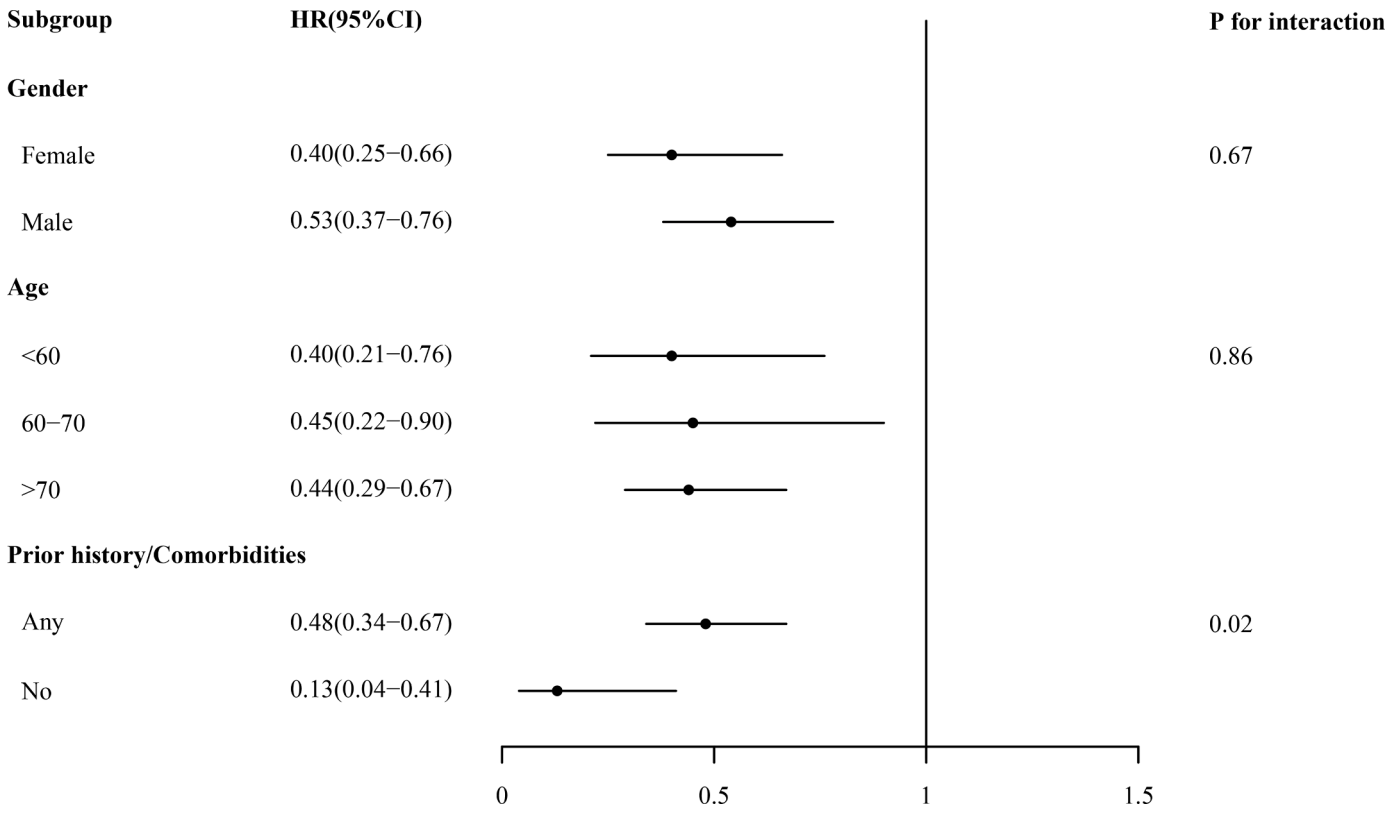

Fig. 3. Hazard ratios of in-hospital mortality across subgroups in inverse probability treatment weighting analysis HR, hazard ratio; 95% CI, 95% confidence interval.

Table 2
Associations between Qingfei Paidu Tang use and mortality in the crude analysis, multivariable analysis, and propensity-score analyses.

Analysis	Mortality
No. of events/no. of patients at risk (%)	
Qingfei Paidu Tang	29 (1.2)
No Qingfei Paidu Tang	303(4.8)
Crude analysis-hazard ratio (95% CI)	0.17(0.11–0.25)
Multivariable analysis- hazard ratio (95% CI)	0.22(0.14–0.34)
Propensity-score analysis- hazard ratio (95% CI)	
With inverse probability weighting	0.49(0.37–0.65)
With matching	0.40(0.22–0.71)
Adjusted as a covariant	0.23(0.15–0.35)

Abbreviations: 95% CI, 95% confidence interval.

immune responses, by regulating the function of cytokines related pathways, such as tumor necrosis factor signaling pathways and mitogen-activated protein kinases signaling pathways (Xu et al.,2020a, 2020b; Wu et al., 2020). Further research is needed to fully investigate the underlying mechanism of the effect of QPT.

In this study, we did not observe the elevated risk of acute liver injury or acute kidney injury among patients receiving QPT. This is consistent with the previous observational studies (Wang et al., 2020; Zhang et al., 2020; Li et al., 2020; Xin et al., 2020). Moreover, our findings are particularly reassuring given the complexity in comorbidities (such as hypertension, diabetes and chronic kidney disease) and concomitant treatments (such as antivirals, corticosteroids and immunomodulators) observed in our cohort. Nevertheless, the long-term safety related to QPT still needs to be verified in future studies.

This study has provided valuable evidence and prospects for the treatment of COVID-19. Currently, there are globally more than 16 million active cases that need treatments (World Health Organization, 2020). However, there is no evidence about any medication that could decrease mortality in COVID-19 except dexamethasone, which has been proved to be able to reduce the 28-day mortality in those who received mechanical ventilation or oxygen alone (Group et al., 2020; Wiersinga et al., 2020). To the best of our knowledge, this is the first study implying that QPT could reduce the mortality risk of patients with COVID-19. Our findings were consistent across subgroups, and robust regardless of analytic methods. It is encouraging that the use of QPT can probably prevent tens of thousands of deaths, if our findings are further confirmed and applied globally.

Limitations

The results of our study should be interpreted in the context of several limitations. First, due to the nature of observational study, we cannot exclude the influence of residual confounders. However, using a propensity score approach, we included most confounders that are commonly taken into account in comparative effectiveness researches. Moreover, after the IPTW, patients who received QPT had higher rates of co-morbidities which was positively related to mortality risk, compared with those who did not receive QPT. Thus, the effectiveness we observed tended to be conservative, and we believed that the improved mortality was largely attributed to the Qingfei Paidu Tang use, rather than other factors. Second, our study was based on real-world data and the quality of data could be affected by the accuracy and completeness of medical records. Therefore, we only included the highly reliable variables on patient characteristics, treatments, and outcomes in the analysis. Third, our study merely collected in-hospital outcomes, therefore, we could not evaluate the long-term effectiveness and safety. Finally, all the patients in our study were from China, and the beneficial effects of QPT in other racially diverse populations still await further validation.

Conclusion

Among the patients hospitalized for COVID-19, the use of QPT was associated with halving the risk of mortality, without raising the risk of acute liver injury or acute kidney injury. Further validation with randomized controlled trials is needed to support the use of QPT worldwide for COVID-19.

Declarations

Ethics approval

The Ethics Committee at the First Affiliated Hospital, Sun Yat-sen University approved the current analysis. Informed consent of individual patients was waived.

Consent for publication

Not applicable

Availability of data and materials

The data sharing needs to be approved by national registry, which is under the supervision of National Health commission. However, on site data audit is allowed under current regulation.

Funding

This project was supported by the Chinese Academy of Medical Sciences Innovation Fund for Medical Science (2020-I2M-Cov19–003) and the National Natural Science Foundation of China under Grants (No. U1611261). The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

CRediT authorship contribution statement

Lihua Zhang: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing. **Xin Zheng:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Xueke Bai:** Data curation, Formal analysis, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Qing Wang:** Data curation, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Bowang Chen:** Data curation, Software, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Haibo Wang:** Data curation, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Jiapeng Lu:** Data curation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Shuang Hu:** Data curation, Formal analysis, Software, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Xiaoyan Zhang:** Data curation, Validation, Writing - original draft, Writing - review & editing. **Haibo Zhang:** Data curation, Validation, Writing - original draft, Writing - review & editing. **Jiamin Liu:** Data curation, Validation, Writing - original draft, Writing - review & editing. **Ying Shi:** Data curation, Supervision, Validation, Writing - original draft, Writing - review & editing. **Zhiye Zhou:** Data curation, Funding acquisition, Supervision, Validation, Writing - original draft, Writing - review & editing. **Lanxia Gan:** Data curation, Supervision, Validation, Writing - original draft, Writing - review & editing. **Xi Li:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Jing Li:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

Dr. Jing Li discloses that she is a recipient of research grants from the government of China, through Fuwai Hospital, for research to improve the management of hypertension and blood lipids, and to improve care quality and patient outcomes of cardiovascular disease; is a recipient of research agreements with Amgen, through National Center for Cardiovascular Diseases (NCCD) and Fuwai Hospital, for a multi-center trial to assess the efficacy and safety of Omecamtiv Mecarbil, and for dyslipidaemia patient registration; is a recipient of a research agreement with Sanofi, through Fuwai Hospital, for a multi-center trial on the effects of sotagliflozin; is a recipient of a research agreement with University of Oxford, through Fuwai Hospital, for a multi-center trial of empagliflozin; and was a recipient of a research agreement, through NCCD, from AstraZeneca for clinical research methods training. The authors declared no other relevant conflict of interest.

Acknowledgments

We appreciate the Bureau of Medical Administration and Medical Service Supervision, National Health Commission of China, for the approval and support on collecting data. We appreciate all the COVID-19 designated hospitals for submitting medical records, and China Standard Medical Information Research Center for the support in collecting and processing data. We appreciate the team in National Center for Cardiovascular Diseases for their multiple contributions in data cleaning and manuscript coordinating.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2021.153531.

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Qingfei Paidu decoction, a Chinese herbal medicine against COVID-19, elevates the blood levels of pro-inflammatory cytokines: An open-label, single-arm pilot study

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Received December 26, 2020; Accepted March 9, 2021

DOI: 10.3892/wasj.2021.96

Abstract. Qingfei Paidu decoction (QFPD) is a Chinese herbal medicine newly formulated for the treatment of COVID-19. QFPD significantly enhances the therapeutic effects of standard pharmacotherapy in mild to critically ill patients with COVID-19. However, limited information is available on the immunological mechanisms underlying the efficacy of QFPD. In addition, the feasibility of the prophylactic administration of QFPD to uninfected individuals remains unconfirmed. To obtain insight into these issues, an open-label, single-arm pilot study was conducted using 19 healthy uninfected individuals as subjects, and the effects of QFPD ingestion at a dose lower than that recommended for therapeutic use on hematological and immunological parameters were examined. QFPD was prepared according to the Chinese official clinical guideline, except that the dose of each herb was reduced to 1/30 and administered orally to the participants twice daily for 3 days. Low-dose QFPD ingestion significantly increased the plasma levels of pro-inflammatory cytokines, tumor necrosis factor (TNF)-α (P=0.000107), interleukin (IL)-1β (P=0.000982), IL-18 (P=0.00105), IL-2 (P=0.0483) and IL-8 (P=0.000191), key mediators of a broad spectrum of antiviral immunity. No apparent adverse effects were observed during the trial. These findings suggest that the clinical efficacy of QFPD against COVID-19 is, at least in part, associated with its immunological activity to mimic the blood cytokine environment produced by early antiviral immune responses, which are shown to be profoundly suppressed during the early stages of COVID-19. The daily ingestion of low-dose QFPD may thus be a possible option for the prevention of COVID-19 during the epidemic. The present study was prospectively registered at the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) under the trial no. UMIN000040341 on May 9, 2020.

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Abbreviations: QFPD, Qingfei Paidu decoction; ssRNA, single-stranded RNA; TLR, Toll-like receptor; NLRP3, NLR family pyrin domain containing 3

Key words: Chinese herbal medicine, COVID-19, inflammation, cytokines, SARS-CoV-2, TNF-α, IL-1β, IL-18, IL-2, IL-8

Introduction

There is an increasing need for the development of diverse prophylactic and therapeutic options against COVID-19. The majority of patients with COVID-19 have been treated with traditional herbal medicine in combination with standard pharmacotherapy in China since the outbreak (1-4). Several newly developed herbal formulas were encouraged for the management of COVID-19 in the latest version of the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) released by the National Health Commission of China (5). One of these is Qingfei Paidu decoction (QFPD, the Chinese word for 'lung cleansing and detoxifying decoction'). QFPD was formulated specifically for the treatment of patients with COVID-19 and has exhibited satisfactory therapeutic efficacy (6-10). QFPD administration combined with the standard of care has been shown to significantly enhance the cure rates and to prevent disease progression in mild to critical cases (6-8). The concomitant administration of QFPD and Western medicine to patients with mild to moderate disease has been shown to achieve greater improvements in blood outcome indicators, such as C-reactive protein, creatine kinase and lactate dehydrogenase (9). The early initiation of treatment with QFPD following symptom onset leads to favorable clinical outcomes, such as a more rapid recovery, earlier viral clearance and a shorter hospitalization (10).

QFPD contains 21 herbal components optimized for the symptoms of COVID-19. Recent network pharmacological studies identified a large number of active natural compounds

contained in the herbal ingredients of QFPD, and predicted the comprehensive molecular, biological and functional networks underlying its pharmacological effects (11-15). The key active compounds include baicalin, glycyrrhizic acid, hesperidin, hyperoside, quercetin, glabridin, gallic acid, genistein and tectorigenin (11-15). These compounds interact with a wide variety of target proteins closely related to the symptoms of COVID-19, and thereby modulate the complex signaling networks involved in immune regulation, anti-inflammatory effects and multi-organ protection (11-15). Molecular docking analyses have revealed that the several active compounds have the potential to directly inhibit SARS-CoV-2 infection by interfering with host-virus protein interaction or downregulating the expression of angiotensin I converting enzyme 2, the viral entry receptor (11-15).

Although the network pharmacological approaches have provided in silico predictions on the possible molecular interaction networks targeted by active chemical compounds (11-15), there is limited information available on the *in vivo* immunological effects of QFPD. Furthermore, the feasibility of QFPD application to uninfected individuals for preventive purposes remains unconfirmed, despite its notable therapeutic benefits against COVID-19. In the present study, to obtain insight into these issues, a pilot study was conducted using uninfected individuals as subjects, and whether QFPD ingestion at a dose lower than that recommended for therapeutic use affects hematological and immunological parameters was examined.

Subjects and methods

Subjects. Participants were recruited through the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) website, the website of our clinic, announcements in an e-mail newsletter and personal contacts. Individuals who met all of the following inclusion criteria were enrolled in the trial: Adults between the ages of 20 and 70, having negative PCR and IgM/IgG antibodies tests for SARS-CoV-2 at study entry. Individuals were excluded from the trial if they met any of the following exclusion criteria: Pregnancy; breastfeeding; duplicate enrollment in other clinical trials; history of infectious disease within the past 6 months; current or past history of chronic inflammatory diseases, immune-related diseases, or malignancy; history of drug use within the past 6 months; underlying conditions associated with a higher risk of infection with COVID-19, including hypertension, cardiovascular disease, cerebrovascular disease, diabetes, obesity [body mass index (BMI) ≥30], chronic obstructive pulmonary disease and chronic kidney disease.

The present study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were reviewed and approved by the Ethics Committees of Takanawa Clinic (approval no. 2020-2). A signed informed consent form was obtained from each participant prior to inclusion in this study.

Preparation and administration of QFPD. A total of 21 types of traditional Chinese herbs for the QFPD formula were purchased from Shanghai Ruisha Comlat Co., Ltd. and mixed in accordance with the Chinese official guidelines (5). The dose of each herb was reduced to 1/30 on the basis of technical advice from herbal medicine specialists, considering possible adverse effects, such as palpitations in healthy subjects and the feasibility of the long-term daily use for prophylactic purposes. QFPD decoction was prepared immediately prior to the first administration. The mixed herbs were soaked in 600 ml of water for 30 min, simmered gently for 1 h, and strained through a tea strainer. The decoction was divided into 6 aliquots and stored at 4˚C during the trial. All procedures for the preparation and delivery of the decoction were performed by a specific pharmacy staff of Takanawa clinic to ensure quality control. The subjects were instructed to take an aliquot of the decoction orally 40 min after breakfast and dinner for 3 days in accordance with the administration protocol of the Chinese official guidelines (5).

Hematological and cytokine analyses. The primary outcome measure was changes in the plasma levels of inflammation-related cytokines after 3 days of low-dose QFPD ingestion compared with the baseline levels. The secondary outcome measure was changes in hematological parameters following low-dose QFPD ingestion for 3 days compared with baseline levels. Peripheral blood samples were obtained from each subject at 12 h prior to the first and after the final administration of QFPD. Hematological and blood biochemical tests were outsourced to SRL, Inc. The concentrations of plasma cytokines were measured using the V-PLEX Proinflammatory Panel 1 Human kit (K15049D-1, Meso Scale Diagnostics) and the human interleukin (IL)-18 ELISA kit (ab215539, Abcam) according to the manufacturers' protocols.

Negative control study. An additional negative control study (without QFPD ingestion) was conducted 9 months after the QFPD trial to exclude the influence of the circadian oscillation of blood cytokine levels and the effect of feeding on blood cytokine levels. Blood samples were re-collected with the same time schedule as that of the QFPD trial from 13 of the 18 subjects who had completed the previous QPPD trial, and plasma cytokines were measured as described above.

Statistical analysis. No outliers were taken into account, and all collected data (n=18) were subjected to statistical analysis. The normality of the data was tested using the normal quantile-quantile plots and the Shapiro-Wilk test. On the basis of the results from these normality tests, the two-tailed Wilcoxon signed-rank test at the significance level (α) of 0.05 was employed for the subsequent statistical analysis of the data. All statistical analyses were performed using EZR version 1.53 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (16). Post hoc power analysis was performed using G*Power version 3.1.9.2 (17).

Results

Participant recruitment began in May 9, 2020 and was completed the following day. A total of 19 volunteers were screened for eligibility, found to be eligible, and enrolled in the trial (Fig. 1). QFPD was administered to all the enrolled

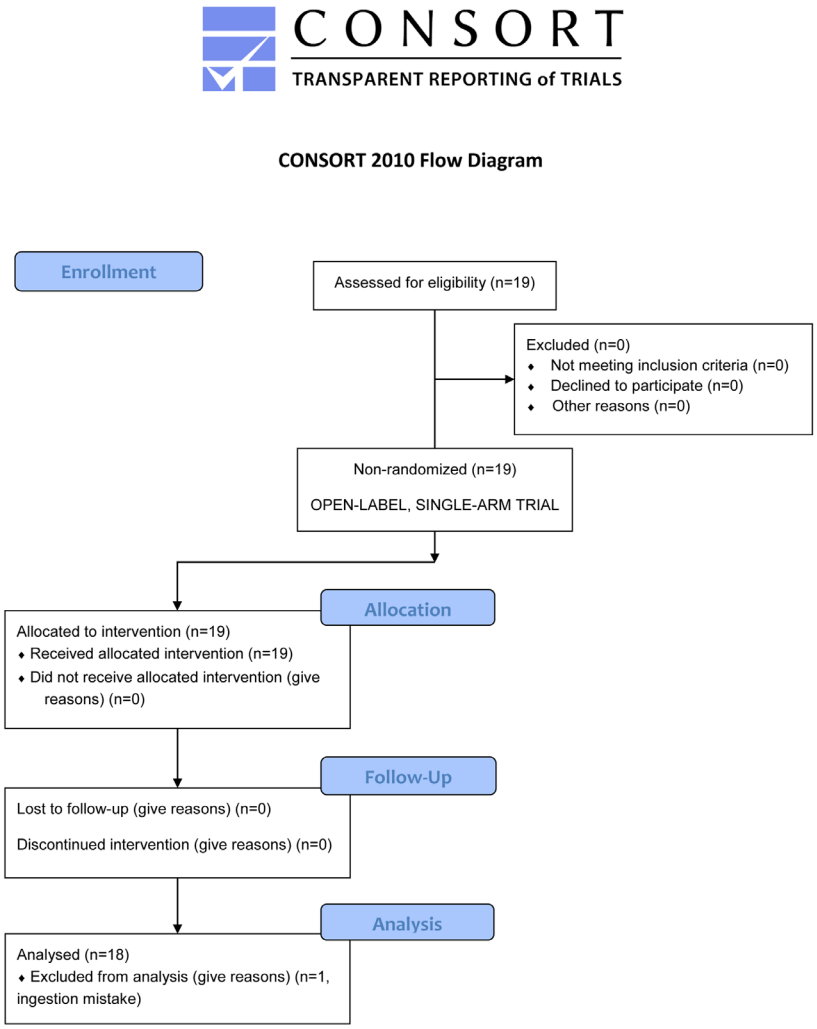

Figure 1. CONSORT flow diagram of the trial.

participants from May 12 to 14, 2020, of which 1 participant was excluded from the main analysis due to an ingestion error. Consequently, 18 subjects [5 males and 13 females; aged, 22-58 years; mean age (SD), 33.8 (10.7) years] completed the intervention, and the data were subjected to statistical analysis.

Marginal changes within reference intervals were found in mean corpuscular volume (Z=3.51, P=0.000454, r=0.827), mean corpuscular hemoglobin concentration (Z=3.07, P=0.00213, r=0.724) and blood urea nitrogen (Z=2.23, P=0.0260, r=0.525); however, there were no significant differences in other hematological and blood biochemical parameters between pre- and post-QFPD ingestion (Table I). Notably, low-dose QFPD ingestion induced significant increases in the plasma levels of the pro-inflammatory cytokines, tumor necrosis factor (TNF)-α (Z=3.46, P=0.000107, r=0.816), IL-1β (Z=3.30, P=0.000982, r=0.881), IL-18 (Z=3.07, P=0.00105, r=0.724), IL-2 (Z=1.98, P=0.0483, r=0.467) and IL-8 (Z=3.38, P=0.000191, r=0.796) (Table I). The levels of these cytokines were increased in 16 (88.9%), 14 (77.8%), 15 (83.3%), 13 (72.2%) and 17 (94.4%) out of the 18 subjects, respectively (Fig. 2). These significant changes in the plasma cytokine levels were not observed in the negative control study without QFPD ingestion (Table II), suggesting that the observed cytokine changes were due to the action of QFPD.

An increase was also observed in the interquartile range of IL-6, an exacerbating factor for COVID-19 (18-20); however, the difference was not statistically significant (Z=1.59, P=0.113, r=0.385). Similarly, the median value and the interquartile range of the anti-inflammatory cytokine, IL-10, were increased, although the difference was not statistically significant (Z=1.76, P=0.0814, r=0.416) (Table I). No apparent adverse effects were observed during the trial.

In addition, post hoc two-tailed power analysis (significance level, α=0.05; sample size, n=18) was performed and statistical powers (1 - β) were obtained of 0.836 (TNF-α), 0.655 (IL-1β), 0.648 (IL-18), 0.361 (IL-2) and 0.923 (IL-8) following the completion of the trial.

Discussion

Inflammation is a host defense mechanism against invading pathogens. Acute inflammatory responses to the infection of single-stranded RNA (ssRNA) viruses, such as SARS-CoV-2 are triggered by the foreign ssRNA sensors, Toll-like receptor (TLR)7, TLR8 and NLR family pyrin domain containing 3 (NLRP3) (21,22). TLR7 and TLR8 are expressed mainly in dendritic cells and macrophages, and induce the production of pro-inflammatory cytokines such as TNF-α and IL-6

Table I. Hematological and cytokine changes in subjects administered low-dose QFPD.

	Pre		Post				
Test parameter	Median	(IQR)	Median	(IQR)	Z	P-value	r
Complete blood count							
Red blood cell count (x10^4/μl)	459	(417-480)	442	(417-482)	0.610	0.542	0.144
Hemoglobin (g/dl)	13.7	(12.6-14.5)	13.1	(12.6-14.6)	0.0711	0.943	0.0167
Hematocrit (%)	41.4	(38.2-43.2)	40.2	(38.8-44.6)	1.26	0.207	0.298
MCV (fl)	89.7	(87.6-93.2)	90.7	(88.7-98.0)	3.51	0.000454[c]	0.827
MCH (pg)	30.1	(29.6-31.5)	30.0	(29.6-31.5)	1.11	0.265	0.263
MCHC (%)	33.4	(32.8-33.8)	32.7	(31.7-33.4)	3.07	0.00213[b]	0.724
White blood cell count (/μl)	5,850	(5600-6650)	5,850	(5300-6180)	1.39	0.163	0.329
Platelet count (x10^4/μl)	24.7	(22.3-27.1)	24.2	(21.7-26.9)	1.18	0.236	0.279
White blood cell differential							
Neutrophils (%)	57.5	(54.5-63.8)	56.8	(52.8–60.6)	0.893	0.372	0.210
Eosinophils (%)	1.35	(0.950-2.43)	1.20	(1.00-2.20)	0.00	1.00	0.00
Basophils (%)	0.450	(0.325-0.500)	0.500	(0.325-0.650)	1.17	0.241	0.276
Monocytes (%)	4.65	(4.23-5.70)	5.15	(4.13-6.10)	0.129	0.897	0.0305
Lymphocytes (%)	36.5	(28.5-37.8)	35.9	(32.3-38.4)	0.479	0.632	0.113
Blood biochemistry							
AST (U/l)	17.0	(16.0-20.0)	18.0	(16.0-22.3)	1.90	0.0574	0.448
ALT (U/l)	15.5	(11.0-22.5)	15.0	(12.0-23.8)	0.945	0.345	0.223
γ-GT (U/l)	19.5	(14.0-31.8)	18.5	(13.3-32.8)	0.699	0.485	0.165
LDH (U/l)	156	(137-163)	155	(143-174)	1.44	0.149	0.340
Albumin (g/dl)	4.75	(4.70-4.88)	4.70	(4.60-4.88)	1.17	0.243	0.275
Urea nitrogen (mg/dl)	13.2	(12.0-15.3)	11.5	(10.9-13.6)	2.23	0.0260[a]	0.525
HDL-cholesterol (mg/dl)	70.0	(58.3-75.5)	67.0	(60.8-76.3)	0.0856	0.932	0.0202
LDL-cholesterol (mg/dl)	115	(75.5-135)	111	(83.3-136)	0.590	0.555	0.139
Triglycerides (mg/dl)	66.5	(48.5-122)	78.5	(51.3-116)	0.174	0.862	0.0411
CRP (mg/dl)	0.0400	(0.0225-0.0500)	0.0400	(0.0300-0.0575)	1.10	0.270	0.260
Cytokines							
IFN-γ (pg/ml)	2.70	(1.39-4.64)	3.78	(2.44-5.43)	1.46	0.154	0.344
IL-6 (pg/ml)	0.443	(0.225-0.509)	0.436	(0.355-1.10)	1.59	0.113	0.385
TNF-α (pg/ml)	1.68	(1.39-1.87)	1.95	(1.65-2.42)	3.46	0.000107[c]	0.816
IL-1β (pg/ml)	0.000	(0.000-0.000)	0.156	(0.0583-0.333)	3.30	0.000982[c]	0.881
IL-18 (pg/ml)	98.2	(71.5-161)	149	(98.8-267)	3.07	0.00105[b]	0.724
IL-2 (pg/ml)	0.326	(0.041-0.765)	0.375	(0.185-0.785)	1.98	0.0483[a]	0.467
IL-8 (pg/ml)	92.3	(22.8-169)	344	(89.8-834)	3.38	0.000191[c]	0.796
IL-10 (pg/ml)	0.175	(0.117-0.271)	0.250	(0.144-0.318)	1.76	0.0814	0.416

Statistically significant results are shown in bold font ([a]P<0.05, [b]P<0.01, [c]P<0.001). MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; LDH, lactate dehydrogenase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; IQR, interquartile range; Z, test statistic for the Wilcoxon signed-rank test; r, Wilcoxon signed-rank test effect size.

in response to viral ssRNA in the endosome (21). NLRP3, when it recognizes viral ssRNA in the cytoplasm, activates the inflammasome-mediated processing of pro-IL-1β and pro-IL-18 to release active IL-1β and IL-18 (21,23). These 'immediate-early' cytokines initiate and coordinate a broad spectrum of downstream antiviral immune cascades (21-23). In the present study trial, low-dose QFPD ingestion induced significant increases in the levels of TNF-α, IL-1β and IL-18. It was therefore speculated that QFPD may partially mimic the blood cytokine environment produced by TLR7/8- and NLRP3-driven early immune responses to ssRNA viruses.

Since systemic hyperinflammation caused by complex immune dysregulation is a hallmark of COVID-19 (19,20,24), the pro-inflammatory activity of QFPD is apparently contra-

Table II. Changes in cytokine levels observed in a negative control study (without QFPD administration).

	Pre		Post				
Test item	Median	(IQR)	Median	(IQR)	Z	P-value	r
IFN-γ (pg/ml)	1.73	(0.689-6.34)	3.83	(1.29-7.16)	0.454	0.685	0.126
IL-6 (pg/ml)	0.00294	(0.000949-1.90)	1.79	(0.00943-1.92)	1.01	0.340	0.281
TNF-α (pg/ml)	0.0119	(0.00653-1.04)	0.580	(0.0165-0.971)	0.734	0.497	0.204
IL-1β (pg/ml)	0.00	(0.00-0.0406)	0.0251	(0.00-0.120)	1.08	0.263	0.300
IL-18 (pg/ml)	126	(108-133)	85.3	(78.6-102)	1.85	0.068	0.514
IL-2 (pg/ml)	0.487	(0.0706-0.737)	0.281	(0.182-0.595)	0.943	0.376	0.262
IL-8 (pg/ml)	133	(12.7-263)	113	(21.4-282)	1.71	0.094	0.475
IL-10 (pg/ml)	0.134	(0.0383-0.275)	0.143	(0.0101-0.345)	0.734	0.497	0.204

IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; IQR, interquartile range; Z, test statistic for the Wilcoxon signed-rank test; r, Wilcoxon signed-rank test effect size.

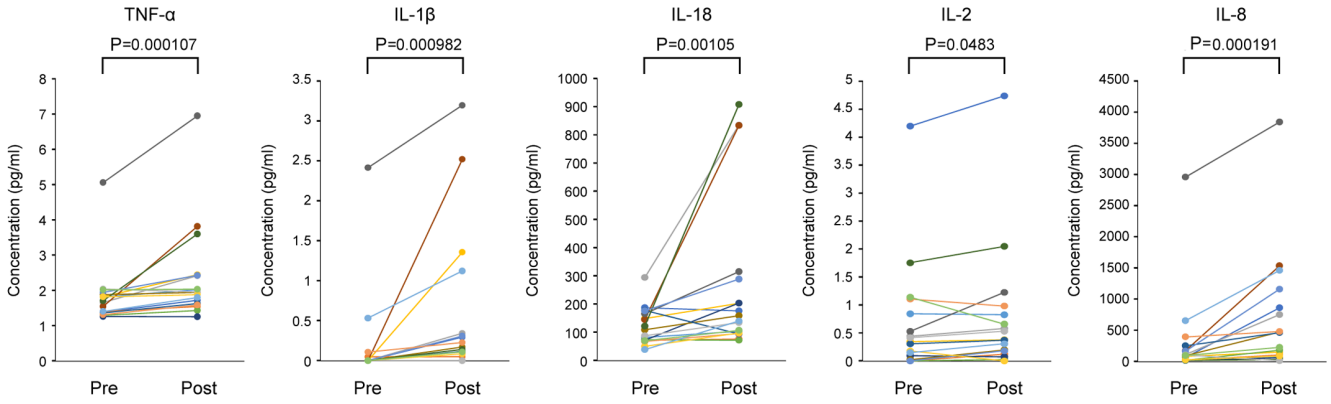

Figure 2. Changes in the plasma levels of TNF-α, IL-1β, IL-18, IL-2, and IL-8 before and after low-dose QFPD ingestion. Data (n=18) were analyzed using the two-tailed Wilcoxon signed-rank test at the significance level (α) of 0.05. Pre, pre-ingestion (baseline); Post, post-ingestion; QFPD, Qingfei Paidu decoction; TNF, tumor necrosis factor; IL, interleukin.

dictory to its clinical benefits against COVID-19. However, recent metagenomics studies have highlighted the importance of the active TLR7/8- and NLRP3-mediated inflammatory pathways in anti- SARS-CoV-2 immunity (25,26). A whole-exome sequencing by van der Made *et al* identified rare loss-of-function variants of the *TLR7* gene in severe COVID-19 patients: A 4-nucleotide deletion and a missense variant that cause impaired TLR7-dependent immune signaling (25). In addition, a comparative transcriptomics study by Mick *et al* demonstrated the suppressed expression of genes involved in innate immunity, including pattern recognition (*TLR8*), inflammasome activation (*NLRP3* and *CASP5*) and inflammatory IL signaling (*IL1A, IL1B, IL18RAP* and *IL1R2*), in the upper airways of patients with COVID-19 (26). Intriguingly, *IL1B* is the most strongly suppressed gene, and the NLRP3-inflammasome and IL-1 immune pathways are particularly non-responsive to SARS-CoV-2 infection early in the course of the disease (26). These transcriptional responses to SARS-CoV-2 lead to impaired neutrophil and macrophage recruitment to the upper airway (26). Collectively, these findings suggest that the TLR7/8- and NLRP3-driven inflammatory responses play important protective roles in the early stages of COVID-19. The QFPD-induced inflammatory tone may therefore be effective in preventing SARS-CoV-2 infection in uninfected individuals.

IL-2 is a type 1 helper T cytokine with diverse regulatory functions in cellular immunity (27). It stimulates $CD8^+$ cytotoxic T lymphocytes, monocytes/macrophages and natural killer cells to eliminate virus-infected cells. IL-2 also promotes the differentiation, expansion and stability of $CD4^+CD25^+Foxp3^+$ regulatory T cells that suppress excessive immune reactions. These opposite functions of IL-2 contribute to the maintenance of immune homeostasis. IL-8 is a potent neutrophil chemotactic factor produced by macrophages, epithelial cells, airway smooth muscle cells and vascular endothelial cells (28). It strongly induces the migration of neutrophils to the sites of infection and activates phagocytic elimination of invading pathogens and infected cells. The activity of low-dose QFPD to upregulate IL-2 and IL-8 may therefore be beneficial in terms of its prophylactic use. However, excessive IL-8 is known to be closely associated with acute lung injury and acute respiratory distress syndrome (29,30), and detailed dose-response trials are required to ensure the safety.

In the present study trial, low-dose QFPD had no significant effects on the plasma levels of IL-6 and IL-10. IL-6 is known to be a critical driver of complex immune dysregulation and systemic hyperinflammation (18-20). There is a definite positive association between the blood IL-6 levels, and COVID-19 severity and mortality (31-33). Similarly, blood IL-10 levels are markedly elevated in patients with severe COVID-19 and are positively associated with disease severity and mortality (34-37). Although IL-10 has been commonly regarded as an immunosuppressive and anti-inflammatory cytokine, there is emerging evidence to indicate that IL-10 can act as an immunostimulatory and pro-inflammatory cytokine in some autoimmune diseases and cancers, which supports the hypothesis that IL-10 may contribute to COVID-19 progression (38). The ineffectiveness of low-dose QFPD on IL-6 and IL-10 may therefore be a suitable pharmacological property for its prophylactic administration to uninfected individuals.

The main limitations of the present study are the small number of participants and the use of uninfected individuals as subjects. Further trials with larger cohorts are essential to confirm the conclusion and determine generalizability. The present study employed uninfected individuals as subjects with the aim of testing the feasibility of the daily use for prophylactic purposes; however, additional studies are required to clarify whether full-dose QFPD induces similar changes in cytokine levels in patients with COVID-19. The safety of long-term, daily use of low-dose QFPD also needs to be confirmed in future longitudinal follow-up studies. QFPD was administered 40 min after breakfast and dinner for 3 days, and blood samples were collected in the evening, 12 h before the first administration of QFPD (shown as 'Pre' in Fig. 2), and in the morning, 12 h after the last administration of QFPD (shown as 'Post' in Fig. 2). Importantly, there are a large number of studies that have reported the circadian oscillation of serum cytokine levels (39,40), as well as the effects of feeding on serum cytokine levels (41,42). A negative control study without QFPD ingestion was also conducted (Table II); however, placebo-controlled trials are essential to adequately examine the immunological efficacy of QFPD.

In conclusion, the findings of the present study suggest that low-dose QFPD may partially mimic the blood inflammatory tone produced by TLR7/8- and NLRP3-dependent early defense responses to ssRNA viruses. Given that the TLR- and NLRP3-driven immune pathways are suppressed early in the course of COVID-19, it was hypothesized that QFPD may also be effective in reducing the risk of SARS-CoV-2 infection in uninfected individuals. The daily intake of low-dose QFPD may therefore be a possible option for the prevention of COVID-19 during the pandemic. Careful prescribing is required when used in uninfected individuals for preventive purposes until a better understanding of the *in vivo* pharmacological actions of QFPD is acquired.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The present study was prospectively registered at the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) under the trial no. UMIN000040341 on May 9, 2020.

Authors' contributions

YK, TE, TA and TN were involved in the conceptualization of the study and in the study methodology. TN was responsible for formal analysis., YK, KA, KK, MM and TE were involved in the investigative aspects of the study and in the provision of resources. YK and TE were involved in data curation. TN was involved in the writing of the original draft and in visualization (figure preparation). YK, KA, KK, MM, TE and TA were involved in the writing, review and editing of the manuscript. YK, TA and TN were involved in study supervision. YK, KA and TE were involved in project administration. YK, TE and TN confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were reviewed and approved by the Ethics Committees of Takanawa Clinic (approval no. 2020-2). A signed informed consent form was obtained from each participant prior to inclusion in this study.

Patient consent for publication

Not applicable.

Competing interests

YK, KA, KK, MM and TE are employees of Takanawa Clinic. TA and TN serve as research advisers to Takanawa Clinic and receive advisory fees.

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Qingfei Paidu decoction for treating COVID-19

A protocol for a meta-analysis and systematic review of randomized controlled trials

Yuan Zhang, MD, Hongyan Xie, MS, Yan Li, MS, Tianhao Li, MS, Haipo Yuan, MS, Xiaoxu Fu, MD, Chunguang Xie, MD*

Abstract

Background: Coronavirus disease 2019 (COVID-19) is one of the infectious diseases that have seriously threatened global public health since its outbreak in 2019. Due to the complicated Pathogenesis, high infectivity and high fatality rate of COVID-19, there is currently no effective treatment for such epidemic disease. Traditional Chinese medicine has a long clinical history for the prevention and treatment of this kind of acute infectious disease. Qingfei Paidu Decoction (QFPD) is widely used in treating COVID-19 in China. However, there is still a lack of comprehensive and systematic evidence on the effectiveness and safety of Qingfei Paidu Decoction.

Methods: We will search each database from the built-in until May 2020. The English literature mainly searches Cochrane Library, PubMed, EMBASE, and Web of Science, while the Chinese literature comes from CNKI, CBM, VIP, and Wangfang database. Simultaneously we will retrieval clinical registration tests and grey literatures. This study only screen the clinical randomized controlled trials (RCTs) about QFPD for COVID-19 to assess its efficacy and safety. The two researchers worked independently on literature selection, data extraction, and quality assessment. The dichotomous data is represented by relative risk (RR), and the continuous is expressed by mean difference (MD) or standard mean difference (SMD), eventually the data is synthesized using a fixed effect model (FEM) or a random effect model (REM) depending on whether or not heterogeneity exists. Total clinical effective rate, improvement rate of lung CT, adverse events were evaluated as the main outcomes. Effective rate of clinical symptoms, treatment time were secondary outcomes. Finally, meta-analysis was conducted by RevMan software version 5.3.

Results: The results of our research will be published in a peer-reviewed journal.

Conclusion: This systematic review aims to provide new evidence of QFPD for COVID-19 in terms of its efficacy and safety.

PROSPERO registration number: CRD42020200894.

Abbreviations: CI = confidence interval, COVID-19 = coronavirus disease 2019, FEM = fixed effect model, MD = mean difference, PRISMA-P = Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol, QFPD = Qingfei Paidu Decoction, RCT = randomized controlled trial, REM = random effect model, RR = relative risk, SMD = standard mean difference.

Keywords: COVID-19, meta-analysis, protocol, Qingfei Paidu decoction, systematic review

1. Introduction

Coronavirus disease 2019 is an acute respiratory infectious disease caused by the new coronavirus type 2 acute respiratory syndrome coronavirus (SARS-CoV-2).[1] COVID-19 patients usually present with respiratory symptoms, such as fever, coughing, sneezing, fatigue, etc. Nearly one-third of patients suffer from at least one coexisting disease.[2,3] Since the first outbreak of the COVID-19 in Wuhan, it has become a key issue that has seriously threatened the public health of people around the world.[4] As this corona virus has the typical characteristics of highly contagious and high fatality rate, until March 2020, the novel coronavirus disease has spread in more than 100 countries around the world.[5] According to reports, a total of 531,630 people worldwide have been infected with the virus, and the death toll is as high as 24,065.[6]

There is no effective treatment to control the new coronavirus disease 2019 according to the World Health Organization (WHO) commentary.[7] According to clinical experience, antiviral drugs and symptomatic and supportive treatment are often used. However, its therapeutic effect still needs further evaluation.[8,9] Therefore, it is imperative that we need to seek new

HX, YL, TL, and ZY contributed equally to this work and are co-first authors.

This project is funded by the projects of Sichuan Provincial Science and technology department (No.2020YFS0012 and 2020YFS0013). The sponsors are not involved in design, execution, or writing the study.

Ethical approval is not needed for this study. The results of this study will be published in peer-reviewed journals.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Zhang Y, Xie H, Li Y, Li T, Yuan H, Fu X, Xie C. Qingfei Paidu decoction for treating COVID-19: A protocol for a meta-analysis and systematic review of randomized controlled trials. Medicine 2020;99:36(e22040).

Received: 31 July 2020 / Accepted: 4 August 2020

http://dx.doi.org/10.1097/MD.0000000000022040

treatment methods and measures to prevent the progression and prevalence of the disease.[10]

However, traditional Chinese medicine and Chinese herbal medicine have accumulated rich clinical experience and effective formulas in the prevention and treatment of epidemics, such as SARS in 2003. In this epidemic, Both of them also played a huge role in the prevention and control of the new coronavirus 2019.[11,12] After the outbreak of the coronavirus disease, the National Health Commission of the People's Republic of China formulated and issued the"Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version)," which is based on the clinical manifestations of the disease, pathology, and accumulated experience in the diagnosis and treatment.[13] Qingfei Paidu Decoction (QFPD) appears in this plan as one of the recommended Chinese medicine prescriptions. Qingfei Paidu Decoction is a traditional Chinese medicine compound first created by Zhang Zhongjing. It is composed of *Ma Xing Shi Gan Decoction, She Gan Ma Huang Decoction, Xiao Chai Hu Decoction,* and *Wu Ling San*. *Herba Ephedrae, Radix Glycyrrhizae, Semen Armeniacae Amarum, Gypsum Fibrosum, Ramulus Cinnamomi, Rhizoma Alismatis, Polyporus Umbellatus, Rhizoma Atractylodis Macrocephalae, Poria, Radix Bupleuri, Radix Scutellariae, Rhizome Pinelliae Preparata, Rhizoma Zingiberis Recens, Radix Asteris, Flos Farfarae, Rhizoma Belamcandae, Herba Asari, Rhizoma Dioscoreae, Fructus Aurantii Immaturus, Pericarpium Citri Reticulatae, Herba Pogostemonis* make up the Qingfei Paidu Decoction. In terms of modern pharmacological analysis, the prescription has multiple functions of antiviral, anti-inflammatory, immune regulation, and antipyretic. It has also been clinically proven to have a good effect on COVID-19.[14] Therefore, we intend to collect randomized controlled trials (RCTs) about QFPD for COVID-19 based on the basis of evidence-based medicine, and conduct a meta-analysis of its efficacy and safety to provide higher quality clinical evidence for Chinese medicine treatment of COVID-19.

2. Methods

2.1. Protocol registration

The systematic review protocol has been registered on the prospero website as CRD42020200894 (https://www.crd.york.ac.uk/prospero/#recordDetails). It is reported following the guidelines of Cochrane Handbook for Systematic Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocol (PRISM).[15] We will update our protocol for any changes in the entire research process if needed.

2.2. Inclusion criteria

2.2.1. Study design. The study only select clinical randomized controlled trials of QFPD for COVID-19 published in both Chinese and English. However, animal experiments, reviews, case reports, and non-randomized controlled trials are excluded.

2.2.2. Participants. This study included patients who had been clearly diagnosed with the new coronavirus disease. Except that participants must be over 18 years old, there were no strict restrictions on gender and severity of the disease.

2.2.3. Intervention. The test group uses QFPD. The control group can be treated with other treatments except QFPD. There are no obvious restrictions on the dosage of therapeutic drugs and specific intervention routes.

2.2.4. Outcomes. The primary outcomes include total clinical effective rate, improvement rate of lung CT, adverse events. Secondary outcomes are mainly composed of effective rate of clinical symptoms treatment time.

2.3. Search methods

2.3.1. Electronic searches. We will retrieve each database from the built-in until May 2021. The English literature mainly searches Cochrane Library, PubMed, EMBASE, and Web of Science. While the Chinese literature comes from CNKI, CBM, VIP, and Wangfang database. We adopt the combination of heading terms and free words as search strategy which decided by all the reviewers. Search terms: Qingfei Paidu Tang, Qingfei Paidu Decoction, 2019 novel coronavirus disease, COVID-19, coronavirus disease 2019, 2019 novel coronavirus infection, 2019-nCoV disease. We will simply present the search process of the Cochrane library (Table 1). Adjusting different search methods according to different Chinese and English databases.

2.3.2. Searching other resources. At the same time, we will retrieve other resources to complete the deficiencies of the electronic databases, mainly searching for the clinical trial registries and grey literature about QFPD for COVID-19 on the corresponding website.

2.4. Data collection and analysis

2.4.1. Selection of studies. Import all literatures that meet the requirements into Endnote X8 software. First of all, two independent reviewers initially screened the literatures that did not meet the pre-established standards of the study by reading the title and abstract. Second, download the remaining literatures and read the full text carefully to further decide whether to include or not. Finally, the results were cross-checked repeatedly by reviewers. If there is a disagreement in the above process, we can reach an agreement by discussing between both reviewers or seek a third party's opinion. Flow chart of the study selection (Fig. 1) will be used to show the screening process of the study.

2.4.2. Data extraction and management. According to the characteristics of the study, we prepare an excel form for data collection before data extraction. Outcome indicators for eligible studies were independently extracted and filled in the data extraction form by two reviewers. The main data extracted are as follows: title, author, year, fund source, sample size, age, sex, duration of disease, interventions, outcome measures, adverse reactions, etc. If there are something unclear, you can not hesitate to contact authors of more detailed information. The above information was finally cross-checked by two reviewers.

2.4.3. Assessment of risk of bias in included studies. The quality assessment of RCTs adopts the risk of bias (ROB) assessment tool provided by the Cochrane Handbook. The following seven items, such as random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other bias, are evaluated by three grades of "low bias," "high bias," and "unclear bias." The discrepancies will get a consistent conclusion by discussing between both reviewers or seeking the third-party consultation.

Table 1

Cochrane library search strategy.

Number	Search terms
1	Mesh descriptor:(Qingfei Paidu Decoction) explode all trees
2	((Qingfei Paidu Decoction*) or (Qingfei Paidu Tang*)or (Qingfei Paidu*)):ti, ab, kw
3	Or 1–2
4	Mesh descriptor:(COVID-19) explode all trees
5	((COVID-19*) or (2019 novel coronavirus disease*)or(coronavirus disease 2019*)or(2019 novel coronavirus infection*) or (2019-nCoV disease*)):ti, ab, kw
6	Or 4–5
7	MeSH descriptor: (randomized controlled trials) explode all trees
8	((random*) or (randomly*) or (allocation*) or (random allocation*) or (placebo*) or (single blind*) or (double blind*) or (clinical trials*) or (randomized control trial*) or (RCT*) or (controlled clinical trials *)):ti, ab, kw
9	Or 7–8
10	3 and 6 and 9

COVID-19 = coronavirus disease 2019, RCT = randomized controlled trial.

2.4.4. Data analysis. Different evaluation methods are selected according to the different efficacy indicators. For the dichotomous data, we will choose the effect scale indicator relative risk (RR) with 95% confidence interval (CI) to represent. While the continuous data is expressed as mean difference (MD) or standardized mean difference (SMD) with 95% CI depending on whether the measurement scale is consistent or not. Review Manager software version 5.3 provided by the Cochrane Collaboration will be performed for data synthesis and analysis. The dichotomous data is represented by RR, continuous data is expressed by MD or SMD. If there is no heterogeneity ($I^2 < 50\%$, $P > 0.1$), the data are synthesized using a fixed effect model. Otherwise ($I^2 \geq 50\%$, $P < 0.1$), a random effect model is used to analyze. Then subgroup analysis will be conducted basing on the

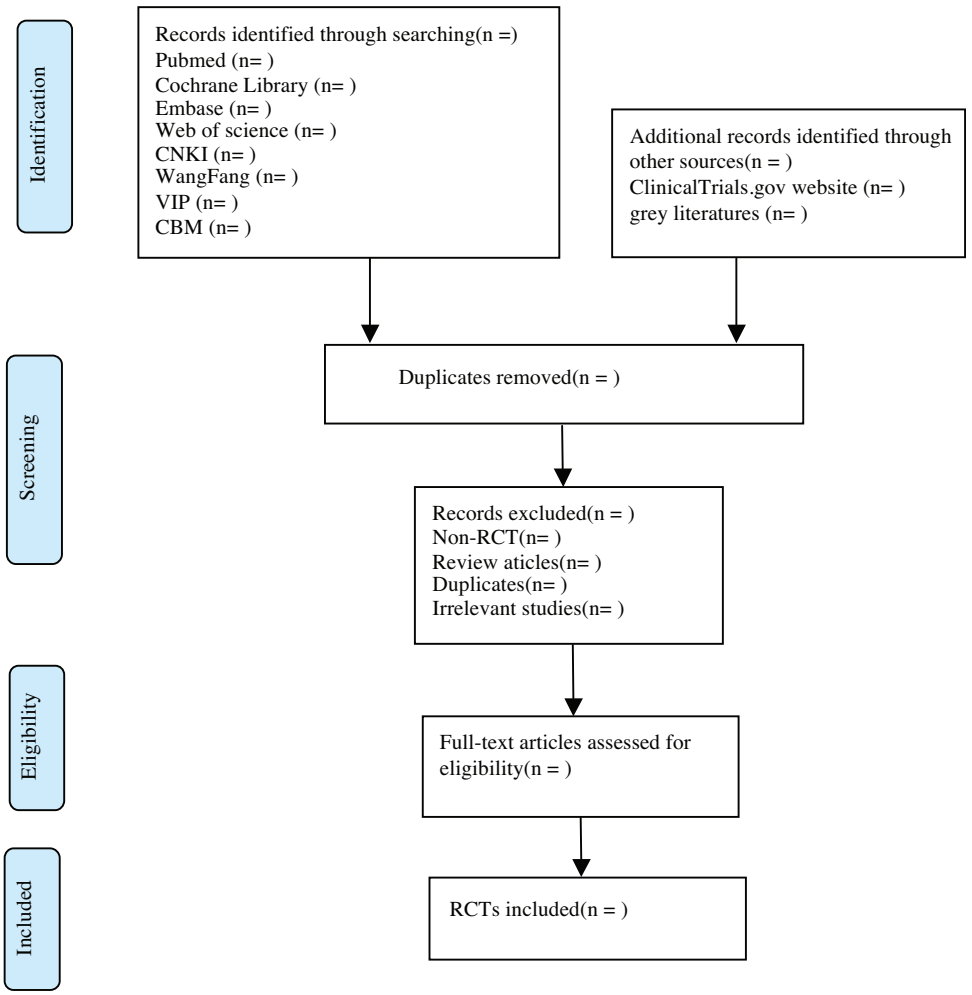

Figure 1. Flow chart of the study selection.

different causes of heterogeneity. If a meta-analysis cannot be performed, it will be replaced by a general descriptive analysis.

3. Discussion

Due to the high lethality and epidemic of the new coronavirus disease, it has attracted the attention of various countries around the world.[16–17] The current clinical treatment is mainly based on antiviral drugs and symptomatic support, and no unified treatment method has been established.[18] Therefore, this pushes us to explore and research novel drugs to fill the current treatment deficiencies.

Traditional Chinese medicine and Chinese herbal medicine have a long history of treating infectious diseases such as malaria in ancient China. It not only has obvious advantages in effective treatment, but also has fewer side effects. During the prevalence of the coronavirus, the clinical efficacy data of the traditional Chinese medicine compound QFPD showed that it has the effect of reducing patient mortality and improving respiratory symptoms. Therefore, this study intends to comprehensively evaluate the safety and safety of the efficacy of QFPD in COVID-19 patients through systematic reviews and meta-analysis. In other words, it can also provide new therapy for early control of the virus and defeat epidemic.

Author contributions

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Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Food and Chemical Toxicology 149 (2021) 111998

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Inhibition of drug-metabolizing enzymes by Qingfei Paidu decoction: Implication of herb-drug interactions in COVID-19 pharmacotherapy

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ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Keywords:
Qingfei Paidu decoction (QPD)
Cytochrome P450 enzymes (CYPs/P450s)
Herb-drug interactions (HDIs)
CYP3A substrate-drugs

ABSTRACT

Corona Virus Disease 2019 (COVID-19) has spread all over the world and brings significantly negative effects on human health. To fight against COVID-19 in a more efficient way, drug-drug or drug-herb combinations are frequently used in clinical settings. The concomitant use of multiple medications may trigger clinically relevant drug/herb-drug interactions. This study aims to assay the inhibitory potentials of Qingfei Paidu decoction (QPD, a Chinese medicine compound formula recommended for combating COVID-19 in China) against human drug-metabolizing enzymes and to assess the pharmacokinetic interactions *in vivo*. The results demonstrated that QPD dose-dependently inhibited CYPs1A, 2A6, 2C8, 2C9, 2C19, 2D6 and 2E1 but inhibited CYP3A in a time- and NADPH-dependent manner. *In vivo* test showed that QPD prolonged the half-life of lopinavir (a CYP3A substrate-drug) by 1.40-fold and increased the AUC of lopinavir by 2.04-fold, when QPD (6 g/kg) was co-administrated with lopinavir (160 mg/kg) to rats. Further investigation revealed that *Fructus Aurantii Immaturus* (Zhishi) in QPD caused significant loss of CYP3A activity in NADPH-generating system. Collectively, our findings revealed that QPD potently inactivated CYP3A and significantly modulated the pharmacokinetics of CYP3A substrate-drugs, which would be very helpful for the patients and clinicians to avoid potential drug-interaction risks in COVID-19 treatment.

1. Introduction

Corona Virus Disease 2019 (COVID-19), a newly emerged infective disease, has spread all over the world, with a long incubation period, high infectivity, and general susceptibility to most people (Hamidian and Hamidianjahromi, 2020; Dima et al., 2020; Niu et al., 2020). COVID-19 has brought significantly negative effects for human health. Now researchers are trying to find effective medications (including western therapeutics and herbal medicines) to treat COVID-19, while some therapeutics and herbal medicines have been used for the treatment or adjuvant treatment of COVID-19 in clinical settings (Luo et al., 2020; Chen et al., 2020; Li et al., 2020a). To fight against COVID-19 in a more efficient way, drug-drug or drug-herb combinations are always used in COVID-19 therapy. In China, several Chinese medicine compound formulas (such as Qingfei Paidu decoction, Jingyin granule and Lianhua Qingwen capsule), have been validated playing an active role in combating this epidemic, especially alleviating the moderate and mild symptoms of some patients (Shi et al., 2020). In most cases, these Chinese Medicines (CMs) are often co-administrated with western therapeutics (such as remdesivir & lopinavir) in COVID-19

Abbreviations: AA, arachidonic acid; AUC, area under the plasma concentration; CES, carboxylesterases; CM, Chinese medicine; CMC-Na, sodium carboxymethyl cellulose; COVID-19, Corona Virus Disease 2019; CYPs/P450s, cytochrome P450 enzymes; DDIs, drug-drug interactions; DMEs, drug-metabolizing enzymes; ESI, electrospray ionization; G-6-P, D-Glucose-6-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase; HDIs, herb-drug interactions; HLMs, human liver microsomes; IC_{50}, half maximal inhibition concentration; PBS, potassium phosphate buffer; QPD, Qingfei Paidu decoction; RLMs, rat liver microsomes; TCM, Traditional Chinese Medicine; TDI, time-dependent inhibition; $t_{1/2}$, half-life.

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https://doi.org/10.1016/j.fct.2021.111998
Received 18 October 2020; Received in revised form 31 December 2020; Accepted 12 January 2021
Available online 19 January 2021

treatment. The concomitant use of CMs or herbal medicines with western therapeutics may trigger clinically relevant herb-drug interactions (HDIs) or adverse reactions, thus it is urgent and essential to assess the potential risks of HDIs in COVID-19 treatment.

Among all recommend Chinese medicines for combating COVID-19 in China, Qingfei Paidu decoction (QPD) has drawn much attention owing to its exact effects in COVID-19 treatment. As the first Chinese medicine compound formula recommended by National Health Commission of the People's Republic of China for COVID-19 therapy, QPD has been used to treat thousands of COVID-19 patients with the total effective rate of 97% (Zhang et al., 2020a; Ni et al., 2020; Meng et al., 2020). Following administration of QPD, the major symptoms and imaging manifestations of more than 60% patients were significantly improved, while the symptoms of 30% patients were stable and did not aggravate (Yang et al., 2020). Notably, QPD is a composite of four classic Chinese medicine prescriptions (including Maxing Shigan decoction, Shegan Mahuang decoction, Xiaochaihu decoction, and Wuling powder) that are used for the treatment of epidemic diseases and the related inflammatory symptoms for thousands of years (Li, 2020; Du and Zhang, 2020). As a super combination of 20 herbs and a mineral drug (Gypsum Fibrosum), QPD is composed by hundreds of ingredients which may interact with a panel of human drug-metabolizing enzymes or drug transporters, and then trigger HDIs or other undesirable effects. Currently, various therapeutic agents (such as antiviral drugs, anti-inflammatory drugs, immunosuppressive agents and other western medicines) have been recommended for treating COVID-19 (Gao et al., 2020; Xin et al., 2020; Wang et al., 2020). These therapeutic agents are more likely to be administrated with QPD in clinical settings. Therefore, it is crucial to investigate the potential interactions between QPD and the commonly used therapeutic drugs for treating COVID-19.

It is well-known that most therapeutic drugs (such as remdesivir, lopinavir, etc) used for treating COVID-19 are substrates of phase I drug-metabolizing enzymes (Warren et al., 2016; Kumar et al., 2004), such as cytochrome P450 enzymes (CYPs or P450s) and carboxylesterase (CES). Subsequently, this study aims to investigate the inhibition/inactivation effects of QPD against human phase I drug-metabolizing enzymes, as well as to assess the potential drug-interaction risks when QPD is co-administrated with CYP substrate-drug(s). Following the testing of a panel of *in vitro* inhibition assays, the results clearly demonstrated that QPD dose-dependently inhibited CYPs1A, 2A6, 2C8, 2C9, 2C19, 2D6 and 2E1 but inhibited CYP3A in a time- and NADPH-dependent manner. *In vivo* pharmacokinetic tests showed that QPD could significantly modulate the pharmacokinetics of lopinavir (a CYP3A substrate-drug), when QPD (6 g/kg) was co-administrated with lopinavir (160 mg/kg) to rats. Further investigation revealed that *Fructus Aurantii Immaturus* (Zhishi) in QPD significantly reduced CYP3A activity in a time- and NADPH-dependent manner, suggesting that this herb is a key culprit responsible for CYP3A reduced activity. All these findings offer new insight into the interactions between QPD and therapeutic agents for treating COVID-19, while the key findings presented here are very helpful for the patients and the clinicians to avoid potential drug-interaction risks in COVID-19 treatment.

2. Materials and methods

2.1. Chemicals and reagents

The water extract of QPD (JZT-QFPDT-0318-PG-0321) and the extract from individual herbs for preparing QPD (the preparation procedure and extraction rate of QPD or 21 individual herbs are shown in Table S1) were provided by Jointown Pharmaceutical Group Co.,Ltd. (Shanghai, China). Lansoprazole was purchased from Hairong (Sichuan, China). Mefenamic acid, 6β-hydroxytestosterone, testosterone, D-glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PDH), and β-$NADP^+$ were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phenacetin, coumarin, paclitaxel, omeprazole, dextromethorphan, chlorzoxazone, lopinavir and ketoconazole were purchased from Meilun Bio. Tech (Dalian, China). Diclofenac was obtained from Ark Pharm (Wuhan, China). D-luciferin methyl ester (DME) and its hydrolytic metabolite D-luciferin were purchased from AAT Bioquest (USA). N-(2-butyl-1,3-dioxo-2,3-dihydro-1H-phenalen-6-yl)-2-chloroacetamide (NCEN) and its hydrolytic metabolite 4-amino-1,8-naphthalimide (NAH) were synthesized by us according to the previously reported scheme (Jin et al., 2015; Wang et al., 2016). $MgCl_2$ and sodium carboxymethyl cellulose (CMC-Na) were obtained on Sinopharm Chemical Reagent (Shanghai, China). Luciferin detection reagent (LDR) was ordered from Promega Biotech (Madison, USA). The pooled human liver microsomes (HLMs, Lot No. H0610) from 50 individual donors were supplied by XenoTech (USA). The pooled rat liver microsomes (RLMs, Lot No. JPXY) were from Research Institute for Liver Diseases (RILD, Shanghai, China). LC grade of methanol, acetonitrile and formic acid were ordered from Fisher Scientific Co. (Fair Lawn, NJ, USA), while ultra-purified water was prepared by a Millipore purification system. Each tested compound was dissolved in acetonitrile and each extract was dissolved in ultra-purified water, then stored at −20 °C until use.

2.2. P450 enzyme inhibition assays

2.2.1. Inhibition of P450s by QPD and its individual herbs

P450 inhibition experiments were carried out in 200 μL reaction mixtures, which included potassium phosphate buffer (PBS, 100 mM, pH 7.4), each P450 substrate, NADPH-generating system (10 mM G-6-P, 1.0 unit/mL G-6-PDH, 1.0 mM β-$NADP^+$ and 4.0 mM $MgCl_2$, HLMs or RLMs, along with inhibitor (Li et al., 2020b; Santori et al., 2020; Salerno et al., 2020; Fang et al., 2020; Zhang et al., 2020b). Each P450 substrate and the details of P450 reactions are shown in Table S2. QPD or its individual herbs (50 μg/mL-5000 μg/mL, final concentrations) were added into reaction mixtures to evaluate the inhibitory potentials against P450s. The final concentration of the organic solvent was less than 1% (v/v). The PBS, inhibitors, HLMs or RLMs, and NADPH-generating system were vortexed and then pre-incubated at 37 °C for 3 min or 33 min. The reactions were initiated by adding individual substrates. Reactions proceeded for 10–30 min at 37 °C, and subsequently 200 μL ice-cold acetonitrile containing internal standard was added to quench the reaction. The mixtures were centrifuged at 20,000×*g*, 4 °C for 20 min, then the supernatant (100 μL) was mixed with ultrapure water (100 μL) in a 1:1 ratio for LC-MS/MS analysis as described in Table S2 (please refer to the details in supplementary material).

2.2.2. Inactivation kinetic analyses for time-dependent inhibition

CYP3A inactivation kinetic experiments were carried out as previous reports (Rowland et al., 2011; Kent et al., 2002; Ji et al., 2015). The incubation mixtures consisted of inactivation groups and activity evaluation groups. The inactivation groups (200 μL) included PBS (pH 7.4), NADPH-generating system, HLMs, and QPD (500–5000 μg/mL, final concentrations). And the activity evaluation groups (180 μL) consisted of PBS (pH 7.4), testosterone, and NADPH-generating system. For inactivation groups, the reactions were initiated by adding NADPH-generating system after pre-incubation for 3 min at 37 °C. The inactivation reaction mixtures (20 μL) were transferred to activity evaluation groups at six time points (0, 5, 10, 15, 20 and 30 min). After the activity evaluation group incubated at 37 °C for 10 min, 200 μL of ice-cold acetonitrile containing internal standard was added to quench the reaction. The procedures for sample preparation and analysis were identical as above. The natural logarithm of the residual activity (hydroxylated rate of testosterone) was plotted against the pre-incubation time. All inactivation data were fitted by the following equations equation (1):

$$K_{obs} = K_{inact} \times I/ (I + K_I) \quad (1)$$

where I is concentration of QPD; K_I is the inactivator (QPD)

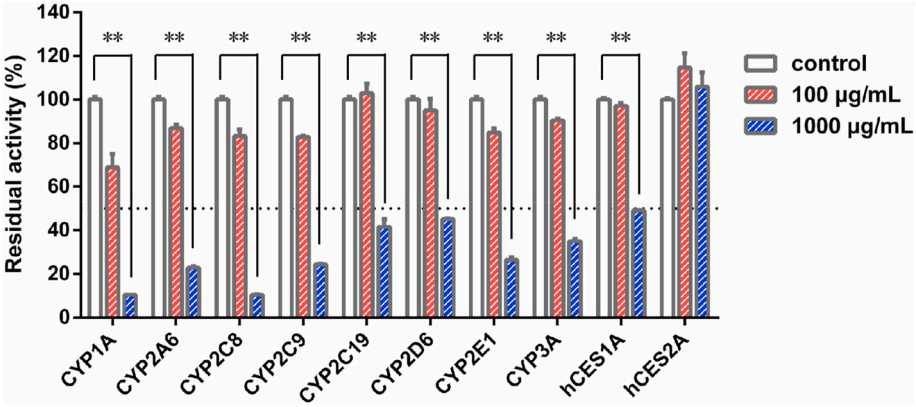

Fig. 1. Inhibition of the major phase I drug-metabolizing enzymes in HLMs by Qingfei Paidu decoction. Data are expressed as mean ± SD (n = 3). **P < 0.01, when compared with the control group.

concentration at half-maximal inactivation; K_{inact} is the maximal inactivation rate constant; K_{obs} is the observed first order inactivation rate constant.

2.3. CES inhibition assays

2.3.1. Inhibition of CES1A-catalyzed DME hydrolysis by QPD

The procedure for hCES1A inhibition assays has been reported previously (Wang et al., 2018; Huo et al., 2020), by using DME as the probe substrate. Briefly, a total of 100 μL incubation system consisted of PBS (pH 6.5), HLMs, DME and QPD (at different concentrations). The final concentration of the organic solvent was less than 1% (v/v). The PBS, QPD, HLMs were vortexed and pre-incubated at 37 °C for 3 or 33 min, then the mixtures were initiated by adding DME and incubated for another 10 min. Then, all reactions were stopped by adding LDR (100 μL). A fluorescence microplate reader (SpectraMax® iD3, Molecular Devices, Austria) was used to measure the hydrolysis rate of DME, *via* monitoring the formation rates of the hydrolytic metabolite D-luciferin. The details for the hCES1A inhibition assays and the detection conditions for D-luciferin were introduced in Table S3.

2.3.2. Inhibition of CES2A-catalyzed NCEN hydrolysis by QPD

The procedure for hCES2A inhibition assays has also been reported previously (Wang et al., 2018; Song et al., 2019a, 2019b), by using NCEN as the probe substrate. In brief, a total of 200 μL incubation system consisted of PBS (pH 7.4), HLMs, NCEN and QPD (at different concentrations). The final concentration of the organic solvent was less than 1% (v/v). The PBS, QPD, HLMs were vortexed and pre-incubated at 37 °C for 3 or 33 min, then the reactions were initiated by adding NCEN. Meanwhile, the fluorescence microplate reader (SpectraMax® iD3, Molecular Devices, Austria) was used to measure the hydrolytic rate of NCEN, *via* monitoring the formation rates of the hydrolytic metabolite 4-amino-1,8-naphthalimide (NAH). The details for the hCES2A inhibition assays and the detection conditions for NAH were shown in Table S3.

2.4. Pharmacokinetic interactions between QPD and lopinavir in rats

Animal tests were ratified by the Animal Care and Use Committee of Shanghai Institute of Food and Drug Control (approval No. SIFDC18096). Male Sprague-Dawley rats (180–200 g, n = 6) were from Shanghai Laboratory Animal Center (Shanghai, China) and were housed at 25 °C in a 12 h light-dark cycles at relative humidity ~ 55%. The rats were fasted overnight before dosing, with water freely, and provided food after finishing the study. QPD was suspended in water and lopinavir was suspended in 0.5% CMC-Na. QPD (6 g/kg, n = 3) or water (6 g/kg, n = 3) was administered intragastrically. After 30 min, lopinavir (160 mg/kg, n = 6) was administered intragastrically (Shi et al., 2013; Ravi and Vats, 2017; Plooy et al., 2011). Blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12 and 24 h and were centrifuged for 10 min at 8000 rpm at 4 °C, and stored at −80 °C until analysis. The plasma (20 μL) was diluted with acetonitrile (containing internal standard) with a ratio of 1:5, and was centrifuged at 20,000×*g* for 30 min at 4 °C. 50 μL supernatant was diluted with 150 μL Millipore water for LC-MS/MS analysis. The quantification of lopinavir (1–5000 ng/mL) was performed in the linear range of the calibration curve. The pharmacokinetic parameters of lopinavir were fitted by standard non-compartmental analyses using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA, USA).

2.5. Data analysis

All assays were performed in triplicates, while all data are shown as mean ± standard deviation (SD). IC_{50} and K_I values were fitted by nonlinear regression in GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, USA).

3. Results

3.1. Chemical profiling of QPD by using UHPLC-Q-Exactive Orbitrap HRMS

Firstly, to elucidate the major constituents in QPD, chemical profiling of QPD was conducted by using UHPLC-Q-Exactive Orbitrap HRMS. As shown in Fig. S1 and Table S4, a total of 340 constituents were identified in QPD, which derived from 20 herbs (except Gypsum Fibrosum) contained in the QPD preparation. These QPD constituents were identified (Fig. S1) *via* comparison with the retention times and MS/MS spectra of the commercially available reference standards, literature and the MS/MS databases of natural products. These constituents could be classified into various classes, including glycosides (111), flavonoids (56), organic acids (37), saponins (34), triterpenoids (24), alkaloids (17), coumarins (10) and others (51). This finding suggests that QPD is a super combination of more than three hundred compounds.

3.2. Inhibitory effects of QPD against DMEs in HLM

Firstly, the inhibitory effects of QPD against ten major human drug-metabolizing enzymes (DMEs) were preliminarily assayed in HLMs, by using three different concentrations (0, 100, and 1000 μg/mL). As shown in Fig. 1, QPD exhibited negligible inhibitory effect on CES2A-catalyzed NCEN hydrolysis in HLMs. In sharp contrast, QPD displayed relatively strong inhibition on all tested P450s and CES1A. To

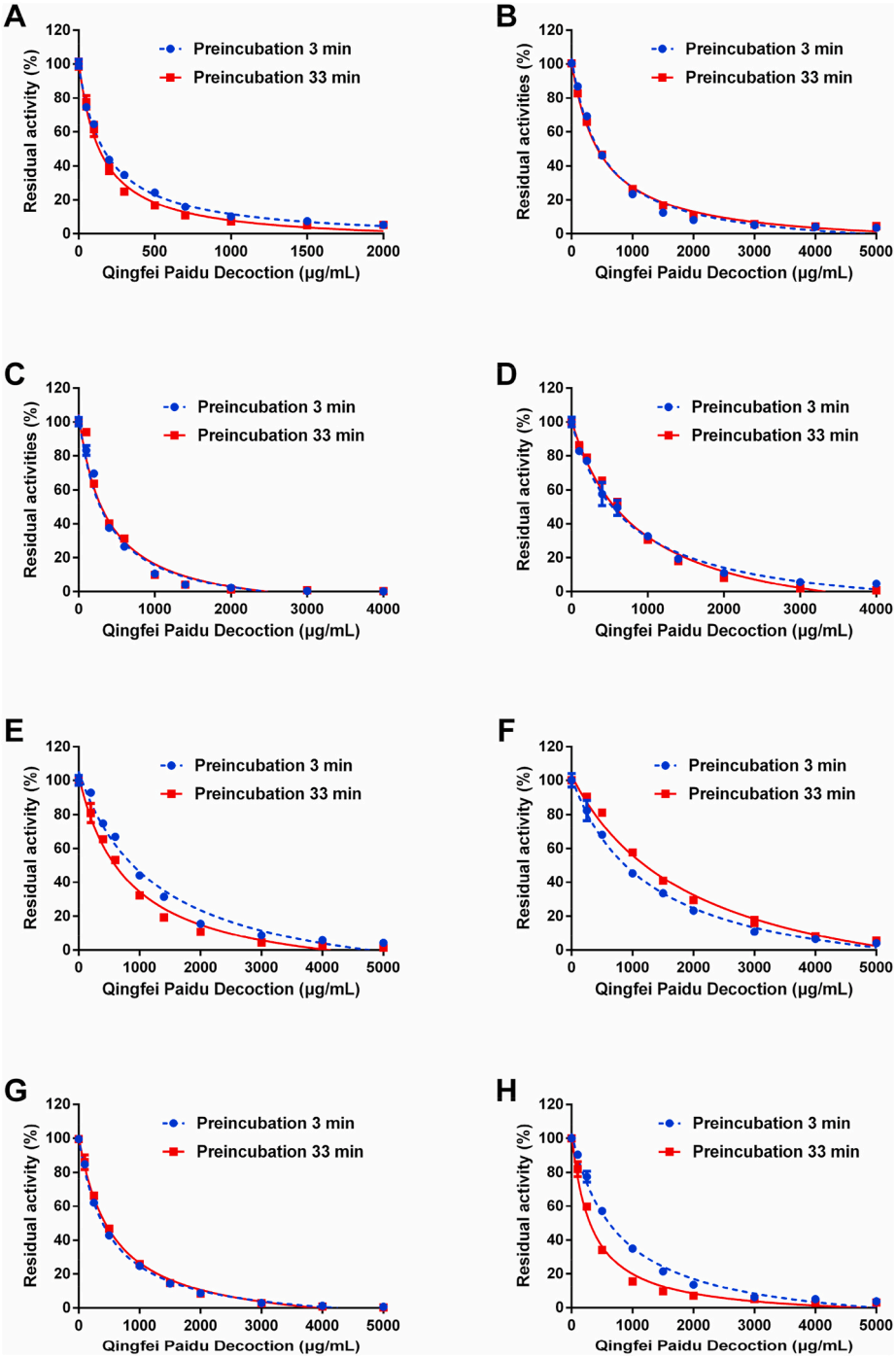

Fig. 2. Dose-inhibition curves of Qingfei Paidu decoction against CYP1A (A), CYP2A6 (B), CYP2C8 (C), CYP2C9 (D), CYP2C19 (E), CYP2D6 (F), CYP2E1 (G) and CYP3A (H) in HLMs, with short (3 min, blue line) or long (33 min, red line) pre-incubation time. Data are expressed as mean ± SD (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

quantitatively measure the inhibitory effects on P450s, dose-inhibition curves of QPD against these DMEs in HLMs were plotted. As shown in Fig. 2, Fig. S2 and Table 1, QPD dose-dependently inhibited CES1A and all tested eight human P450s, with the calculated IC_{50} values as 1336 μg/mL, 174.4 μg/mL, 498.3 μg/mL, 337.7 μg/mL, 706.8 μg/mL, 1244.0 μg/mL, 1253.0 μg/mL, 469.3 μg/mL, 762.1 μg/mL for CES1A, CYP1A, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, respectively.

To investigate whether QPD inhibited the activity of DMEs in a time-dependent manner, the effects of different pre-incubation periods on the remaining enzyme activities were assayed in human microsomal incubations containing QPD. As shown in Fig. 2, Fig. S2 and Table 1, following 33 min pre-incubation at 37 °C, QPD dose-dependently inhibited CES1A and eight tested human P450s, with IC_{50} values of 1436 μg/mL, 143.3 μg/mL, 484.5 μg/mL, 355.2 μg/mL, 846.7 μg/mL, 790.3 μg/mL, 1991.0 μg/mL, 541.5 μg/mL, 324.4 μg/mL, respectively. It is evident from these results that co-incubation of QPD with HLMs in the NADPH-generating system for a long period of time could result in significant loss of CYP3A activity (the IC_{50} value was decreased from 762.1 μg/mL to 324.4 μg/mL). By contrast, following 33 min pre-incubation, the inhibition potency of QPD against other human CYPs and CES1A became weaker or did not change noticeably (IC_{50} ratio <

Table 1
Inhibitory effects of Qingfei Paidu decoction on major P450s and CES1A (with 3 min or with 33 min pre-incubation) in HLMs.

Probe reaction	Target enzyme	Time-dependent inhibition IC_{50} (μg/mL)		Ratio
		Pre-incubation for 3 min	Pre-incubation for 33 min	
Phenacetin O-deethylation	CYP1A	174.4 ± 7.7	143.3 ± 13.0	1.21
Coumarin 7-hydroxylation	CYP2A6	498.3 ± 50.6	484.5 ± 25.3	1.03
Paclitaxel 6α-hydroxylation	CYP2C8	337.7 ± 43.3	355.2 ± 57.9	0.95
Diclofenac 4′-hydroxylation	CYP2C9	706.8 ± 71.1	846.7 ± 92.6	0.83
Omeprazole 5-hydroxylation	CYP2C19	1244.0 ± 201.3	790.3 ± 84.2	1.70
Dextromethorphan O-demethylation	CYP2D6	1253.0 ± 105.7	1991.0 ± 245.5	0.63
Chlorzoxazone 6-hydroxylation	CYP2E1	469.3 ± 24.3	541.5 ± 34.1	0.87
Testosterone 6β-hydroxylation	CYP3A	762.1 ± 65.6	324.4 ± 33.6	2.35
DME-hydrolysis	CES1A	1336.0 ± 173.2	1436.0 ± 192.2	0.93

2.0). These findings suggest that QPD contains naturally occurring CYP3A inactivators, which might inactivate CYP3A activity *in vivo* thus resulting in undesirable effects.

3.3. Inactivation kinetic of QPD against CYP3A in HLMs

Next, the inactivation kinetic analyses of QPD against CYP3A were performed in HLMs, while the inactivation parameters (including K_I and k_{inact} values) were determined in HLMs according to the previously reported method (Fang et al., 2010). In the NADPH-generating system, QPD inactivated CYP3A activity in a dose- and time-dependent manner (Fig. 3). As calculated from the plots present in Fig. 3, the inactivation kinetic constants of QPD against CYP3A, including the K_I and K_{inact} were determined as 1641 μg/mL and 0.032 min^{-1}, respectively. These results clearly demonstrated that QPD inactivates CYP3A activity in a dose-, NADPH- and time-dependent manner, suggesting that QPD may result in undesirable effects *via* inactivation of CYP3A.

3.4. Inactivation of QPD against CYP3A in RLMs

To explore whether QPD shows similar inactivation effects in RLMs as those in HLMs, the inhibitory effects of QPD against CYP3A in RLMs were investigated following pre-incubation at 37 °C during different periods (3 min or 33 min). As shown in Fig. S3, long pre-incubation (33 min) of QPD with RLMs resulted in significant loss of CYP3A activity, the IC_{50} value was decreased from 706.4 μg/mL to 318.0 μg/mL, resulting in IC_{50} ratio of 2.22-fold. These findings clearly demonstrated that QPD inactivates CYP3A in RLMs with very similar inactivation effects as that in HLMs. The pharmacokinetic interactions between QPD and CYP substrate-drugs *in vivo* were studied using rat as a surrogate model for herb-drug interactions in humans.

3.5. Pharmacokinetic interactions between QPD and lopinavir in rats

Encouraged by the above mentioned findings, the *in vivo* effects of QPD on the pharmacokinetic behavior of CYP3A substrate-drug(s) were investigated in rats. Considering that some CYP3A substrate-drugs (such as the antiviral agent lopinavir) are more likely co-administrated with QPD in clinical settings, the pharmacokinetic interactions between QPD and lopinavir were investigated in rats. As shown in Fig. 4 and Table 2. Following co-administration of QPD and lopinavir, the metabolic half-life ($t_{1/2}$) of lopinavir in rats could be prolonged by 40% (from 1.90 h to 2.66 h), while the area under the plasma concentration of lopinavir in rats increased by 2.04-fold (from 6092 ng/mL·h to 12429 ng/mL·h). Moreover, the C_{max} value of lopinavir in rat plasma was slightly increased from 1140 ng/mL to 1190 ng/mL. It is evident from these

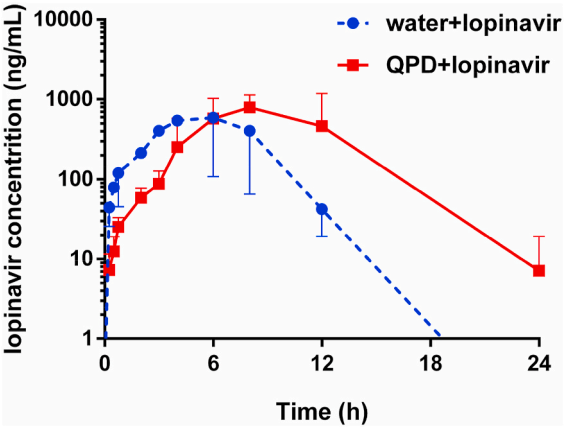

Fig. 4. The mean plasma concentration-time curves of lopinavir (160 mg/kg, i.g.) in control group (water, n = 3) and experimental group (6 g/kg of QPD, i.g., n = 3).

Table 2
Influence of Qingfei Paidu decoction on the pharmacokinetics of lopinavir in rats. Mean ± SD of triplicate rats.

Group	$AUC_{(0\text{-}inf)}$ (ng/mL·h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)
Water + lopinavir	6092	1140	1.90	6.00
QPD + lopinavir	12429	1190	2.66	8.00
Ratio	2.04	1.04	1.40	1.33
Increasing (%)	104	4	40	33

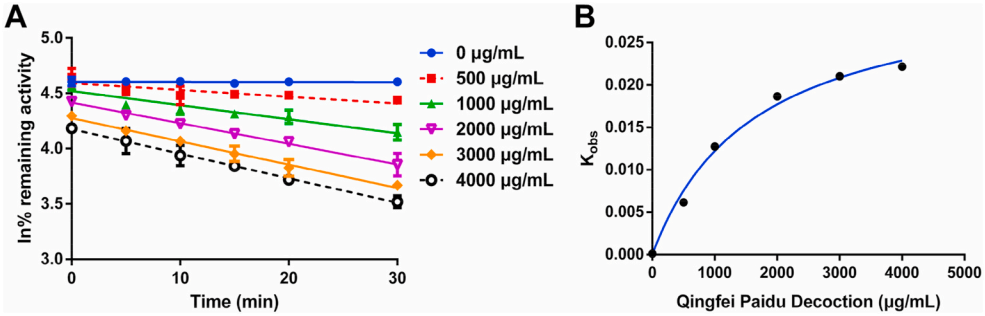

Fig. 3. Time-dependent inhibition of CYP3A by Qingfei Paidu decoction. (A) Time- and dose-dependent inhibition of CYP3A by Qingfei Paidu decoction. (B) The hyperbolic plot of k_{obs} of CYP3A vs. Qingfei Paidu decoction concentrations. Data are expressed as mean ± SD (n = 3).

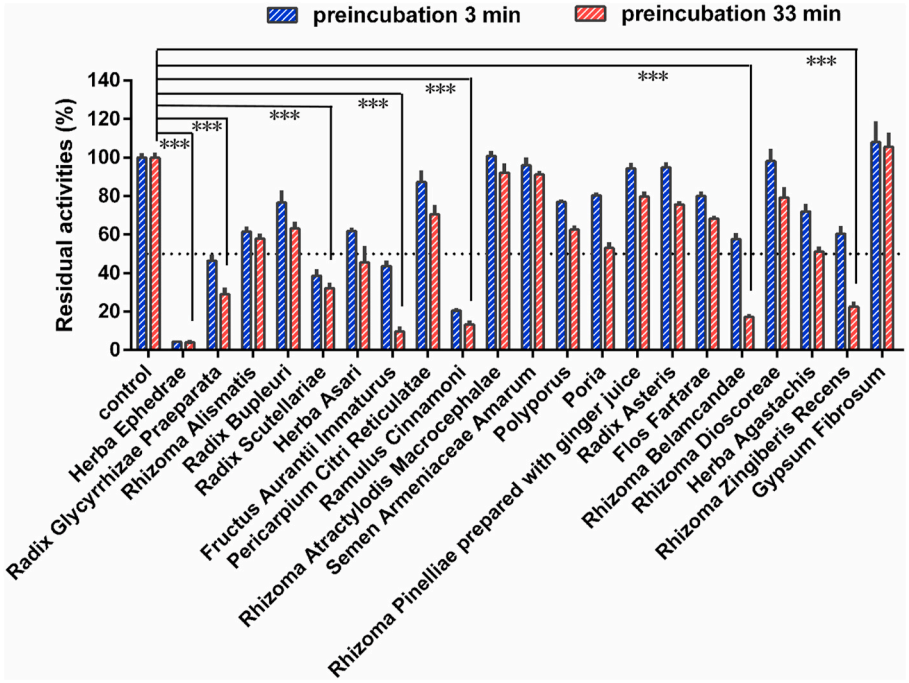

Fig. 5. The inhibitory effects of individual extracts from 21 different herbs used to prepare Qingfei Paidu decoction against CYP3A-catalyzed testosterone 6β-hydroxylation. Data are expressed as mean ± SD (n = 3). ***P < 0.001, when compared with the control group.

findings that QPD could strongly modulate the pharmacokinetics of lopinavir in rats, *via* increasing the plasma exposure to lopinavir prolonging its plasma half-life.

3.6. Inactivation of CYP3A by individual herbs in QPD preparation

To find the key herbs in QPD that caused CYP3A inactivation, time-dependent inhibition of CYP3A by the extract from Gypsum Fibrosum (250 μg/mL, final concentration) and 20 individual herbs for preparing QPD were conducted. As shown in Fig. 5, seven herbs (including *Herba Ephedrae*, *Radix Glycyrrhizae Praeparata*, *Radix Scutellariae*, *Fructus Aurantii Immaturus*, *Ramulus Cinnamoni*, *Rhizoma Belamcandae*, *Rhizoma Zingiberis Recens*) displayed relatively strong CYP3A inhibition activities, with the residual activities less than 50% in 250 μg/mL. In this case, the inhibition and inactivation effects of these seven individual herbs for CYP3A were investigated in HLMs. As shown in Fig. 6 and Table 3, *Fructus Aurantii Immaturus*, *Ramulus Cinnamoni*, *Rhizoma Belamcandae*, and *Rhizoma Zingiberis Recens* could inhibit CYP3A-catalyzed testosterone 6β-hydroxylation in HLMs *via* a time- and NADPH-dependent manner, with IC_{50} ratio 7.03-fold, 2.07-fold, 3.11-fold, and 2.82-fold, respectively. Among all tested herbs, *Fructus Aurantii Immaturus* (Zhishi) displayed the most potent CYP3A inactivation potency, with a dramatic 7.03-fold change in IC_{50} value. This finding suggests that *Fructus Aurantii Immaturus* (Zhishi) is a key herb in QPD resulting in significant loss of CYP3A activity in NADPH-generating system.

4. Discussion

Currently, to fight against COVID-19 in a more efficient way, various drug-drug or drug-herb combinations have been recommended for treating COVID-19 in clinical settings. The concomitant use of drug-herb combinations or CM-drug combinations may trigger clinically relevant drug/herb-drug interactions resulting in adverse drug reactions. As the most popular used Chinese medicine compound formula for combating COVID-19, QPD has been frequently used with other medications (such as antiviral agents) to treat COVID-19 patients. As an extremely complicated CM prescription, QPD contains hundreds of ingredients which are more likely to interact with a panel of human drug-metabolizing enzymes or drug transporters (Zhao et al., 2020a; Ge, 2019), which in turn may modulate the treatment outcomes (including efficacy and safety) of co-administrated agents and trigger clinically relevant HDIs. Therefore, it is urgent and essential to investigate the inhibition/inactivation potential of QPD against human drug-metabolizing enzymes (DMEs), as well as to assess the potential changes in the pharmacokinetics of co-administrated drug(s) when QPD is co-administrated with western drug(s).

As listed in Table S5, a variety of western drugs including antiviral drugs (such as remdesivir, favipiravir, chloroquine, hydroxychloroquine, nafamostat, camostat, lopinavir and ritonavir) have been recommended for treating COVID-19, these agents are more likely to be co-administrated with QPD in clinical settings. Notably, most of the recommended antiviral drugs are substrates of phase I drug-metabolizing enzymes (such as CYPs and CES). We first investigated the potential interactions between QPD and the key drug-metabolizing enzymes (DMEs) in humans. The results clearly demonstrate that QPD inhibits hCES1A and a series of human P450s. Among all tested DMEs, QPD strongly inhibits CYP1A in a reversible manner, while this Chinese medicine potently inhibits CYP3A in a time- and NADPH-dependent manner. Considering that CYP1A participates in the oxidative metabolism of some important drugs (such as doxofylline) for treating respiratory diseases (Zhao et al., 2020b), QPD may extend the duration time or enhance the plasma exposure of these CYP1A-substrate drugs *in vivo* and modulate their treatment outcomes. Meanwhile, in view of the fact that doxofylline display relatively high safety profiles, inhibition of CYP1A by QPD may enhance the therapeutic efficacy of these agents and hardly trigger serious events of adverse drug reactions. In future, it is necessary to investigate the influence of QPD on the pharmacokinetic profiles of doxofylline or other therapeutic agents (including CYP1A and CYP2C19 substrate drugs) for treating COVID-19, by using suitable *in vivo* surrogate models.

By contrast, inhibition of CYP3A by QPD may trigger clinically relevant HDIs. It is well-known that CYP3A metabolizes ~50% therapeutic agents including many antiviral drugs (such as lopinavir and ritonavir) and some key agents with narrow therapeutic windows (Wu

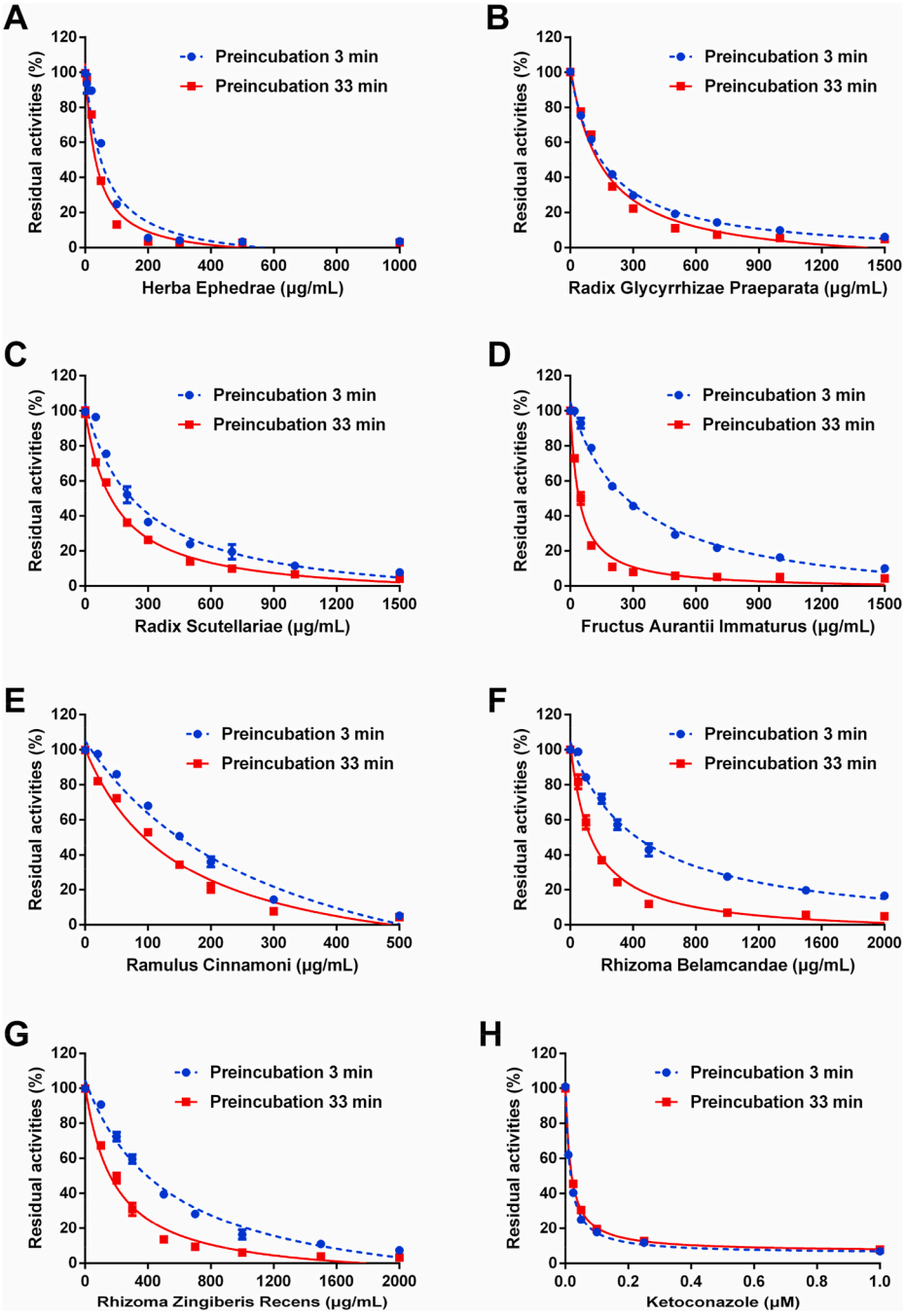

Fig. 6. The inhibitory effects of Herba Ephedrae (A), Radix Glycyrrhizae Praeparata (B), Radix Scutellariae (C), Fructus Aurantii Immaturus (D), Ramulus Cinnamoni (E), Rhizoma Belamcandae (F), and Rhizoma Zingiberis Recens (G) against CYP3A-catalyzed testosterone 6β-hydroxylation. Figure H depicts the effects of a positive inhibitor ketoconazole against CYP3A-mediated testosterone 6β-hydroxylation in HLMs. Data are expressed as mean ± SD (n = 3).

et al., 2021). As a consequence, strong inhibition/inactivation of this key enzyme by QPD may trigger clinically relevant HDIs. Notably, to validate the modulatory effect of QPD on the pharmacokinetic behavior of CYP3A substrate drug(s), an *in vivo* pharmacokinetic test was conducted in the present study. The results showed that QPD (6 g/kg) strongly modulates the pharmacokinetic behavior of lopinavir (160 mg/kg), a CYP3A substrate-drug, *via* prolongation of the plasma half-life and increase of the plasma exposure (AUC) to this antiviral agent in rats. Meanwhile, it was also clear from Fig. 4 that QPD could affect the adsorption of lopinavir in circulation system. As a result, the T_{max} value of lopinavir was delayed from 6 h to 8 h, when QPD was co-administrated with lopinavir to rats. Such findings could be explained by the non-specific binding of lopinavir with the crude extract of QPD in the gastrointestinal system, which in turn, delaying the T_{max} value of lopinavir. More recently, a clinical case study reported that QPD could result in hyperkalemia in patients with COVID-19, when QPD is co-administrated with lopinavir/ritonavir (Han et al., 2020). These findings clearly demonstrated that QPD could trigger *in vivo* HDI *via* inhibition of CYP3A, suggesting that much attention should be paid when QPD was co-administrated with CYP3A substrate drugs. Notably, lopinavir has been reported with various adverse drug reactions (such as diarrhea, nausea, vomiting, blurred vision, epistaxis, hypertriglyceridemia, and Hypercholesterolemia) in clinical settings, thus the dose-related lopinavir ADRs that might become more serious when QPD

Table 3
IC_{50} values of seven individual herbs for preparing Qingfei Paidu decoction with strong CYP3A inhibition activities.

Herbs	Dose (g)	Extraction rate (%)	Time-dependent inhibition[a] IC_{50} (μg/mL)		Ratio
			Pre-incubation for 3 min	Pre-incubation for 33 min	
Herba Ephedrae	9	11.6	55.8 ± 12.9	34.1 ± 6.7	1.64
Radix Glycyrrhizae Praeparata	6	18.5	157.7 ± 7.1	150.3 ± 19.3	1.05
Radix Scutellariae	6	31.2	254.4 ± 38.9	141.4 ± 8.7	1.80
Fructus Aurantii Immaturus	6	14.5	295.9 ± 30.2	42.1 ± 4.4	7.03
Ramulus Cinnamoni	9	5.1	310.7 ± 66.1	150.3 ± 24.9	2.07
Rhizoma Belamcandae	9	9.8	424.1 ± 51.1	136.5 ± 16.3	3.11
Rhizoma Zingiberis Recens	9	4.2	532.8 ± 74.6	188.9 ± 23.1	2.82

[a] The IC_{50} values were determined in HLM following short (3 min) or long (33 min) pre-incubation.

was continuously administrated with lopinavir for several days. Furthermore, the drug reactions may become more serious when QPD is co-administered with some CYP3A-substrate drugs with very narrow therapeutic windows, such as digoxin, warfarin and some anti-cancer agents that are predominantly metabolized by CYP3A.

Notably, inhibition of human P450s is always a double-edged sword. For some agents with very narrow therapeutic indices (such as warfarin and digoxin), inhibition of P450s will trigger undesirable drug/herb-drug interactions. But for some agents with improved safety profiles or wide therapeutic windows, inhibition of the key P450s responsible for metabolic clearance of these agents may extend the metabolic half-lives and increase the plasma exposure of these P450 substrate-drugs *in vivo*, which will be beneficial for the patients. Furthermore, some P450s (such as CYP1A and CYP3A) have been validated as the key enzymes participating in the oxidative metabolism of arachidonic acid (AA) and other fatty acids (Arnold et al., 2010; Kroetz and Zeldin, 2002), while the oxidative metabolites of AA have been recognized as the key chemical mediators of inflammation (Tallima and Ridi, 2017; Fishbein et al., 2020). Thus, potent inhibition on CYP1A and CYP3A by QPD may partially block the formation of the oxidative metabolites of AA, thereby alleviating the systemic inflammation in patients with COVID-19. Thus, at least in part, QPD may exert its anti-inflammatory effects by inhibiting human P450s (mainly on CYP1A and CYP3A). In view of the fact that a set of anti-inflammatory drugs (such as dexamethasone) have been recommended for treating COVID-19 and part of them are CYP substrate-drugs (Tomlinson et al., 1997), the potential interactions between QPD and these anti-inflammatory agents should be carefully investigated from the respects of both pharmacodynamics and pharmacokinetics.

Although this study reports that QPD may inhibit CYP3A both *in vitro* and *in vivo*, it is very difficult to find the key ingredients in QPD that are responsible for CYP3A inhibition. As mentioned above, QPD is a super combination of 21 herbs that composed of hundreds of ingredients, thus it is unfeasible to test the CYP3A inhibition activities of each ingredient in QPD. For this reason, we assayed the inhibitory effects of the water extract of individual herbs on human CYP3A. The results demonstrated that seven herbs in QPD, including *Herba Ephedrae*, *Radix Glycyrrhizae Praeparata*, *Radix Scutellariae*, *Fructus Aurantii Immaturus*, *Ramulus Cinnamoni*, *Rhizoma Belamcandae*, *Rhizoma Zingiberis Recens*, strongly inhibited CYP3A in a dose-dependent manner. Further investigation demonstrated that *Fructus Aurantii Immaturus*, *Ramulus Cinnamoni*, *Rhizoma Belamcandae*, and *Rhizoma Zingiberis Recens* inhibited CYP3A-catalyzed testosterone 6β-hydroxylation in time-dependent manners, implying that these herbs may contain CYP3A inactivators. Particularly, *Fructus Aurantii Immaturus* (Zhishi) displayed potent CYP3A inactivation potency and triggered a dramatic IC_{50} shift (7.03-fold change) following different pre-incubation times in NADPH-generating system, suggesting that this herb might be the major culprit in QPD resulting in significant loss of CYP3A activity. Thus, in future, the key ingredients in *Fructus Aurantii Immaturus* (Zhishi) and QPD responsible for CYP3A inhibition/inactivation should be identified and carefully characterized, which will be very helpful for preparing a new herbal remedy to reduce the risks of herb-drug interactions.

5. Conclusion

In summary, the study investigated the inhibitory potentials of QPD against human phase I drug-metabolizing enzymes and assessed the modulatory effects of QPD on the pharmacokinetics of lopinavir *via* inhibiting P450s. The results clearly demonstrated that QPD displayed relatively strong inhibition on all tested P450s and CES1A. Time-dependent inhibition assays showed that QPD inhibit CYP3A-catalyzed testosterone 6β-hydroxylation in a time-dependent manner in liver microsomes of both humans and rats. Further investigation showed that QPD inactivated CYP3A in a dose- and NADPH-dependent manner, with the K_I and K_{inact} (the inactivation kinetic constants) of 1641 μg/mL, and 0.032 min^{-1}, respectively. *In vivo* assays demonstrated that QPD prolonged the half-life of lopinavir by 40% and increased the $AUC_{(0\text{-}inf)}$ (ng/mL·h) of lopinavir by 104%, when QPD (6 g/kg) was co-administrated with lopinavir (160 mg/kg) in rats. In addition, time-dependent inhibition assays of the individual extract from single herbs for preparing QPD demonstrated that *Fructus Aurantii Immaturus* (Zhishi), *Ramulus Cinnamoni* (Guizhi), *Rhizoma Belamcandae* (Shegan), *Rhizoma Zingiberis Recens* (Shengjiang) inhibited CYP3A-catalyzed testosterone 6β-hydroxylation in a time-dependent manner. Among all tested herbs, *Fructus Aurantii Immaturus* (Zhishi) displayed the most potent inactivation effect, suggesting this herb is a key culprit responsible for CYP3A inactivation. Collectively, our findings revealed that QPD could significantly modulate the pharmacokinetic behavior of CYP3A substrate-drugs *via* inactivation of CYP3A in a time- and NADPH-dependent manner, which would help the patients and clinicians to avoid potential drug-interaction risks in COVID-19 treatment. Meanwhile, the key findings present here are also helpful for the developer to optimize the constituted herbs and their ratios, to slow down the risks of herb-drug interactions.

CRediT authorship contribution statement

Feng Zhang: performed the experiments, wrote the manuscript. **Jian Huang:** performed the experiments. **Wei Liu:** performed the experiments. **Chao-Ran Wang:** performed the experiments. **Yan-Fang Liu:** analyzed the data. **Dong-Zhu Tu:** analyzed the data. **Xin-Miao Liang:** analyzed the data. **Ling Yang:** revised the manuscript. **Wei-Dong Zhang:** revised the manuscript. **Hong-Zhuan Chen:** revised the manuscript. **Guang-Bo Ge:** conceived and designed the study, wrote the manuscript, analyzed the data.

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.

Acknowledgement

We'd like to thank Jointown Pharmaceutical Group Co.,Ltd. (China)

for providing QPD extract and 21 single herbs. This work was supported by the National Key Research and Development Program of China (2020YFC0845400, 2017YFC1700200, 2017YFC1702000), the NSF of China (81922070, 81973286), Shanghai Science and Technology Innovation Action Plans (20S21901500; 20S21900900) supported by Shanghai Science and Technology Committee, Program of Shanghai Academic/Technology Research Leader (18XD1403600), the Three-year Action Plan of Shanghai TCM Development (ZY-(2018–2020)-CCCX-5001), Shuguang Program (18SG40) supported by Shanghai Education Development Foundation and Shanghai Municipal Education Commission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.111998.

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Pharmacological Research 161 (2020) 105290

Contents lists available at ScienceDirect

Pharmacological Research

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Association between early treatment with Qingfei Paidu decoction and favorable clinical outcomes in patients with COVID-19: A retrospective multicenter cohort study

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Abbreviations: QFPDD, Qingfei Paidu Decoction; COVID-19, coronavirus disease 2019; SARS-COV-2, severe acute respiratory syndrome-coronavirus 2; RT-PCR, reverse transcription-polymerase-chain-reaction; WISP, work information system platform; HR, hazard ratio; CI, confidence interval; No., number of patients; COPD, chronic obstructive pulmonary disease; WBC, white blood cell; RBC, red blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SpO2, blood oxygen saturation; PO2, partial pressure of oxygen; Ref., reference; Adj., adjusted; Unadj., unadjusted; CT, computed tomography; IFN, interferon.

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https://doi.org/10.1016/j.phrs.2020.105290

Available online 9 November 2020

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ARTICLE INFO

Keywords:
COVID-19
Qingfei Paidu decoction
Early treatment

ABSTRACT

The coronavirus disease 2019 (COVID-19) epidemic has been almost controlled in China under a series of policies, including "early diagnosis and early treatment". This study aimed to explore the association between early treatment with Qingfei Paidu decoction (QFPDD) and favorable clinical outcomes. In this retrospective multicenter study, we included 782 patients (males, 56 %; median age 46) with confirmed COVID-19 from 54 hospitals in nine provinces of China, who were divided into four groups according to the treatment initiation time from the first date of onset of symptoms to the date of starting treatment with QFPDD. The primary outcome was time to recovery; days of viral shedding, duration of hospital stay, and course of the disease were also analyzed. Compared with treatment initiated after 3 weeks, early treatment with QFPDD after less than 1 week, 1-2 weeks, or 2-3 weeks had a higher likelihood of recovery, with adjusted hazard ratio (HR) (95 % confidence interval [CI]) of 3.81 (2.65–5.48), 2.63 (1.86-3.73), and 1.92 (1.34-2.75), respectively. The median course of the disease decreased from 34 days to 24 days, 21 days, and 18 days when treatment was administered early by a week ($P < 0.0001$). Treatment within a week was related to a decrease by 1-4 days in the median duration of hospital stay compared with late treatment ($P<0.0001$). In conclusion, early treatment with QFPDD may serve as an effective strategy in controlling the epidemic, as early treatment with QFPDD was associated with favorable outcomes, including faster recovery, shorter time to viral shedding, and a shorter duration of hospital stay. However, further multicenter, prospective studies with a larger sample size should be conducted to confirm the benefits of early treatment with QFPDD.

1. Introduction

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has severely impacted public health and has become a global pandemic [1, 2]. More than 8.7 million confirmed cases of COVID-19 have been reported in over 200 countries, areas, and territories, with over 461 thousand deaths due to COVID-19 as of June 21, 2020 [3].

Initially, many studies have reported the clinical characteristics of

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patients with COVID-19 [4–7]. Some studies have explored risk factors for the illness that may influence the prognosis [8–10], all of which helped toward a better understanding of the physiology and pathology of COVID-19 for the further development of potential effective vaccines and drugs. Clinical trials on potential drugs are still ongoing, and some preliminary results have been reported [11–16]. Many vaccine candidates were in phase II and one in phase III of clinical trials [17]. However, no effective medication is currently confirmed, and the vaccine is still months to years away from being used in clinical practice. Current treatments are supportive of relieving the severity of symptoms and avoiding critical illness. In China, traditional Chinese medicine has been included as an option for the treatment of COVID-19, and Qingfei Paidu decoction (QFPDD) is recommended for use in patients in the seventh edition of the COVID-19 guideline [18].

To date, few studies have focused on efficient policies for COVID-19 management. As the crisis has progressed, there has been an enormous burden on medical sources and health systems due to increasing demands. Hence, efficient policies for the management of COVID-19 are of great importance in coping with the challenges posed to people's health and medical systems.

Different policies have been implemented. "Self-isolation" and "self-care" were recommended to keep people with minor ailments at home [19]. Policies, including "early diagnosis and early treatment", have been implemented in China as preventive and therapeutic strategies, under which the COVID-19 epidemic has been almost controlled [18]. The benefits of early treatment, if proven, could help reduce hospital stay, lower intensive care use, and relieve the burden on the public health system, especially for those living in source-limited areas [20]. However, very few studies have provided data on the correlation between early treatment and clinical outcomes. This study aimed to examine whether early treatment with QFPDD results in favorable clinical outcomes.

2. Methods

2.1. Participants

A retrospective follow-up study design was adopted. Patients aged 18-87 years with confirmed COVID-19 from 54 hospitals in nine provinces of China (Anhui, Fujian, Guangxi, Hebei, Heilongjiang, Shaanxi, Sichuan, Shanxi, and Chongqing) were enrolled from January 21, 2020 to March 10, 2020. The final follow-up was conducted on March 17, 2020. Patients were eligible for inclusion if they self-reported signs and symptoms and were diagnosed with confirmed SARS-CoV-2 by the nasopharyngeal reverse transcription-polymerase chain reaction (RT-PCR) test [18].

This study was supported by the National Administration of Traditional Chinese Medicine, Administration of Traditional Chinese Medicine of the nine provinces, and the institutional board of the 54 participating hospitals. The study was approved by the ethics committee of the Chinese Clinical Trial Registry (ChiECRCT20200123). Due to the urgency of COVID-19 treatment, the requirement for written informed consent from the study participants was replaced by verbal consent.

2.2. Treatment

All patients were treated with QFPDD. QFPDD is a Chinese formula comprising 21 herbs: má huáng (*Herba Ephedrae*) 9 g, zhì gān cǎo (*Radix et Rhizoma Glycyrrhizae Praeparata cum Melle*) 6 g, xìng rén (*Semen Armeniacae Amarum*) 9 g, shí gāo (*Gypsum Fibrosum*) 15-30 g (fried first), guì zhī (*Ramulus Cinnamomi*) 9 g, zé xiè (*Rhizoma Alismatis*) 9 g, zhū líng (*Polyporus*) 9 g, bái zhú (*Rhizoma Atractylodis Macrocephalae*) 9 g, fú líng (*Poria*) 15 g, chái hú (*Radix Bupleuri*) 16 g, huáng qín (*Radix Scutellariae*) 6 g, jiāng bàn xià (*Rhizoma Pinelliae Praeparatum*) 9 g, shēng jiāng (*Rhizoma Zingiberis Recens*) 9 g, zǐ wǎn (*Radix et Rhizoma Asteris*) 9 g, kuǎn dōng huā (Flos Farfarae) 9 g, shè gān (*Rhizoma Belamcandae*) 9 g, xì xīn (Radix et Rhizoma Asari) 6 g, shān yào (*Rhizoma Dioscoreae*) 12 g, zhǐ shí (*Fructus Aurantii Immaturus*) 6 g, chén pí (*Pericarpium Citri Reticulatae*) 6 g, and huò xiāng (Herba Agastachis) 9 g. The quality of the herbs conformed to the criteria set by the 2015 Chinese Pharmacopoeia. Hazardous substances, including pesticide residues, heavy metals, and microbial contamination, were detected in all herbs to ensure safety, and the results met the criteria in China. QFPDD was prepared by a pharmacist according to the standardized procedure in each hospital. The dose and mode of administration were based on the *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia* [18].

Additionally, antiviral drugs, antibiotics, corticosteroids, α-IFN inhalation, and symptomatic treatments were used based on the patients' needs. The dose and mode of administration were based on the *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia* [18]. Supplemental oxygen was given to those with oxygen saturation levels dropping below 93 % or in patients who felt obvious chest tightness.

2.3. Laboratory procedures

Laboratory blood examinations included hematologic indices and infection-related indices. Chest imaging results were separately reviewed by two radiologists to assess image progression or absorption. Two evaluators independently assessed the chest computed tomography (CT) features of the patients without access to clinical or laboratory findings. Disagreements, if any, were resolved by discussion and consensus. Physicians determined the timings of all the above examinations based on the patients' condition during hospitalization.

2.4. Definitions

Recovery was defined as: (1) body temperature returned to normal for more than 3 days; (2) respiratory symptoms clearly improved; (3) pulmonary imaging showed obvious absorption of inflammation; and (4) nucleic acid tests showed negative results twice consecutively with an interval of at least 24 h [18]. The primary outcome was time to recovery, which was defined as the interval from the first date of onset of symptoms to the date of recovery.

The secondary outcomes were days of viral shedding, course of the disease, and duration of hospital stay. Days of viral shedding was defined as the interval from the first positive RT-PCR test to the date of the second consecutive negative test. The course of the disease was calculated from the day of onset of symptoms to the day of discharge. The duration of hospital stay was calculated from the day of admission to the day of discharge.

The exposure variable was defined as the treatment initiation time, which was the interval from the onset of symptoms to the start of the treatment with QFPDD. Accordingly, patients were divided into four groups: the $\leq$1 week group ($\leq$7 days), 1-2 week group ($>$7 days and $\leq$14 days), 2-3 week group ($>$14 days and $\leq$21 days), and $>$3 week group ($>$21 days).

The clinical classification of patients included mild, moderate, severe, and critical cases [18]. Mild cases were defined as those with mild clinical symptoms without any signs of pneumonia on imaging. Moderate cases were defined as those showing fever and respiratory symptoms with radiological findings of pneumonia. Severe cases were defined as those meeting any of the following criteria: (1) respiratory distress ($\geq$30 breaths/min), (2) oxygen saturation $\leq$93 % at rest, and (3) arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) $\leq$300 mmHg (1 mmHg = 0.133 kPa). Cases that met any of the following criteria were defined as critical cases: (1) respiratory failure and requiring mechanical ventilation, (2) shock, and (3) other organ failure requiring ICU care. In our study, mild and moderate cases were combined into a non-severe group, and severe and critical cases were combined into a severe group. Comorbidities included previously diagnosed diseases such as diabetes, hypertension, coronary heart disease, and cerebrovascular disease.

2.5. Data collection

A work information system platform (WISP) was developed for data collection and management. Demographic and clinical characteristics and treatment information were collected through WISP; the laboratory and imaging tests were scanned or photographed and uploaded to WISP. Demographic and clinical characteristics and treatment information were extracted from WISP using a standardized data collection form. Laboratory data were input by researchers in duplicate, and imaging data were reviewed by radiologists independently. If core data were missing, we contacted coordinators on the site who would contact the attending physicians.

2.6. Statistical analysis

Continuous and categorical variables are presented as medians (interquartile ranges) and percentages (%), respectively. The univariate analysis for demographic and clinical characteristics at baseline was conducted using the Chi-square test or Fisher's exact method for categorical data; continuous variables were compared using ANOVA if data were normally distributed and by the Kruskal-Wallis test otherwise. Survival curves were generated by the Kaplan-Meier method. Univariate and multivariable Cox proportional hazard ratio (HR) models were used to estimate unadjusted and adjusted HRs and 95 % confidence intervals (CIs) for the association between treatment initiation time and clinical outcomes. As recovery was a beneficial event, an HR value >1 would increase the likelihood of an event, whereas an HR value <1 meant the factor would decrease the likelihood of an event. Proportional assumptions for the Cox proportional hazard model were examined using scaled Schoenfeld residuals. Similar analyses were then conducted in subgroups of patients with different classifications.

Multiple linear regression models were used to model the relationships between the continuous outcomes with logarithm transformation and treatment initiation time, adjusted by propensity score. A repeated measures ANOVA was used to estimate the trend and possible difference in body temperature among the four groups. For the categorical data, a multinomial logistic regression model was used.

To adjust for the potential bias inherent in the retrospective studies, such as unbalanced baseline clinical characteristics, propensity scores were derived from multivariable logistic regression that included covariates, including age, sex, clinical classification, history of visiting Wuhan in the past 14 days, days from onset of symptoms to hospital admission, fever and cough on admission, comorbidities, antiviral use, expectorant use, and CT imaging outcomes, which were selected by the stepwise selection method ($P = 0.05$). The propensity score was then included in the regression models as a continuous variable, along with the treatment groups and other significant covariates, but not selected in the stepwise selection method at baseline in the univariate analysis, to adjust the heterogeneity at the baseline among the four groups with different treatment initiation times.

All tests were two-sided, and a *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R software, version 4.0.0 (R Foundation for Statistical Computing).

3. Results

3.1. Demographic and clinical characteristics

A total of 943 patients were recruited, and 782 patients (male, 52 %; median age, 46 years) from 54 hospitals in nine provinces were included in our analysis till the cut-off date of March 17, 2020. Patients were divided into ≤1 week (321 patients, 41 %), 1-2 week (221 patients, 28 %), 2-3 week (123 patients, 16 %), and >3 week (117 patients, 15 %) groups (Fig. 1).

The patients included were predominantly non-severe (91 %); 34 % of the patients reported that they visited Wuhan within the past 14 days, and 66 % had contacted patients with confirmed COVID-19. The most common coexisting diseases included hypertension (15 %), diabetes (7%), and coronary heart disease (3%). The most common signs and symptoms on admission were cough (52 %), followed by fever (49 %), fatigue (31 %), and dry cough (29 %). The median body temperature

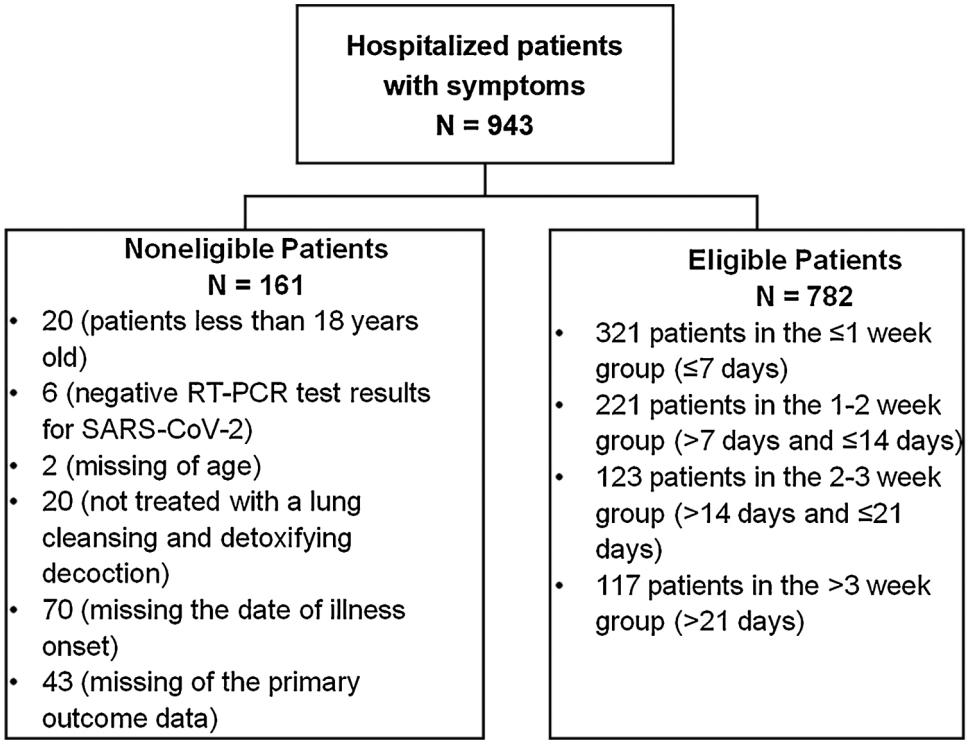

Fig. 1. Patient inclusion/exclusion criteria.

was 37.7 °C. A total of 641 (90 %) patients had abnormal findings with unilateral or bilateral multiple mottling and ground-glass opacity of the lung. All 782 patients were treated with QFPDD (100 %); over 90 % of the patients received antiviral therapy, including arbidol, lopinavir/ritonavir, ribavirin, ganciclovir, and others. Antibiotics (53 %), α-IFN inhalation (43 %), corticosteroids (15 %), antipyretic drugs (10 %), and expectorants (4%) were also used. At baseline, there were significant differences among the four groups based on the clinical classification ($P = 0.0016$); history of visiting Wuhan ($P = 0.0002$); signs and symptoms on admission, including fever ($P = 0.0022$), sore throat ($P = 0.0357$), cough ($P = 0.0008$), shortness of breath ($P < 0.0001$), and fatigue ($P = 0.0423$); median body temperature of patients on admission ($P = 0.0088$) and during hospitalization ($P = 0.0486$); and the imaging results of CT ($P = 0.0088$), and use of expectorant ($P = 0.0005$) (Table 1). Significant differences were observed among the four groups in hematologic test results, including lymphocyte count ($P = 0.0007$), RBC count ($P = 0.0012$), hemoglobin ($P < 0.0001$), and platelet count ($P< 0.0001$), as well as in infection-related indices, including C-reactive protein ($P = 0.0018$), procalcitonin ($P = 0.0017$), and erythrocyte sedimentation rate ($P = 0.0136$) (Table 2).

3.2. Primary outcome

The univariate Cox HR models showed that groups with different treatment initiation times with QFPDD were statistically associated with recovery ($P < 0.0001$). Compared with treatment initiated after 3 weeks, the patients who received treatment within a week after the onset of symptoms showed more likelihood of recovery (unadjusted HR 4.56, 95 % CI: 3.61-5.76). Similar higher odds were also found for those in the groups treated at 1-2 weeks (unadjusted HR 3.36, 95 % CI: 2.62-4.30) and 2-3 weeks (unadjusted HR 2.24, 95 % CI: 1.71-2.93) (Table 3). This negative association between treatment initiation time and recovery was also observed in the subgroup of non-severe patients (≤1 week vs. > 3 weeks: unadjusted HR 4.34, 95 % CI: 3.40-5.55; 1-2 weeks vs. > 3 weeks: unadjusted HR 3.16, 95 % CI: 2.44-4.08; 2-3 weeks vs. > 3 weeks: unadjusted HR 2.26, 95 % CI: 1.69-3.01). In the subgroup of severe patients, there were significant differences between the ≤1 week group and the >3 week group (unadjusted HR 5.59, 95 % CI: 2.02-15.48) and between the 1-2 week group and the >3 week group (unadjusted HR 5.80, 95 % CI: 1.83-18.45); however, the difference between the 2-3 week group and the >3 week group (unadjusted HR 2.39, 95 % CI: 0.95-5.98) was not significant (Sup Table 1).

In multivariable Cox HR models adjusted for age, sex, clinical classification, history of visiting Wuhan within the past 14 days, days from the onset of symptoms to hospital admission, whether fever or cough were present on admission, any comorbidities, treatment with antiviral therapy and expectorant therapy, and imaging results of CT selected by the stepwise selection method, treatment initiation time still showed a significant association with recovery. Compared with the >3 week group, the ≤1 week group, 1-2 week group, and the 2-3 week group showed two to three times more likelihood of recovery (adjusted HR 3.16, 95 % CI: 2.27-4.41; adjusted HR 2.75, 95 % CI: 2.00-3.79; adjusted HR 2.08, 95 % CI: 1.50-2.89, respectively) (Table 3).

Furthermore, after adjusting for significant baseline variables and propensity score, treatment initiation time also showed a statistically significant association with a clinically effective outcome, suggesting that a longer time from the onset of symptoms to the start of treatment with QFPDD was negatively associated with the time to recovery ($P < 0.0001$) (Table 3). The Kaplan-Meier plot showed that the median time to recovery of the ≤1 week, 1-2 week, 2-3 week, and >3 week groups were 19 days (95 % CI: 17-20), 22 days (95 % CI: 21-23), 26 days (95 % CI: 24-27), and 35 days (95 % CI: 33-36), respectively (Fig. 2). Compared with the patients in the >3 week group, the patients in the ≤1 week, 1-2 week, and 2-3 week groups demonstrated approximately two to three times higher odds of recovery as adjusted by propensity score and other covariates (adjusted HR 3.81, 95 % CI: 2.65-5.48; adjusted HR

Table 1
Demographic and clinical characteristics of patients infected with coronavirus disease 2019 at baseline, China.

Study population	No. (%)	Time from onset of symptoms to treatment with QFPDD[#]				P*
		≤1 week	1–2 weeks	2–3 weeks	>3 weeks	
No. of patients	782	321 (41 %)	221 (28 %)	123 (16 %)	117 (15 %)	–
Age, median, years	46.0 (23.0)	44.0 (24.0)	47.0 (22.0)	45.0 (24.0)	46.0 (18.0)	0.5429
Sex						0.4246
Male	405 (52 %)	156 (49 %)	120 (54 %)	69(56 %)	60(51 %)	
Female	377 (48 %)	165 (51 %)	101 (46 %)	54(44 %)	57(49 %)	
Clinical classification						0.0016
Nonsevere[a]	710 (91 %)	301 (94 %)	205 (93 %)	107 (87 %)	97(83 %)	
Severe[b]	72 (9%)	20 (6%)	16 (7%)	16(13 %)	20(17 %)	
Exposure to source of transmission within the past 14 days						
Recently visited Wuhan, China						0.0002
No	518 (66 %)	231 (72 %)	154 (70 %)	71(58 %)	62(53 %)	
Yes	263 (34 %)	90(28 %)	66(30 %)	52(42 %)	55(47 %)	
Had contact with the confirmed COVID-19 patients						0.5594
No	266 (34 %)	104 (32 %)	74(34 %)	42(36 %)	46(40 %)	
Yes	508 (66 %)	216 (68 %)	146 (66 %)	76(64 %)	70(60 %)	
Comorbidities						
Hypertension	120 (15 %)	54(17 %)	39(18 %)	12(10 %)	15(13 %)	0.1738
Diabetes	51 (7%)	22 (7%)	17 (8%)	4(3%)	8(7%)	0.4350
Coronary heart disease	24 (3%)	7(2%)	10 (5%)	3(2%)	4(3%)	0.4508
Cerebrovascular disease	7(1%)	4(1%)	1(0%)	2(2%)	0	0.4349
Liver disease	8(1%)	3(1%)	1(0%)	2(2%)	2(2%)	0.6322
COPD	14 (2%)	4(1%)	4(2%)	5(4%)	1(1%)	0.1916
Signs and symptoms on admission						
Fever	383 (49 %)	132 (41 %)	117 (53 %)	72(59 %)	62(53 %)	0.0022
Chills	39 (5%)	15 (5%)	10 (5%)	10 (8%)	4(3%)	0.3424
Nasal congestion	37 (5%)	13 (4%)	12 (5%)	6(5%)	6(5%)	0.8926
Shed tears	27 (3%)	8(2%)	6(3%)	7(6%)	6(5%)	0.2526
Sore throat	86(11 %)	23 (7%)	31(14 %)	15(12 %)	17(15 %)	0.0357
Cough	409 (52 %)	140 (44 %)	130 (59 %)	69(56 %)	70(60 %)	0.0008
Dry cough	228 (29 %)	88(27 %)	67(30 %)	37(30 %)	36(31 %)	0.8461

(continued on next page)

Table 1 (*continued*)

Study population	No. (%)	Time from onset of symptoms to treatment with QFPDD[#]				*P*[*]
		≤1 week	1–2 weeks	2–3 weeks	>3 weeks	
Shortness of breath	102 (13 %)	23 (7%)	26(12 %)	21(17 %)	32(27 %)	<0.0001
Apocleisis	98(13 %)	39(12 %)	31(14 %)	12(10 %)	16(14 %)	0.6828
Diarrhea	34 (4%)	13 (4%)	8(4%)	7(6%)	6(5%)	0.7879
Fatigue	245 (31 %)	83(26 %)	74(33 %)	46(37 %)	42(36 %)	0.0423
Vital signs at admission						
Respiratory rate, median, rpm	20.0 (1.0)	20.0 (1.0)	20.0 (1.0)	20.0 (1.0)	20.0 (1.5)	0.6215
Temperature at admission						
median, °C	37.7 (1.3)	37.5 (1.2)	37.8 (1.2)	37.8 (1.4)	37.8 (1.5)	0.0088
<37.3 °C	212 (32 %)	94(35 %)	60(31 %)	32(29 %)	26(27 %)	0.1258
37.3–38.0 °C	261 (39 %)	111 (41 %)	76(39 %)	36(32 %)	38(40 %)	
38.1–39.0 °C	179 (27 %)	57(21 %)	54(28 %)	42(38 %)	26(27 %)	
>39.0 °C	21 (3%)	9(3%)	5(3%)	2(2%)	5(5%)	
Highest temperature during hospitalization						
median, °C	37.4 (1.1)	37.3 (1.1)	37.5 (1.2)	37.5 (0.8)	37.4 (0.9)	0.0486
<37.3 °C	319 (42 %)	155 (50 %)	84(40 %)	32(27 %)	48(41 %)	
37.3–38.0 °C	296 (39 %)	102 (33 %)	81(38 %)	58(49 %)	55(48 %)	
38.1–39.0 °C	118 (16 %)	46(15 %)	38(18 %)	24(20 %)	10 (9%)	
>39.0 °C	25 (3%)	8(3%)	9(4%)	5(4%)	3(3%)	
CT imaging						
Normal[c]	68 (9%)	37(12 %)	22(10 %)	4(3%)	5(4%)	0.0088
Abnormal[d]	641 (90 %)	248 (87 %)	184 (89 %)	106 (96 %)	103 (95 %)	
Medication						
QFPDD	782 (100 %)	321 (100 %)	221 (100 %)	123 (100 %)	117 (100 %)	1.0000
Anti-virus[e]	712 (91 %)	284 (88 %)	202 (91 %)	115 (94 %)	111 (95 %)	0.1301
Antibiotics	411 (53 %)	161 (50 %)	126 (57 %)	62(50 %)	62(53 %)	0.4335
α-IFN inhalation	339 (43 %)	153 (48 %)	93(42 %)	52(42 %)	41(35 %)	0.1154
Corticosteroid	118 (15 %)	51(16 %)	37(17 %)	11 (9%)	19(16 %)	0.2234
Antipyretic drugs	81(10 %)	33(10 %)	18 (8%)	17(14 %)	13(11 %)	0.4189
Expectorant	32 (4%)	7(2%)	10 (5%)	13(11 %)	2(2%)	0.0005

Abbreviations: QFPDD, Qingfei Paidu decoction; No., number of patients; COVID-19, coronavirus disease 2019; COPD, chronic obstructive pulmonary disease; CT, computed tomography; IFN, interferon. Data are expressed as *n* (%). Totals do not add up to 100 % because of rounding or missing data.

[*] *P*-values were calculated using the Chi-square test, Fisher's exact method, or Kruskal-Wallis test. Bold indicates statistically significant <0.05.

[#] Patients were divided into four groups: the ≤1 week group (≤7 days), 1–2 week group (>7 days and ≤14 days), 2–3 week group (>14 days and ≤21 days), and >3 week group (>21 days).

[a] Including mild and moderate cases.

[b] Including severe and critical cases.

[c] Without unilateral or bilateral abnormal lung lesions.

[d] Unilateral or bilateral multiple mottling and ground-glass opacity of the lung.

[e] Including arbidol, lopinavir/ritonavir, ribavirin, ganciclovir, etc.

2.63, 95 % CI: 1.86-3.73; adjusted HR 1.92, 95 % CI: 1.34-2.75) (Fig. 2). This negative impact of treatment initiation time on recovery was consistent in the subgroup of non-severe patients (≤1 week vs. > 3 weeks: adjusted HR 3.75, 95 % CI: 2.56-5.49; 1-2 weeks vs. > 3 weeks: adjusted HR 2.47, 95 % CI: 1.72-3.56; 2-3 weeks vs. > 3 weeks: adjusted HR 1.80, 95 % CI: 1.23-2.64). However, there was no significant association in the subgroup of severe patients ($P > 0.05$) (Sup Table 1).

3.3. Secondary outcomes

The multivariate linear regression models showed that there was a significant difference among the four groups in days of viral shedding ($P = 0.0137$), duration of hospital stay ($P < 0.0001$), and course of the disease ($P<0.0001$), along with early treatment with QFPDD after the onset of symptoms. There was no significant difference in the CT imaging results among the four groups ($P = 0.3065$) (Table 4). Approximately half of the patients (49 %) had a fever on admission; the results demonstrated that the body temperature of the patients in the four groups significantly decreased after treatment ($P < 0.0001$), showing a trend of better remission of fever in the early treatment group, with no significant difference among the four groups ($P > 0.05$) (Fig. 3).

Favorable secondary outcomes were shown in the case of early treatment with QFPDD in the subgroup of non-severe patients in days of viral shedding ($P = 0.0279$), duration of hospital stay ($P < 0.0001$), and course of the disease ($P<0.0001$); however, the differences were not significant in the subgroup of severe patients ($P = 0.0750$; $P = 0.2145$; $P = 0.4562$, respectively) (Sup Table 2).

As of March 17, 2020, 715 patients (91 %) had recovered, 37 patients (5%) continued treatment in a stable condition, 28 patients (4%) deteriorated and were transferred to the ICU or superior hospitals to receive invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO), and 2 patients died. The median age for the 28 deteriorated patients was 55 years; 12 (43 %) were males, and 20 (71 %) were severe cases on admission. The coexisting diseases were hypertension, diarrhea, coronary heart disease, and chronic obstructive pulmonary disease. The allocation of deteriorated patients was 10 (10/321, 3%) in the ≤1 week group, 9 (9/221, 4%) in the 1-2 week group, 5 (5/123, 4%) in the 2-3 week group, and 4 (4/117, 3%) in the >3 week group. Two female patients died; the first patient was 72 years old with a severe case on admission combined with diarrhea, deteriorated 2 days after admission, and was transferred to the ICU. The patient died of respiratory failure and multiple organ failure 6 days later. The second patient's condition—who was 75 years old with a severe case on admission, combined with diarrhea and coronary heart disease—deteriorated 13 days after admission, and died of respiratory failure and multiple organ failure 2 days after transfer to the ICU. Two deaths in the study were not treatment-related adverse events judged by clinical experts.

4. Discussion

We analyzed data from 782 patients with COVID-19 from nine

Table 2
Baseline laboratory indices of patients infected with coronavirus disease 2019, China.

Tests in the study population	Total (n = 782)		Time from onset of symptoms to treatment with QFPDD[#]								P
			≤1 week (n = 321)		1−2 weeks (n = 221)		2−3 weeks (n = 123)		>3 weeks (n = 117)		
	No.	Value, median	No.	Value, median	No.	Value, median	No.	Value, median	No.	Value, median	
Hematologic											
Neutrophils, $\times10^9$/L	708	3.4(2.4)	308	3.4(2.4)	203	3.2(2.3)	108	3.2(2.7)	109	3.6(2.1)	0.3459
Lymphocyte, $\times10^9$/L	712	1.3(0.8)	309	1.4(0.8)	206	1.3(0.8)	108	1.2(0.7)	109	1.5(0.8)	0.0007
RBC count, $\times10^{12}$/L	675	4.5(0.8)	278	4.61(0.8)	201	4.5(0.7)	106	4.5(0.7)	108	4.3(0.8)	0.0012
Hemoglobin, g/L	672	136.0(24.0)	276	140.0(25.0)	201	136.0(25.0)	106	132.0(19.0)	107	129.0(26.0)	<0.0001
WBC count, $\times10^9$/L	710	5.4(2.8)	308	5.4(3.0)	205	5.2(2.6)	108	5.1(2.7)	109	5.8(2.4)	0.0530
Platelet count, $\times10^9$/L	672	197.5(94.0)	279	190.0(84.0)	198	195.0(96.0)	105	202.0(108.0)	108	233.0(74.5)	<0.0001
Infection-related indices											
CRP, mg/L	534	10.0(15.2)	237	5.1(10.0)	148	10.0(26.4)	86	10.0(23.3)	80	10.0(13.6)	0.0018
Procalcitonin, ng/mL	445	0.1(0.1)	198	0.1(0.1)	133	0.1(0.1)	66	0.1(0.1)	57	0.1(0)	0.0017
ESR, mm/hr	299	27.0(34.0)	104	20.5(24.5)	87	26.0(37.0)	58	30.5(39.0)	59	34.0(43.0)	0.0136
Arterial blood gas analysis											
SpO_2, %	266	97.0(3.4)	85	97.0(3.0)	75	97.0(4.0)	47	97.3(4.0)	64	97.1(4.0)	0.4312
PO2, mmHg	213	87.0(28.5)	74	87.0(24.9)	54	86.5(28.0)	38	85.8(29.0)	51	91.8(42.3)	0.2552

Abbreviations: QFPDD, Qingfei Paidu decoction; No., number of patients tested; WBC, white blood cell; RBC, red blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SpO_2, blood oxygen saturation; PO2, partial pressure of oxygen. The P-values of the comparison groups were calculated using the Kruskal-Wallis method. Bold indicates statistically significant <0.05.

[#] Patients were divided into four groups: the ≤1 week group (≤7 days), 1−2 week group (>7 days and ≤14 days), 2−3 week group (>14 days and ≤21 days), and >3 week group (>21 days).

Table 3
Cox proportional hazards models for the primary outcome of recovery in patients infected with coronavirus disease 2019, China.

Time from onset of symptoms to treatment with QFPDD[#]	Univariate		Multivariable			
	Unadj. HR (95 % CI)	P[a]	Adj. HR (95 % CI)	P[b]	Adj. HR (95 % CI)	P[c]
≤1 week	4.56(3.61,5.76)	<0.0001	3.16(2.27,4.41)	<0.0001	3.81(2.65,5.48)	<0.0001
1−2 weeks	3.36(2.62,4.30)	<0.0001	2.75(2.00,3.79)	<0.0001	2.63(1.86,3.73)	<0.0001
2−3 weeks	2.24(1.71,2.93)	<0.0001	2.08(1.50,2.89)	<0.0001	1.92(1.34,2.75)	0.0004
>3 weeks	ref. = 1		ref. = 1		ref. = 1	

Abbreviations: QFPDD, Qingfei Paidu decoction; ref., reference; Unadj., unadjusted; Adj., adjusted; CI, confidence interval; HR, hazard ratio. HRs and their 95 % CIs were calculated using the Cox proportional risk model. The proportional hazards assumption was not violated (P > 0.05). Bold indicates statistically significant <0.05.

[#] Patients were divided into four groups: the ≤1 week group (≤7 days), 1−2 week group (>7 days and ≤14 days), 2−3 week group (>14 days and ≤21 days), and >3 week group (>21 days).

[a] Unadjusted result.

[b] Adjusted by covariates at baseline, including age, sex, clinical classification, history of visiting Wuhan in the past 14 days, days from the onset of symptoms to hospital admission, fever and cough on admission, any comorbidity, antiviral, expectorant, and computed tomography imaging, which were selected by the stepwise selection method (P = 0.05).

[c] Adjusted by propensity score and other covariates. Propensity scores were derived from multivariable logistic regression that included covariates selected by the stepwise selection method at baseline. The covariates that were significant in univariate analysis but not selected in the stepwise method were included in the model as other covariates.

provinces in China to examine the association between various treatment timings with QFPDD and clinical outcomes. We found that early treatment with QFPDD is linked to favorable clinical outcomes of quicker recovery, a shorter period of viral shedding, a shorter course of the disease, and a shorter duration of hospital stay. Meanwhile, the mortality in our study was 0.3 % (2/754), lower than the global mortality of 5.3 % (461,715/8,708,008)[3], indicating that the combination treatment with QFPDD against COVID-19 in China served an advantage.

The present study showed that compared with treatment initiated 3 weeks after the onset of symptoms, treatment initiated within 2-3 weeks, 1-2 weeks, or less than 1 week had two to three times more likelihood of quicker recovery; the median days decreased from 35 days to 26 days, 22 days, and 19 days, respectively. The early treatment group (within 7 days) had a lower proportion of patients with severe COVID-19 than the other three treatment groups. These findings are similar to those of the previous studies; one showed that treatment with lopinavir/ritonavir within 12 days from the onset of symptoms could promote the recovery of patients with COVID-19 [12]. One study showed that treatment with remdesivir within 10 days from the onset of symptoms could promote clinical improvement [11]; another revealed that treatment initiated during the first 10 days had higher odds of recovery than treatment initiated 10 days after the onset of symptoms [14]. The present study also explored the relationship between treatment initiation time and recovery in the severe and non-severe subgroups of patients. Similar results of early treatment with QFPDD having favorable outcomes were found in patients with non-severe COVID-19, suggesting that for patients with mild and moderate COVID-19, early treatment with QFPDD was related to a shorter time to recovery.

Early treatment with QFPDD was also found to be associated with fewer days of viral shedding, a shorter course of the disease, and a shorter duration of hospital stay. Compared with patients in whom the treatment was initiated after 3 weeks, the median days of viral shedding decreased by approximately 5 days in patients in whom the treatment was initiated within 3 weeks. A similar trend of a decrease in the days of viral shedding from 12 days to 7 days after early triple treatment was found by Hung et al. in a subgroup analysis of an open-label, randomized, phase II trial [13]. Early treatment was related to a shortened course of the disease by 10 days or more. Compared with late treatment, treatment within a week after the onset of symptoms was related to a decrease of 1-4 days in the duration of hospital stay, consistent with a

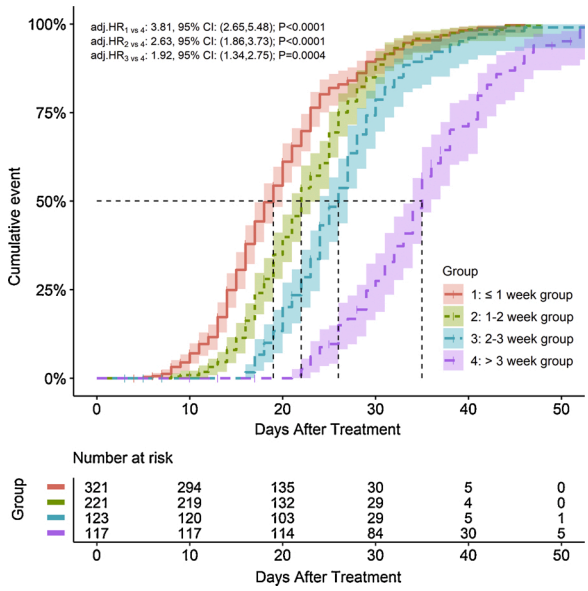

Fig. 2. Kaplan-Meier plot for the primary outcome of time to recovery in patients infected with coronavirus disease 2019 by time from the onset of symptoms to treatment groups with 95 % CIs. Abbreviations: COVID-19, coronavirus disease 2019; Adj., adjusted; CI, confidence interval; HR, hazard ratio. The primary outcome was time to events, which was defined as the days from the onset of COVID-19 disease symptoms to clinical effectiveness for the treatment. If no event had occurred at the time of the last record, the patient's survival time was censored at that time. Covariates were selected by the stepwise selection method ($P = 0.05$) and then were used to estimate propensity scores in multivariable logistic regression. HR and its 95 % CI were calculated using the Cox proportional risk model adjusted by propensity score and other significant covariates in the univariate analysis.

previous study that reported a decrease by 5 days [13]. The correlation between early treatment and fewer days of viral shedding, a shorter course of the disease, and a shorter duration of hospital stay were also found in patients with non-severe COVID-19. The differences were not significant in patients with severe COVID-19, possibly due to the small sample size of severe patients in our study. Symptoms of fever and CT images of the patients improved significantly at the end of the treatment and showed a trend of better outcomes in relation to early treatment.

As included in the core outcome set of COVID-19, the time to recovery, duration of hospital stay, and days of viral shedding were considered as significant clinical outcomes for patients [21]. The improvement in these outcomes may indicate better effects of treatment and prognosis of patients with COVID-19. A shorter duration of the disease and hospital stay could result in a decreased demand for hospital beds and relieve the burden on health systems.

Our study has some notable strengths. First, the current study is a large sample multicenter study and is one of the few studies to address the relationship between treatment initiation time and clinical outcomes. Second, we applied the propensity score method to balance the patients' baseline demographic and clinical characteristics to reduce the bias caused by these potential confounding factors. Third, we revealed that early treatment with QFPDD resulted in favorable clinical outcomes.

However, there were several limitations to the present study. First, the data did not include patients who were unable to be hospitalized at the early stage of the outbreak or were not treated with QFPDD during hospitalization, thereby affecting the representativeness of the sample and statistical results. Second, the percentage of patients with severe COVID-19 in the present study was 9%, which is lower than the 19 % reported by the Chinese Center for Disease Control and Prevention in February 2020 [22]. The lower proportion of patients with severe COVID-19 may be explained by our study, as data from Wuhan city was

Table 4
Multivariate analysis for the secondary outcomes in patients infected with coronavirus disease 2019, China.

Outcomes	All patients	Time from onset of symptoms to treatment with QFPDD[#]				*P* value
	No. (%) (*n* = 782)	≤1 week (*n* = 321)	1–2 week (*n* = 221)	2–3 week (*n* = 123)	>3 week (*n* = 117)	
Days of viral shedding[a]						
median, days	13.0 (9.0)	12.0 (7.0)	12.0 (7.0)	13.0 (8.0)	17.0 (10.0)	0.0137
Duration of hospital stay[a]						
median, days	15.0 (9.0)	14.0 (8.5)	15.0 (9.0)	15.0 (9.0)	18.0 (12.0)	<0.0001
Course of disease[a]						
median, days	22.0 (12.0)	18.0 (8.0)	21.0 (10.0)	24.0 (9.0)	34.0 (12.0)	<0.0001
CT imaging[b]						
Foci absorption, or no abnormal lesions	457(77 %)	176 (75 %)	126 (74 %)	78(80 %)	77(83 %)	0.3065
No significant change	103(17 %)	37(16 %)	34(20 %)	16(16 %)	16(17 %)	
Progress	37(6%)	22 (9%)	11 (6%)	4 (4%)	0	

Abbreviations: QFPDD, Qingfei Paidu decoction; CT, computed tomography; No., number of patients. Totals do not add up to 100 % because of rounding or missing data. Bold indicates statistically significant <0.05.

[#] Patients were divided into four groups: the ≤1 week group (≤7 days), 1–2 week group (>7 days and ≤14 days), 2–3 week group (>14 days and ≤21 days), and >3 week group (>21 days).

[a] Outcomes were used by logarithm transformation and *P*-values were calculated using a multiple linear regression model. Covariates were selected by the stepwise selection method ($P = 0.05$) and were then used to estimate propensity scores in multivariable logistic regression. *P-values* were adjusted by propensity score and other significant covariates at baseline in the univariate analysis.

[b] *P-values* were calculated using a multinomial logistic regression model adjusted by propensity score and other covariate variables.

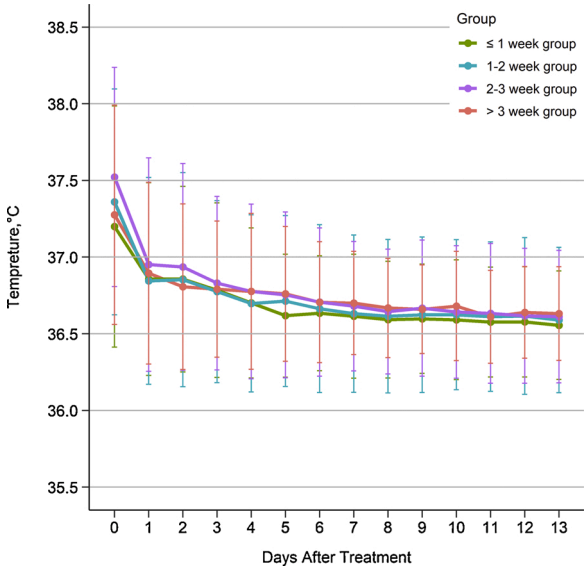

Fig. 3. Daily temperature (°C) variations of patients infected with coronavirus disease 2019 by time from the onset of symptoms to treatment during a 14-day hospitalization period. Data are expressed as the Mean ± SD. Abbreviation: SD, standard deviation.

not included, where there were more patients with severe COVID-19 than in other provinces. Additionally, because of the retrospective study design, not all laboratory tests, including lactate dehydrogenase and IL-6, were performed in all patients, and certain laboratory reports were not uploaded to the system and could not be obtained after contacting the coordinators. Moreover, deteriorated patients in our study were transferred to a superior hospital, and we could not obtain data on their outcomes at that time when physicians invested more energy on patient care and were unable to follow-up. Lastly, although we have tried to use certain statistical methods to control the influence of the confounding variables on the results, it cannot be eliminated; the evidence from this retrospective study is therefore at a lower level when compared with evidence from a well-conducted prospective and randomized controlled trial, as this design could only detect association between factors and outcomes, while the latter can confirm the association.

Early treatment with QFPDD is associated with favorable outcomes for patient recovery, viral shedding, hospital stay, and course of the disease. It demonstrated that early treatment with QFPDD could be an effective strategy for controlling the epidemic and can provide evidence that government and international organizations should adopt such COVID-19 policies. Further multicenter, prospective studies with larger samples should be conducted to confirm the benefits of early treatment with QFPDD.

Contributors

YW, YW, HZ, WW, GL, NS, BL, NL, YM, YG and HW participated the study design. JB, HC, LC, QF, TG, YH, GH, XH, YH, JH, QH, SH, LJ, JW, HJ, XL, CL, JL, ML, QL, XL, HL, JL, ZL, YM, YM, LM, HN, FS, SS, DW, JW, MW, XW, YW, YW, GW, WW, LW, YX, HX, HX, SX, RX, CY, KY, PY, SY, GZ, JZ, LZ, SZ, WZ, KZ, YZ, JZ and TZ were responsible for recruiting patients. WB, RC, YF, HG, RH, LJ, JW, YL, HL, LL, JL, SL, ZS, YT, LT, ZW, YX, CZ, YZ and XZ were responsible for inputting and checking data. HY, HG, YK, ST and YZ participated in data management and statistical analysis. YW, HZ, GL, NS, BL, NL, YM, YG and HW participated in drafting the manuscript. YW, YW, HZ and WW revised the final paper. All authors reviewed and approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding

This study was supported by “National Science and Technology Major Project” (2018ZX10101001-005-003, 2018ZX10101001-005-004).

Disclaimer

The funder of the study had no role in the study design, data collection, statistical analysis, results interpretation or reports writing. The corresponding authors had full access to all the data and had final responsibility for the decision to submit for publication.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

All relevant data to the study are included in the article or uploaded as supplementary information. Data are available upon reasonable request.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We acknowledged all participants’ works in the study. And particularly we thank Prof. Dongshan Zhu for language editing on the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.105290.

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基于网络药理学和分子对接技术初步探索“清肺排毒汤”抗新型冠状病毒肺炎作用机制

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摘要: 利用中药网络药理学和分子对接技术初步探索“清肺排毒汤”抗新型冠状病毒肺炎 (coronavirus disease 2019, COVID-19) 作用机制。结合中国药典 (2015版) 以及中药系统药理学数据库及分析平台 (Traditional Chinese Medicine Systems Pharmacology, TCMSP)、OMIM (Online Mendelian Inheritance in Man)、GeneCard、STRING等在线数据库, 进行一系列网络构建、核心靶点筛选以及信号通路富集分析, 最后对重要化合物进行分子对接预测。结果发现, 清肺排毒汤化合物−肺炎靶点网络包含292个化合物和相应靶点214个, 核心靶点涉及AKT1 (AKT serine/threonine kinase 1)、IL6 (interleukin 6)、MAPK8 (mitogen-activated protein kinase 8)、MAPK1 (mitogen-activated protein kinase 1) 和JUN (jun proto-oncogene) 等。应用GO (Gene Ontology) 数据库功能富集分析得到858个GO条目, 应用KEGG (Kyoto Encyclopedia of Genes and Genomes) 数据库富集筛选得到122条有关通路, 其中包含低氧诱导因子-1 (hypoxia inducible factor-1, HIF-1) 通路、Toll样受体 (Toll-like receptors, TLRs) 通路等已报道与肺炎相关的通路, 也包括T细胞受体 (T-cell receptor, TCR) 通路等与肺损伤保护相关的通路。分子对接结果显示, 清肺排毒汤中药材部分核心化合物对新型冠状病毒 (2019-nCoV) 的3C类似蛋白酶 (3C-like protease, 3CLpro) 和血管紧张素转换酶2 (angiotensin-converting enzyme 2, ACE2) 蛋白具有一定的亲和力。本文初步探索了清肺排毒汤抗COVID-19的作用机制, 且预测了其药效物质基础, 期待本结果能为进一步确证清肺排毒汤抗COVID-19有效成分和作用机制提供帮助。

关键词: 新型冠状病毒肺炎; 清肺排毒汤; 网络药理学; 分子对接技术; 机制初探

中图分类号: R285 **文献标识码**: A **文章编号**: 0513-4870(2020)03-0374-10

Preliminary exploration of the mechanism of Qingfei Paidu decoction against novel coronavirus pneumonia based on network pharmacology and molecular docking technology

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Abstract: Traditional Chinese medicine (TCM) network pharmacology and molecular docking technology

收稿日期: 2020-02-18; 修回日期: 2020-02-22.
基金项目: 十堰市科技局指导项目 (19Y30).
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DOI: 10.16438/j.0513-4870.2020-0136

were applied to explore the mechanism of anti-coronavirus pneumonia (coronavirus disease 2019, COVID-19) of Qingfei Paidu decoction. The Chinese Pharmacopoeia (2015 edition) and Traditional Chinese Medicine Systems Pharmacology (TCMSP), OMIM (Online Mendelian Inheritance in Man), GeneCard, STRING, and others online databases are used for building a series of network, and selecting the core target and analyzing the signal pathway. Finally, we make molecular docking predictions for the important compounds. The results showed that the Qingfei Paidu decoction compound-pneumonia target network contained 292 compounds and 214 corresponding targets, and the core targets involved AKT1 (AKT serine/threonine kinase 1), IL6 (interleukin 6), MAPK8 (mitogen-activated protein kinase 8), MAPK1 (mitogen-activated protein kinase 1), and JUN (jun proto-oncogene). GO (Gene Ontology) function enrichment analysis yielded 858 GO entries, and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment screening yielded 122 related pathways, including hypoxia inducible factor-1 (HIF-1) and Toll-like receptor (TLRs) signaling pathways related to pneumonia, as well as T-cell receptor (TCR) signaling pathway related to lung injury protection. The molecular docking results showed that some core compounds of the Chinese herbal medicine of Qingfei Paidu decoction have a certain degree of affinity for 2019-novel coronavirus (2019-nCoV) main protease (3C-like protease, 3CLpro) and angiotensin-converting enzyme 2 (ACE2). In this paper, we preliminarily explored the potential therapeutic mechanism for Qingfei Paidu decoction to against COVID-19 and predicted the active ingredients. We hope that the results will help to the further study on the active ingredients and mechanism of Qingfei Paidu decoction to COVID-19.

Key words: COVID-19; Qingfei Paidu decoction; network pharmacology; molecular docking technology; preliminary exploration

截至目前为止, 新型冠状病毒 (2019-novel coronavirus, 2019-nCoV) 在我国已经肆虐了近两月, 波及全球 20 余个国家及地区, 累计确诊新型冠状病毒肺炎 (coronavirus disease 2019, COVID-19) 患者已 7 万多人, 然而至今仍缺乏有效遏制该病毒的临床药物。尽管基础研究实验室捷报频传[1-4], 但世人仍对新型冠状病毒疫苗、新药 (remdesivir 等) 临床试验或研发进展觉得缓不济急[5]。与此同时, 祖国医学在抗疫中的表现也可圈可点, 国内多个地区发布了中医药干预方案[6,7], 已有诸多中医药实验室开展相关基础研究、临床观察及分子对接技术等[8-10]实验手段进行中药单体或者复方的快速筛选。尽管中医药的疗效和安全性在华夏五千年的历史长河中已久经考验, 但随着现代疾病谱的快速改变, 中医药遣方用药也应推陈出新。在当前紧迫阶段, 基于实验室开展天然药物化合物或中药复方针对新型冠状病毒的直接抑制药理实验有可能应用于临床的速度依然缓慢。而针对临床反馈的治疗新型冠状病毒诱导的肺炎效果良好的复杂中药复方进行有效成分识别和作用机制初探, 从而进行快速的处方优化和二次开发, 可能是中医药精简增效应对临床急症的一条出路。

2020 年 2 月 7 日, 国家中医药管理局发布关于《推荐在中西医结合救治新型冠状病毒感染的肺炎中使用“清肺排毒汤”的通知》[国中医药办医政函(2020)22 号], 据介绍, 清肺排毒汤来源于中医经典方剂组合, 包括麻杏石甘汤、射干麻黄汤、小柴胡汤和五苓散, 四方皆出自《伤寒杂病论》, 性味平和, 其处方组成主要由麻黄、炙甘草、杏仁、生石膏等 21 味中药组成。根据中医理论等[11]对清肺排毒汤抗新型冠状病毒肺炎进行方解分析: 新型冠状病毒肺炎以湿毒为病, 首先犯肺, 继而碍脾, 甚则伤肾, 湿性黏滞, 阻碍气机, 郁久化热。因此, 治疗需要清肺排毒、宣通气机、建运化湿为本。病势剧猛, 合方而治。以麻杏石甘汤加紫菀冬花射干清肺排毒, 缓解肺部症状; 以五苓散加细辛温阳化饮, 防止水饮凌肺; 以小柴胡汤调理气机, 促进三焦通利; 以山药陈皮枳实霍香健脾行气, 斡旋中焦运化。多方相合, 宣畅三焦, 既能清除肺部热毒, 又给湿以出路, 标本同治。据统计, 目前运用清肺排毒汤救治确诊病例, 总有效率达 90% 以上, 其中 60% 以上患者症状和影像学表现改善明显, 30% 患者症状平稳且无加重。在国家中医药管理局发布通知后, 陕西、山西、河北、黑龙江等省纷纷跟进, 广泛推荐使用。

清肺排毒汤由 21 味中药组成, 处方量较大, 根据常理, 其临床患者给药顺应性可能较差, 有必要对该方进行进一步的精简优化, 以提质增效。因此, 尽快开展并初步探索清肺排毒汤抗新型冠状病毒肺炎的活性成分及作用机制势在必行。结合生物信息学手段, 可快速初步研究中药复方的体内生物学特征, 为后续基础实验研究和临床研究奠定基础。中药网络药理学[12]整合了系统生物学、多向药理学、计算生物学和网络分析等学科, 从整体角度探索中药与疾病间的关联性, 提供了一种从系统水平寻找中药复方潜在活性成分和作用

靶点的新策略, 契合于中药多成分−多靶点的作用关系, 与中药从整体水平调控机体并发挥治疗作用的观点相吻合。分子对接技术[13]是采用计算机辅助进行化药直接设计的一种成熟技术, 其采用计算机技术, 通过化学计量学方法模拟分子的几何结构和分子间作用力, 寻找小分子 (或配体) 与已知结构的大分子 (或受体) 活性位点的低能结合模式的过程。本文通过对当前临床报道的抗COVID-19有效中药大处方清肺排毒汤进行网络药理学分析, 推测其相应作用的生物学途径, 并通过分子对接技术对复方中关键化合物与新型冠状病毒肺炎密切相关的靶点[14,15], 如2019-nCoV的3C类似蛋白酶 (3C-like protease, 3CLpro) 和COVID-19相关蛋白血管紧张素转换酶2 (angiotensin-converting enzyme 2, ACE2) 蛋白的结合能进行评估, 进行逆向分析, 评估采用网络药理学进行该复方活性物质的筛选和机理初探的可靠性, 以及考察分子对接技术在中医药领域的适用性, 期待本文能为清肺排毒汤的快速二次开发提供一些帮助。

材料与方法

清肺排毒汤有效成分筛选 应用中药系统药理学数据库及分析平台 (Traditional Chinese Medicine Systems Pharmacology, TCMSP) 数据库 (http://tcmspw.com/index.php), 以清肺排毒汤中的20味药材 (除生石膏) 为关键词进行检索, 对检索到的每味药材以口服利用度 (oral bioavailability, OB) 与类药性 (drug-like, DL) 为指标进行筛选, 其中OB≥30%, DL≥0.18, 筛选后得到每味药材的有效成分。为避免个别认可度较高的有效成分被筛选掉, 故同时查阅中国药典 (2015版) 与CNKI中相关文献, 得到此类成分并合并至TCMSP数据库筛选结果中。从TCMSP数据库下载这些化合物的靶点信息。

构建药理网络 在OMIM数据库 (https://omim.org/) 与GeneCard数据库 (https://www.genecards.org/) 中分别以“pneumonia”作为关键词进行检索, 得到肺炎相关靶点, 与所得有效化合物靶点取交集, 以得到清肺排毒汤治疗肺炎的潜在作用靶点。使用R语言生成“清肺排毒汤−药材−化合物−靶点−肺炎”的药理网络文本, 将文本录入到Cytoscape软件中进行可视化处理, 从而构建清肺排毒汤的药理网络。对构建好的药理网络进行拓扑分析以得到各个节点的中心度值 (betweenness centrality)、亲中心度值 (closeness centrality) 和等级值 (degree)。

蛋白质相互作用 (protein-protein interaction, PPI) 网络的构建 将得到的清肺排毒汤治疗肺炎的潜在作用靶点录入STRING网站 (https://string-db.org/cgi/input.pl), 得到潜在作用靶点的PPI网络。将其数据导入Cytoscape软件中进行拓扑分析, 并使用Cytoscape的MCODE功能对PPI网络进行模块分析, 设置*K*-core值需大于4。

GO (Gene Ontology) 富集分析与KEGG (Kyoto Encyclopedia of Genes and Genomes) 通路分析 通过DAVID网站 (https://david.ncifcrf.gov/) 对得到的潜在作用靶点进行GO富集分析和KEGG信号通路富集分析, 输入潜在作用靶点并限制物种为人, 得到的分析结果以*P*值小于0.05为指标进行筛选。使用R语言对的结果进行可视化处理。

分子对接 从清肺排毒汤的有效成分中选取每味药材的标志性成分与2019-nCoV的3C类似蛋白酶 (3CLpro) 和天然人类血管紧张素转换酶相关的羧肽酶 (ACE2) 进行分子对接, 从RCSB数据库 (https://www.rcsb.org/) 下载相关蛋白的结构, 使用Pymol软件去除溶剂分子与配体, 使用AutoDock软件进行加氢、加电子等操作。从ChemicalBook数据库 (https://www.chemicalbook.com/) 中下载化合物结构, 并使用AutoDock软件进行加氢、加电子、加ROOT等操作。完成后进行分子对接其中蛋白结构的设置为刚性大分子, 使用算法为Local Search Parameters, 得到结果中每个蛋白结合能最低的两组使用Pymol对得到的最佳结果进行绘图。

结果

1 活性化合物筛选结果

通过TCMSP数据库检索后, 符合条件OB≥30%, DL≥0.18的化合物共有310个, 另从药典与相关文献[16-18]中得到每味药物的指标性成分, 共计347个。其中藿香含有12个, 陈皮含有6个, 枳实含有23个, 山药含有16个, 细辛含有9个, 射干含有18个, 冬花含有22个, 紫菀含有19个, 生姜含有11个, 姜半夏含有14个, 黄芩含有36个, 柴胡含有38个, 茯苓含有15个, 白术含有7个, 猪苓含有12个, 泽泻含有10个, 桂枝含有8个, 杏仁含有20个, 甘草含有92个, 麻黄含有25个。考虑到甘草作为使药, 含有符合OB及DL条件成分过多, 故单独设计条件OB≥60%, DL≥0.36对甘草化合物进行筛选, 结果发现该条件与原条件筛选出来的靶点数量差异很小, 故维持原条件。

2 药理网络

建立了清肺排毒汤−药材−有效成分−靶点−肺炎的药理网络 (图1)。

其中粉色节点代表药材, 绿色节点代表有效化合

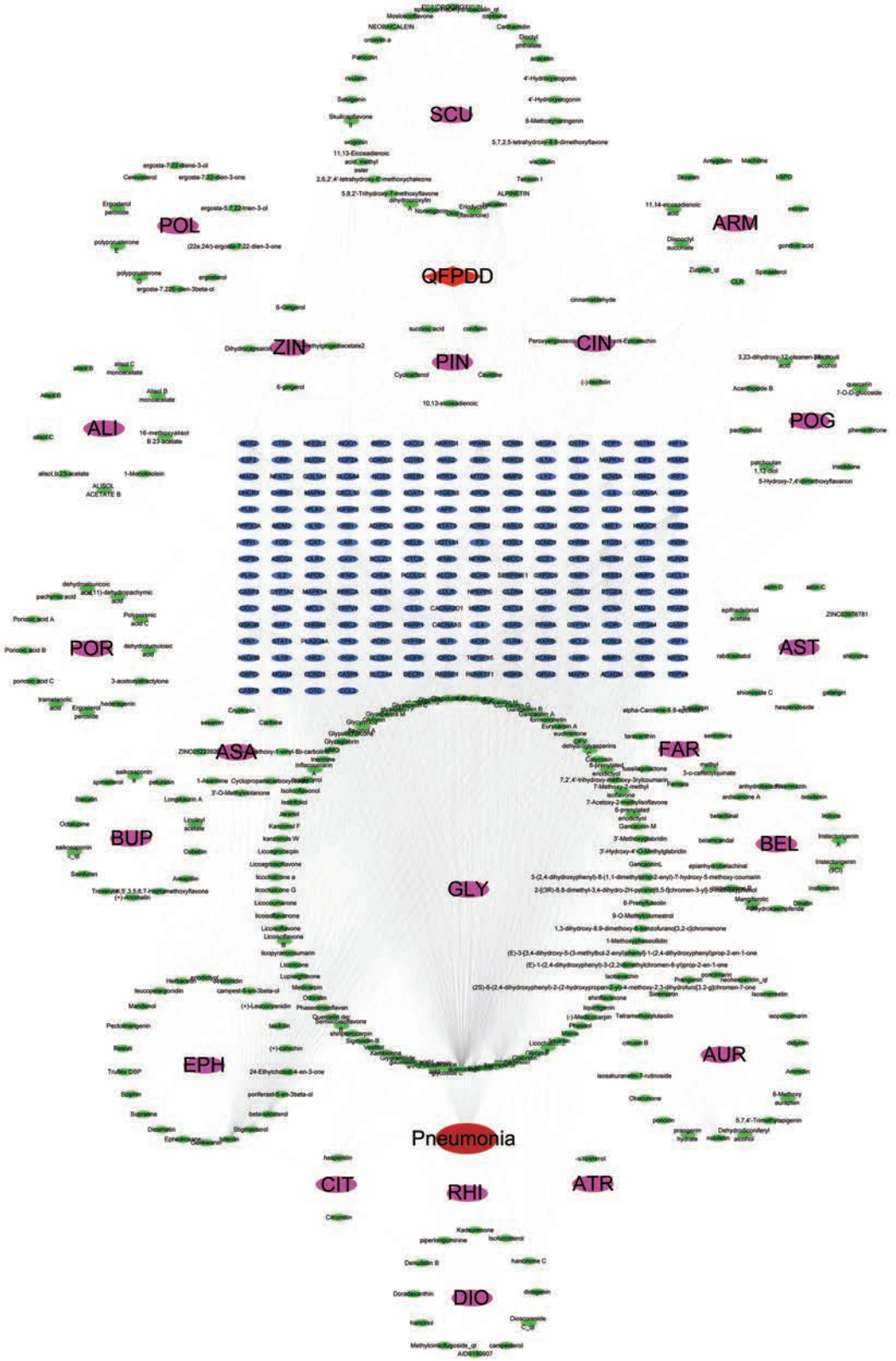

Figure 1　The compound-target network diagram of Qingfei Paidu decoction (QFPDD). The pink labels represent herbs, the blue labels represent targets, the green labels represent compounds. ATR: Atractylodis macrocephalae; BUP: Bupleuri radix; CIT: Citri reticulatae pericarpium; FAR: Farfarae flos; POR: Poria; CIN: Cinnamomi ramulus; SCU: Scutellariae radix; POG: Pogostemonis herba; PIN: Pinelliae rhizoma praeparatumcum zingibere et alumine; EPH: Ephedrae herba; BEL: Belamcandae rhizoma; ZIN: Zingiberis rhizoma recens; RHI: Rhizoma gypsum fibrosum; DIO: Dioscoreae rhizoma; ARM: Armeniacae semen amarum; ASA: Asari radix et rhizoma; GLY: Glycyrrhizae radix et rhizoma praeparata cum melle; POL: Polyporus; AUR: Aurantii fructus immaturus; AST: Asteris radix et rhizoma; ALI: Alismatis rhizoma

物, 蓝色节点代表潜在靶点。在这张图中包含了292个化合物节点和214个潜在靶点节点。在网络图中删除药物、疾病和药材3种要素的节点后得到的化合物−潜在靶点网络图, 可发现平均每个化合物连接了6.32个靶点, 同时每个靶点连接了9.24个化合物, 这样多个化合物同时作用于同一个靶点且单个化合物又作用于多个靶点的现象也体现了中药多成分作用于多个靶点的治疗特点。从化合物角度来看, 有3.83%的化合物拥有超过20个作用靶点, 说明在这个网络中应存在着能够作用于清肺排毒汤大部分靶点的少数关键化合物。通过分析这个网络图的拓扑性质, 及对中心度值、亲中心度值、等级值3个值进行分析, 得到了在整个网络中起到了枢纽作用的化合物, 其中排名最靠前的5个化合物分别是槲皮素、山奈酚、木犀草素、β-谷固醇和柚皮苷。基于网络药理学算法理论, 从单个化合物−靶点网络的角度考虑, 这5个化合物可能代表这个组方的大部分疗效, 即可能是清肺排毒汤的主要疗效物质。从靶点的角度来看, 单个靶点受到越多的化合物影响, 则清肺排毒汤对这个靶点的调控性可能越高, 有13.55%的靶点有不小于10个的化合物于其交互, 其中有8个靶点链接了100个以上的化合物。

3 PPI网络

使用STRING网站对得到的潜在靶点进行分析, 得到了PPI网络图 (图2)。

其中每个节点的大小代表了其等级值 (degree), 颜色由冷到暖代表了中心度值 (betweenness centrality),

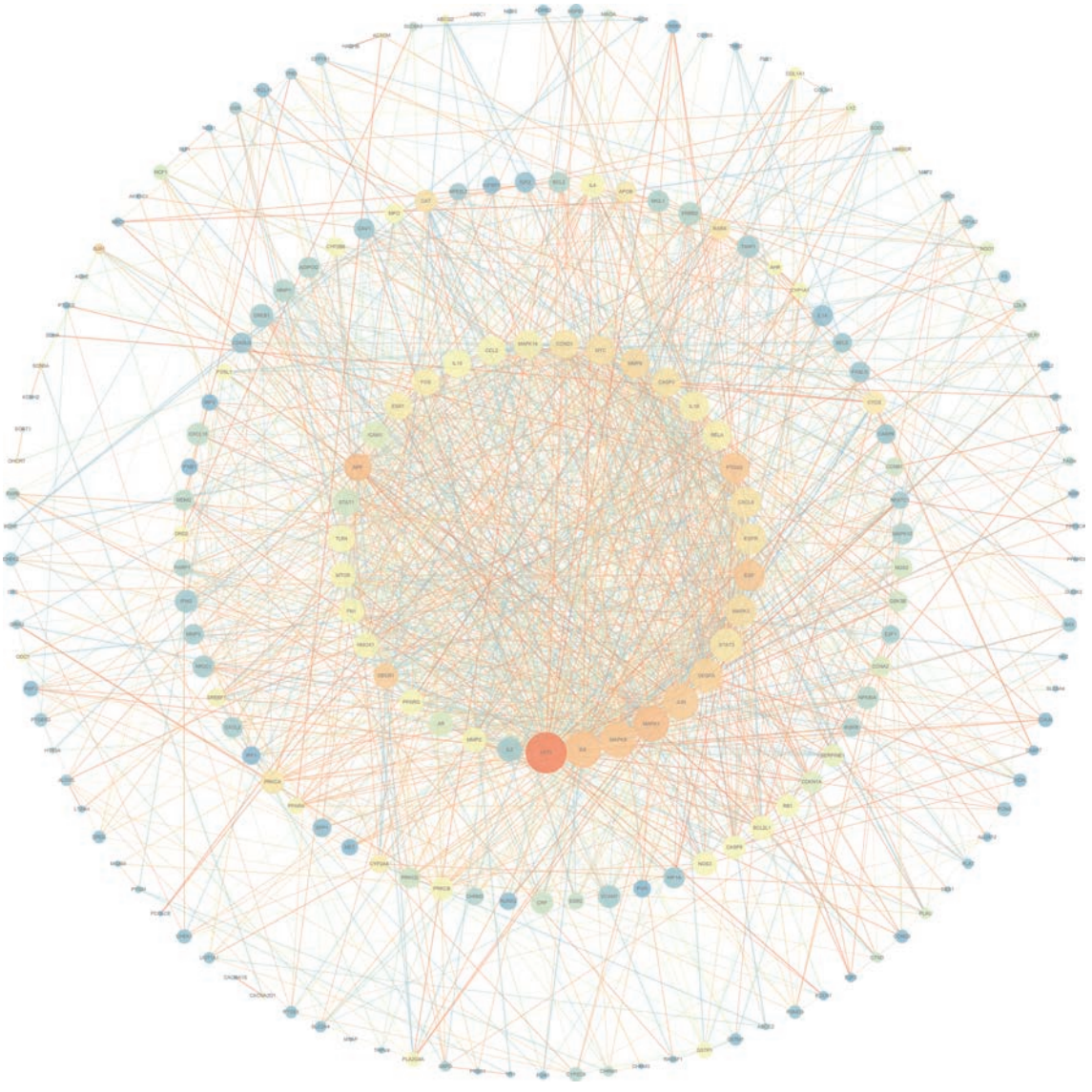

Figure 2 The protein-protein interaction (PPI) network of potential targets in Qingfei Paidu decoction. The size of each label represents its degree, the color from cold to warm represents the value of betweenness centrality, and the thickness and color of the lines represent Edge betweenness and combine-score

连线的粗细和颜色分别代表了 Edge betweenness 和 combine-score。对 PPI 网络进行拓扑分析能够得到这个网络中的枢纽蛋白, 其中等级值排名前 5 位的分别为 AKT1 (AKT serine/threonine kinase 1)、IL6 (interleukin 6)、MAPK8 (mitogen-activated protein kinase 8)、MAPK1 (mitogen-activated protein kinase 1) 和 JUN (jun proto-oncogene)。同时使用 Cytoscape 的 MCODE 功能对 PPI 网络进行了模块分析, 其中 *K*-core 值大于 4 的模块一共有 5 个, 表明其中的蛋白有着更为紧密的联系, 它们可能共同执行着某个生物过程, 如模块 5 与细胞周期调控有关、模块 4 与信号传导和免疫系统相关。在对模块 1 的分析中发现其包含大量白介素相关靶点和干扰素相关靶点, 能够调控免疫与炎症反应, 其中更有许多病毒和细胞启动子中存在的序列 CREB1 (CAMP responsive element binding protein 1) 和具有抗病毒活性和重要免疫调节功能的 IFNG (interferon gamma) (图 3)。

4　GO 富集分析与 KEGG 通路分析结果

使用 DAVID 网站对潜在靶点进行 GO 富集分析, 得到了 $P<0.05$ 的 GO 条目共计 858 个, 其中包含生物过程 (biological process, BP) 条目 681 个, 细胞组成 (cell composition, CC) 条目 72 个, 分子功能 (molecular function, MF) 条目 105 个, 各类别前 20 的通路如图 3 所示。通过 KEGG 通路分析得到了 $P<0.05$ 的通路共 122 条, 包括 non-small cell lung cancer、small cell lung cancer、HIF-1 signaling pathway、Toll-like receptor signaling pathway、T cell receptor signaling pathway 等已报道[19]与肺炎相关的通路, 同样包括 TNF signaling pathway、PI3K-Akt signaling pathway、MAPK signaling pathway、B cell receptor signaling pathway、apoptosis 等与肺损伤保护相关的通路。其中排名前 30 的通路如图 4 所示。

对 KEGG 分析的结果在 DAVID 网站中进行聚类分析, 使用 Function Annotation Clustering 功能, Classification Stringency 设置为 Medium, 可得到 13 类, 得分前 5 类如表 1 所示。

5　清肺排毒汤各药材指标性成分与两种蛋白的分子对接结果

选取了清肺排毒汤中 19 种药材中 24 种化学成分与 3CLpro 和 ACE2 的对接结果如表 2 所示。其中每个蛋白对接最好的前 2 位化合物的对接结果如图 5 所示, 其中麦角甾醇 (ergosterol) 能够与 2019-nCoV 的 3C 类似蛋白酶形成氢键, 其他 3 种对接的最佳结果均未形成氢键。

讨论

本文采用中药网络药理学方法, 在充分考虑化合

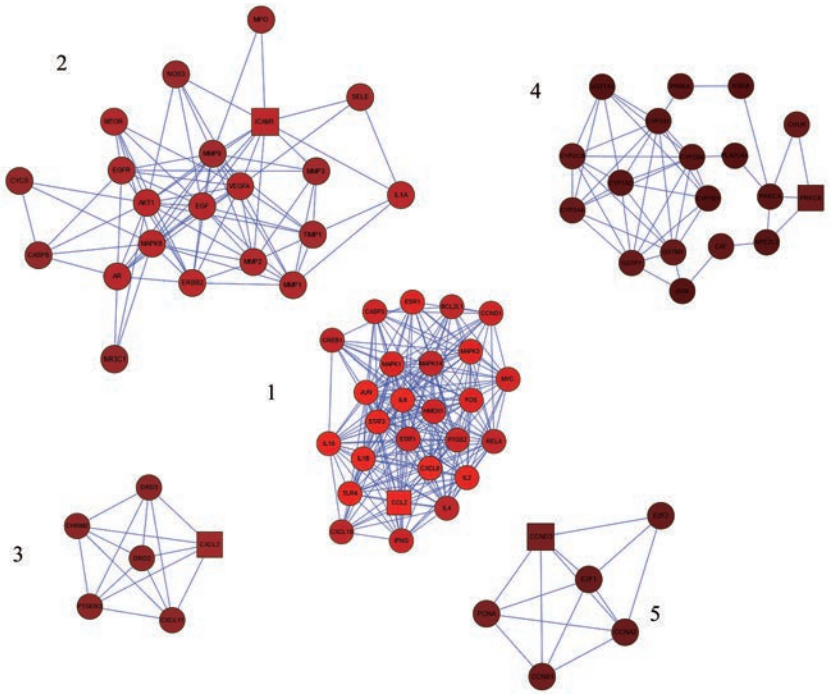

Figure 3　Module analysis results of PPI network. The quadrilateral label is a seed node, and MCODE expands from this node to find nodes that meet the requirements in its neighbor nodes. Eligible nodes are represented by round labels. The shade of each node's color represents its MCODE-score. (1. Score17.8, Nodes26, Edges222; 2. Score8.4, Nodes21, Edges84; 3. Score6, Nodes6, Edges15; 4. Score5.4, Nodes18, Edges46; 5. Score5.2, Nodes6, Edges13)

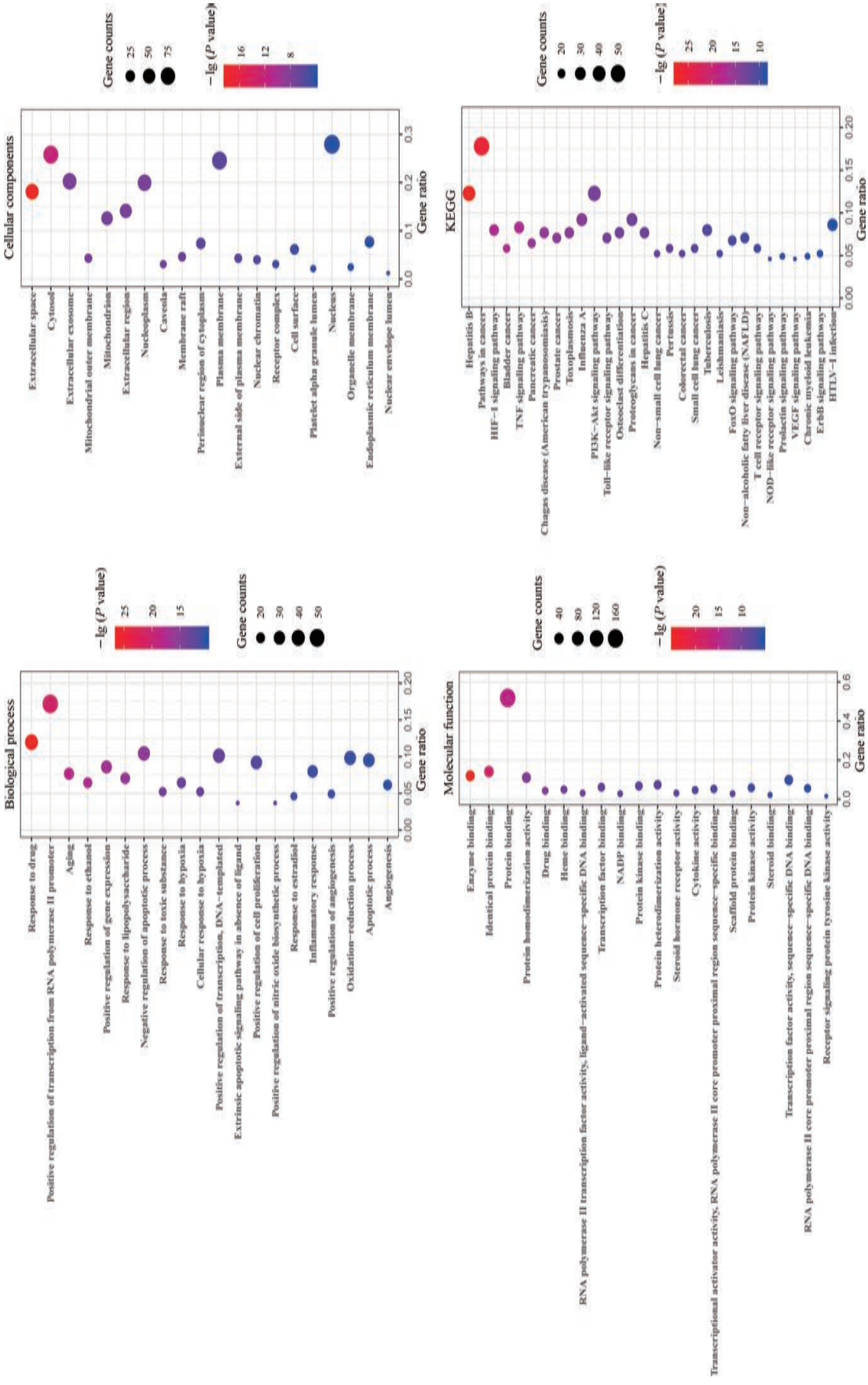

Figure 4　GO enrichment analysis and KEGG pathway analysis of potential targets in Qingfei Paidu decoction. The size of the bubbles in each bubble chart represents the gene counts of this entry, and the colors from cold to warm represent the *P* values from large to small. Each bubble chart is sorted by *P* value. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes

Table 1　Cluster analysis of KEGG analysis results. FDR: False discovery rate

Cluster	KEGG signaling pathway	Count	*P*-value	FDR/%
1	Chagas disease (American trypanosomiasis)	25	5.24E-16	7.11E-13
	Osteoclast differentiation	25	1.42E-13	1.82E-10
	Leishmaniasis	17	7.66E-11	9.85E-08
	T cell receptor signaling pathway	19	2.55E-10	3.28E-07
	B cell receptor signaling pathway	13	4.11E-07	5.28E-04
2	Pancreatic cancer	21	3.71E-16	4.33E-13
	Hepatitis C	25	2.01E-13	2.59E-10
	FoxO signaling pathway	22	1.16E-10	1.49E-07
	Ras signaling pathway	21	5.33E-06	6.86E-03
3	Bladder cancer	19	7.04E-18	9.06E-15
	Pancreatic cancer	21	3.71E-16	4.33E-13
	Prostate cancer	23	1.63E-15	2.14E-12
	Non-small cell lung cancer	17	1.45E-12	1.86E-09
	Chronic myeloid leukemia	16	1.00E-09	1.29E-06
	Glioma	15	2.33E-09	2.99E-06
	Thyroid hormone signaling pathway	17	1.20E-07	1.54E-04
	Melanoma	13	5.67E-07	7.30E-04
	Endometrial cancer	11	1.49E-06	1.92E-03
4	TNF signaling pathway	27	7.32E-18	9.41E-15
	Chagas disease (American trypanosomiasis)	25	5.24E-16	7.11E-13
	Toxoplasmosis	25	2.27E-15	2.85E-12
	Influenza A	30	4.45E-15	5.72E-12
	Toll-like receptor signaling pathway	23	1.07E-13	1.38E-10
	Pertussis	19	1.48E-12	1.90E-09
	Tuberculosis	26	1.84E-11	2.36E-08
	Leishmaniasis	17	7.66E-11	9.85E-08
	NOD-like receptor signaling pathway	15	2.77E-10	3.56E-07
	Salmonella infection	16	8.01E-09	1.03E-05
	Epithelial cell signaling in Helicobacter pylori infection	12	2.30E-06	2.96E-03
	RIG-I-like receptor signaling pathway	12	3.59E-06	4.62E-03
	Herpes simplex infection	19	3.67E-06	4.72E-03
	Shigellosis	10	7.02E-05	9.03E-02
	Cytosolic DNA-sensing pathway	9	4.10E-04	5.26E-01
5	Chronic myeloid leukemia	16	1.00E-09	1.29E-06
	Acute myeloid leukemia	13	3.59E-08	4.62E-05
	B cell receptor signaling pathway	13	4.11E-07	5.28E-04

物的成药性和物质的可存在性的基础上, 基于清肺排毒汤各药材中近千种已知化合物的OB与DL值, 结合各种药材的法定质量控制指标, 筛选整合分析并建立可靠的化合物库。以肺炎为疾病模型, 构建相应的药理网络。通过常规网络药理学算法得出主要核心化合物为槲皮素、山奈酚、木犀草素、*β*-谷固醇和柚皮苷, 该结果与部分文献[10]采用网络药理学筛选其他抗新型冠状病毒知名汤剂得出的核心化合物较为一致, 且文献对这些化合物进行分子对接评分, 其相应的结合能与临床抗病毒化学药物结合能极其接近, 但是不同的中药方剂筛选出来却得到同样的核心化合物, 理论上其合理性可能有待商榷, 可能缺乏处方特异性。因此在此基础上, 本文特别注重选用清肺排毒汤中所有药材(除生石膏、山药) 的质量控制指标或文献报道的重要成分进行后续的分子对接分析。

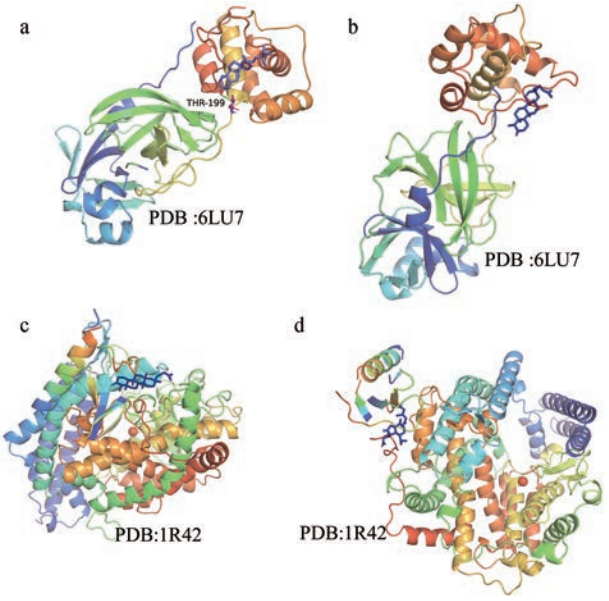

Figure 5　Four best docking results. a: Ergosterol and 3CLpro (3C-like protease); b: Shionone and 3CLpro; c: Shionone and ACE2 (angiotensin-converting enzyme 2); d: Tussilagone and ACE2. The PDB number of 3CLpro is 6LU7, and the PDB number of ACE2 is 1R42. The structure of the compound is represented by a stick, and different branches of the protein are represented by different colors. Because only the hydrogen bond is formed in figure a, the green dotted line is used to represent its hydrogen bond, and the position of the hydrogen bond with the compound in the protein is marked

为了进一步合理确证清肺排毒汤抗COVID-19的药效物质基础, 本文采用分子对接技术对清肺排毒汤组方药材的法定质量控制指标或文献报道的重要成分进行对接验证。在进行分子对接时, 一般认为配体与受体结合的构象稳定时能量越低, 发生的作用可能性越大。以结合能≤−5.0 kJ·mol^{-1}作为筛选标准, 与2019-nCoV的3C类似蛋白酶 (3CLpro) 结合能小于−5.0的有百秋李醇 (藿香)、柴胡皂苷b (柴胡)、麦角甾醇 (猪苓)、紫菀酮 (紫菀)、23-乙酰泽泻醇B (泽泻), 即该类化合物可能直接作用于新型冠状病毒3CLpro, 从而阻断病毒增殖; 与ACE2结合能小于−5.0的有百秋李醇 (藿香)、款冬酮 (冬花)、麦角甾醇 (猪苓)、细辛脂素 (细辛)、盐

Table 2 Docking results of quality control compounds in Qingfei Paidu decoction

Qingfei Paidu decoction	Compound	Molecular formula	CAS	Binding energy values (3CLpro)	Binding energy values (ACE2)
Pogostemonis herba	Patchouli alcohol	$C_{15}H_{26}O$	5986-55-0	−5.68	−5.09
Bupleuri radix	Saikosaponin A	$C_{42}H_{68}O_{13}$	20736-09-8	−2.69	−1.62
	Saikosaponin B	$C_{42}H_{68}O_{13}$	58558-08-0	−6.19	−4.57
Citri reticulatae pericarpium	Hesperidin	$C_{28}H_{34}O_{15}$	520-26-3	−1.6	−0.55
Belamcandae rhizoma	Irisflorentin	$C_{20}H_{18}O_{8}$	41743-73-1	−4.83	−4.09
Poria	Pachymic acid	$C_{33}H_{52}O_{5}$	29070-92-6	−4.67	−3.5
Glycyrrhizae radix et rhizoma Praeparata cum melle	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	1405-86-3	−4.82	−3.2
Cinnamomi ramulus	*trans*-Cinnamaldehyde	$C_{9}H_{8}O$	14371-10-9	−4.04	−3.71
Pinelliae rhizoma praeparatumcum zingibere et alumine	Succinic acid	$C_{4}H_{6}O_{4}$	110-15-6	−2.57	−1.26
Scutellariae radix	Baicalin	$C_{21}H_{18}O_{11}$	21967-41-9	−2.97	−1.58
Zingiberis rhizoma recens	6-Gingerol	$C_{17}H_{26}O_{4}$	23513-14-6	−2.67	−2.38
	8-Gingerol	$C_{43}H_{32}O_{20}$	30462-35-2	−1.07	−0.95
	10-Gingerol	$C_{21}H_{34}O_{4}$	23513-15-7	−1.58	−1.04
Farfarae flos	Tussilagone	$C_{23}H_{34}O_{5}$	104012-37-5	−3.29	−5.54
Armeniacae semen amarum	Amygdalin	$C_{20}H_{27}NO_{11}$	29883-15-6	−2.13	−0.77
Polyporus	Ergosterol	$C_{28}H_{44}O$	57-87-4	−6.46	−6.1
Aurantii fructus immaturus	Synephrine	$C_{9}H_{13}NO_{2}$	94-07-5	−4.14	−4.31
Asari radix et rhizoma	Asarinin	$C_{20}H_{18}O_{6}$	133-05-1	−5	−5.46
Ephedrae herba	Ephedrine hydrochloride	$C_{10}H_{16}ClNO$	50-98-6	−3.57	−5.09
	(1S,2S)-(+)-Pseudoephedrine hydrochloride	$C_{10}H_{16}ClNO$	345-78-8	−3.54	−3.93
Asteris radix et rhizoma	Shionone	$C_{30}H_{50}O$	10376-48-4	−6.85	−6.76
Alismatis rhizoma	23-O-Acetylalisol B	$C_{32}H_{50}O_{5}$	19865-76-0	−5.78	−4.57
Atractylodis macrocephalae	Atractylon	$C_{15}H_{20}O$	6989-21-5	−	−
	Atractylenolide-1	$C_{15}H_{18}O_{2}$	73069-13-3	−	−
Rhizoma gypsum fibrosum	−	−	−	−	−
Dioscoreae rhizoma	−	−	−	−	−

酸麻黄碱 (麻黄)、紫菀酮 (紫菀), 表明该类化合物直接作用于宿主人体细胞, 提高机体免疫力, 阻断病毒侵袭; 同时与 3CLpro 和 ACE2 结合能小于−5.0 的有百秋李醇、麦角甾醇及紫菀酮, 即藿香、紫菀、猪苓可能同时具有较好的抗 2019-nCoV 及其诱导的 COVID-19。本文基于临床有效处方清肺排毒汤中的化合物库中的质量标志物进行同一水平的横向结合能比较, 评价这些化合物抗新型冠状病毒肺炎的贡献大小, 对于后续的优化处方等二次开发可能具有一定的意义, 缺点在于没有评价其他的非质控指标的化合物, 其他化合物也可能具有类似的功效, 但是作者仍觉得中药发挥药效可能还是其中的主要物质, 其他含量很少的物质可能活性挺好, 但是基于物质的可存在性等因素, 它们发挥效应的贡献率可能微乎其微。当然上述所有结论均为基于分子模拟对接得到的结果, 其科学合理性有待进一步的临床验证。

综上所述, 本文采用生物信息学手段进行清肺排毒汤的抗 2019-nCoV 及 COVID-19 的初步预测, 除了生石膏、山药缺乏特征化合物, 其余的 19 种药材通过网络药理学和分子对接技术, 初步探索了清肺排毒汤抗 2019-nCoV 诱导的肺炎的生物学途径, 也初步探索了该方发挥药效的主要药效物质, 为后续临床中医遣方用药以及该方的优化提供了一定的参考, 但其结果的准确性仍需要临床进一步验证。

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