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Efficacy and safety of ReDuNing injection as a treatment for COVID-19 and its inhibitory effect against SARS-CoV-2

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ABSTRACT

Background: Although the rapid emergence of coronavirus disease 2019 (COVID-19) poses a considerable threat to global public health, no specific treatment is available for COVID-19. ReDuNing injection (RDN) is a traditional Chinese medicine known to exert antibacterial, antiviral, antipyretic, and anti-inflammatory effects. In addition, RDN has been recommended in the diagnosis and treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated pneumonia by the National Health Council and the National Administration of Chinese Medicine. However, there is no information regarding its efficacy against COVID-19.

Aim of study: This study was designed to determine the clinical efficacy of RDN in patients with COVID-19 and characterize its antiviral activity against SARS-CoV-2 in vitro.

Materials and methods: A total of 50 adults with COVID-19 were included in this study, and the primary endpoint was recovery from clinical symptoms following 14 days of treatment. General improvements were defined as the disappearance of the major symptoms of infection including fever, fatigue, and cough. The secondary endpoints included the proportion of patients who achieved clinical symptom amelioration on days 7 and 10, time to clinical recovery, time to a negative nucleic acid test result, duration of hospitalization, and time to defervescence. Plaque reduction and cytopathic effect assays were also performed in vitro, and reverse-transcription quantitative PCR was performed to evaluate the expression of inflammatory cytokines (TNF-α, IP-10, MCP-1, IL-6, IFN-α, IFN-γ, IL-2 and CCL-5) during SARS-CoV-2 infection.

Results: The RDN group exhibited a shorter median time for the resolution of clinical symptoms (120 vs. 220 h, P < 0.0001), less time to a negative PCR test result (215 vs. 310 h, P = 0.0017), shorter hospitalization (14.8 vs. 18.5 days, P = 0.0002), and lower timeframe for defervescence (24.5 vs. 75 h, P = 0.0001) than the control group. In addition, time to improved imaging was also shorter in the RDN group than in the control group (6 vs.8.9 days, P = 0.0273); symptom resolution rates were higher in the RDN group than in the control group at 7 (96.30% vs. 39.13%, P < 0.0001) and 10 days (96.30% vs. 56.52%, P = 0.0008). No allergic reactions or anaphylactic responses were reported in this trial. RDN markedly inhibited SARS-CoV-2 proliferation and viral plaque formation in vitro. In addition, RDN significantly reduced inflammatory cytokine production in infected cells.

Conclusions: RDN relieves clinical symptoms in patients with COVID-19 and reduces SARS-CoV-2 infection by regulating inflammatory cytokine-related disorders, suggestion that this medication might be a safe and effective treatment for COVID-19.

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1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the third coronavirus to affect humans in the past 20 years (Vincent et al., 2020 and Nirmal et al., 2020). This epidemic was preceded by SARS-CoV and the Middle East Respiratory Syndrome Coronavirus (MERS), epidemics in 2003 and 2012, respectively (De et al., 2016; C. Huang et al., 2020). Both are extremely contagious and can lead to high mortality rates (Paules et al., 2020; Song et al., 2019). COVID-19 is prevalent in most countries worldwide, and its high transmissibility and associated mortality rates have presented unprecedented challenges to its control (Chen et al., 2020; Phelan et al., 2020; Li et al., 2020; Marta et al., 2021).

Given its rapid spread, severity of symptoms, and high proportion of asymptomatic carriers (Nissen et al., 2020), effective treatment for COVID-19 and the reduction of its mortality rate are foremost priorities in clinical treatment. To date, although there are several vaccines with emergency use approvals in use worldwide, the development of specific and effective drug treatments for COVID-19 remain scarce (Beigel et al., 2020; Harrison et al., 2020). Traditional Chinese medicine (TCM) has a long history of treating viral respiratory diseases (Duan et al., 2011), and certain TCM preparations have been evaluated for their efficacy against COVID-19, including Lianhuaqingwen capsules, Liu Shen capsules, and Xuebijing injection (Hu et al., 2020; Runfeng et al., 2020; Q. Ma et al., 2020; Ma et al., 2020, 2020; Qinbai Ma et al., 2020).

ReDuNing injection (RDN), approved by the Chinese Food and Drug Administration (China FDA) in 2005, is prepared from Lonicerae Japonicae Flos (Lonicera japonica Thunb), Artemisiae Annuae Herba (Artemisiae carvi) Buch. Ham. Ex Roxb.), and Gardeniae Fructus (Gardenia jasminoides J. Ellis) and is used to treat a variety of infectious diseases characterized by chills, high fever, myalgia, headache, and cough with phlegm. RDN has been shown to have antibacterial, antiviral, antipyretic (Zhang et al., 2013a,b; Chen et al., 2014), and anti-inflammatory effects (Tang et al., 2014a,b; Gao et al., 2011; Chang et al., 2014). It has also been highly recommended for the diagnosis and treatment of SARS-CoV-2-associated pneumonia by the National Health Council and National Administration of Chinese Medicine (Trial Seventh Edition, 2020). However, its clinical efficacy and safety in patients with COVID-19 are yet to be established. To provide a useful reference for the treatment of patients with COVID-19, we examined the therapeutic effects of RDN in 50 COVID-19 patients and its efficacy against SARS-CoV-2 in vitro.

2. Materials and methods

2.1. Patients

We enrolled 50 adults with COVID-19 hospitalized in the Lianyungang Fourth People’s Hospital affiliated to Kangda College, Nanjing Medical University and The Third People’s Hospital of Yichang in China in this study. This was a randomized, open-labeled, multicenter, controlled trial of patients with COVID-19 who met the diagnostic criteria established by the “Diagnosis and Treatment Program for Novel Coronavirus Infection Pneumonia (Trial Fifth Edition).” Patients were enrolled in this study between February 6th and March 23rd, 2020, and the eligibility criteria were as followed: 1) Laboratory-confirmed infection with COVID-19 as established by the National Health Commission (General Office Of The National Health and Health Commission, 2020); 2) Presenting as symptomatic (having fever, cough, or fatigue) and exhibiting radiological abnormalities consistent with pneumonia; 3) 18 years or older of either sex. This study was approved by the Ethics Committee at the Tianjin University of Traditional Chinese Medicine and conducted in accordance with GCP guidelines and the Declaration of Helsinki. Written informed consent was obtained from the patients or their legal representatives, and the study was registered with the requisite authorities (ChiCTR2000029589).

The sex, age, height, weight, admission temperature, disease grade (mild, moderate, severe, and critical as described by the “Diagnosis and Treatment Program for Novel Coronavirus Infection Pneumonia (Trial Fifth Edition)”), clinical symptoms (fever, fatigue, cough, wheezes, and sputum), and medication (antiviral drugs, antibacterial drugs, and hormones) were all recorded.

Patients were included if they were 18 years or older, diagnosed with COVID-19 and showed one or more symptoms, i.e., fever, cough, and fatigue at the time of enrollment, with a time from onset to diagnosis of not more than 48 h.

Patients were excluded for any of the following reasons: (1) pregnant or lactating women, (2) severe pneumonia requiring mechanical ventilation, (3) previously consumed immunosuppressive medications within 3 months of admission or recipients of a solid organ or bone marrow transplant, (4) known allergies to the investigational medications, (5) mental illness or lack of self-knowledge, (6) estimated survival time of no more than 48 h from screening, or (7) other conditions judged by the investigators to warrant exclusion.

2.2. Study treatment

RDN was prepared according to the guidelines proposed in the Pharmacopoeia of the People’s Republic of China, and the bioactive constituents were evaluated in a previous study using high-performance liquid chromatography (HPLC). HPLC revealed that RDN contained neoehlorogenic acid, chlorogenic acid, caffeic acid, crypto chlorogenic acid, geniposide, oxidation of brucine, isochlorogenic acid B, isochlorogenic acid C, and isochlorogenic acid A (Fig. 1) (Liu et al., 2017).

A simple randomization was used to group the participants based on the last digit of their ID card. Those with an even number were entered into the RDN group, while those with an odd number were placed in the control group. Participants in the RDN group received routine treatment + RDN, while those in the control group received routine treatment only. All routine treatments complied with the guidelines described in the “Diagnosis and Treatment Program for Novel Coronavirus Infection Pneumonia (Trial Fifth Edition)”, including supportive (oxygen), antiviral, and symptomatic treatments. In the case of the RDN treatment group, 20 mL of RDN was diluted in 250 mL of saline and then administered via intravenous infusion once a day for 14 days. Adherence to treatment, clinical outcomes, use of concomitant medications, and adverse events were recorded. This was an open-label trial, and there was no blinding.

2.3. Measuring efficacy

The primary endpoint of this study was recovery from clinical symptoms on day 14 of treatment. The criterion for symptom amelioration was the disappearance of the major symptoms, such as fever, fatigue, and cough. The secondary endpoints included the proportion of patients who achieved clinical symptom amelioration on days 7 and 10, time to clinical recovery, time to a negative nucleic acid test result (defined as two consecutive negative nucleic acid tests, at least 1 day apart for respiratory tract pathogens), time to the first negative test result, duration of hospitalization (the criteria for release from quarantine, and discharge were established using the “Diagnosis and Treatment Program for Novel Coronavirus Infection Pneumonia (Trial Fifth Edition)”, and time to defervescence (time required to reach a temperature of <37.4°C and maintain this temperature for at least 24 h after starting therapy).

2.4. Cell lines and the virus

Human hepatocellular carcinoma cell lines (Huh-7) and African green monkey kidney epithelial (Vero E6) cells were purchased from the American Type Culture Collection (ATCC), and cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) supplemented with 10%
fetal bovine serum (FBS, Gibco, USA), 100 μg/mL streptomycin (Gibco, USA), and 100 U/mL penicillin (Gibco, USA). SARS-CoV-2 was isolated from clinical samples deposited at the First Affiliated Hospital of Guangzhou Medical University and propagated as previously described (Zhu et al., 2020). The 50% tissue culture infective dose (TCID<sub>50</sub> = 10<sup>-7</sup>/100 μL) was determined using the Reed–Muench method, and the samples were collected and stored at −80 °C.

2.5. Cytotoxicity assay

The 50% toxicity concentration (TC<sub>50</sub>) for RDN in both Huh-7 and Vero E6 cells was determined using the MTT assay (Park et al., 2011). Different dilutions of RDN were incubated with Vero E6 (5 × 10<sup>4</sup> cells/well) and Huh-7 (5 × 10<sup>4</sup> cells/well) cells in 96-well plates for the cytotoxicity assay, and the TC<sub>50</sub> values for RDN causing 50% cell death were calculated.

2.6. Cytopathic effect (CPE) inhibition assay

The 50% inhibition concentration (IC<sub>50</sub>) of virus-induced CPE was used to investigate the efficacy of RDN against SARS-CoV-2. Briefly, a monolayer of Vero E6 cells was inoculated with 100 TCID<sub>50</sub> of virus at 37 °C for 2 h and then incubated with different concentrations of RDN after the removal of the inoculum. Infected cells were observed under a microscope after 72 h of incubation, and the IC<sub>50</sub> value and the selectivity index (SI) were determined using the ratio of TC<sub>50</sub> to IC<sub>50</sub> (Reed et al., 1938).

2.7. Plaque reduction assay

Vero E6 cell monolayers were cultivated in 6-well plates and treated with 100 plaque-forming units (PFUs) of SARS-CoV-2. These cells were then incubated for 2 h as previously described before they were overlaid with 2 mL of an agar/basic medium mixture, containing 0.8% agar and different concentrations of RDN. Cells were fixed in 4% formalin for 30 min and stained with 0.1% crystal violet for 3 min after incubation at 37 °C for 72 h. The plaques were visualized and counted as previously described (Reed et al., 1938).

2.8. RNA isolation and reverse transcription quantitative PCR analysis (RT-qPCR)

To further evaluate the possible anti-inflammatory effects of RDN, we used several concentrations in our subsequent experiments. The primers used for the amplification of MCP-1, TNF-α, IP-10, CCL-5, IL-6, IFN-γ, IFN-α, IL-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Table 1) were designed using Primer 5.0. Huh-7 cells were exposed to the virus at a multiplicity of infection of 1 for 2 h and treated with different concentrations of RDN. After 48 h, the cells were harvested for RNA isolation as described by the protocol supplied with the RNA reagent (Invitrogen, MA, USA). Total RNA was quantified using a PrimeScript<sup>™</sup> RT Master Mix kit (Takara Bio, Japan), and reverse transcribed into cDNA as per the manufacturer’s instructions. RT-qPCR was performed on the resulting cDNA using SYBR Premix Ex Taq<sup>™</sup> II (Takara Bio, Japan). PCR data were analyzed using the detection system (ABI PRISM<sup>®</sup> 7500 Real-time PCR system, Applied Biosystems Co., USA) under the following cycling conditions: 30 s at 95 °C, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The expression of the target mRNA was normalized relative to that of the GAPDH gene. Relative expression of the target genes was calculated using the 2−∆∆Ct method as previously described (Pfaffl, 2001).

2.9. Statistical analysis

Results are presented as the mean ± standard deviation. Data were analyzed using analysis of variance (ANOVA) in SPSS ver. 19.0 (IBM Corp., Armonk, NY, USA). Differences between the groups were evaluated using one-way ANOVA and Tukey’s honestly significant difference test. The Wilcoxon rank-sum test was used for data that did not conform to a normal distribution and count data are presented as a percentage. Comparisons of the count data were completed using a χ<sup>2</sup> test. Survival data were compared between groups using the log rank test. Differences were considered significant at P < 0.05.

Fig. 1. The HPLC profile of RDN at 220 nm. (A) The HPLC profile of RDN. (B) The HPLC profile of the reference solution. 1. Neochlorogenic acid; 2. Chlorogenic acid; 3. Cryptochlorogenic acid; 4. Caffeic acid; 5. Geniposide; 6. Oxidation of brucine; 7. Isocholorgenic acid B; 8. Isocholorgenic acid A; 9. Isocholorgenic acid C.
3. Results

3.1. Patient characteristics at baseline

A total of 50 patients (age: 21–88 years; male: 28 [56.0%]; pre-existing diseases [including hypertension, diabetes mellitus, coronary heart disease]: 26 [52.0%]) with confirmed COVID-19 infection were enrolled and randomized into treatment groups. Patient characteristics are presented in Table 2. These 50 patients were then randomly assigned to their treatment groups leaving 27 individuals in the RDN group and 23 individuals in the control group. None of the patients who received RDN experienced allergic reactions or suffered functional damage to the heart, liver, kidneys, or other organs.

3.2. Evaluating both primary and secondary efficacy indicators

Symptoms were more efficiently reduced in the RDN group after 7 days compared to those in the control (96.30% vs. 39.13%, \( P = 0.0001 \)). Similar results were also obtained at the 10-day evaluations (96.30% vs. 39.13%, \( P = 0.0001 \)) (Fig. 3), shorter periods of hospitalization (14.8 vs. 18.5 days, \( P = 0.0001 \)) (Fig. 5A), and a shorter time to defervescence (24.5 vs. 75 h, \( P < 0.0001 \)) (Fig. 4), and a shorter time to improved imaging (24.5 vs. 75 h, \( P < 0.0001 \)) (Fig. 2), less time to improved imaging was also shorter in the RDN group than in the control group (6 vs. 8.9, \( P = 0.0273 \)) (Fig. 5B). Collectively, these observations indicate that RDN improved resolution rates after 7 and 10 days of treatment, and its application was characterized by a shorter time to symptom resolution, less time to a negative nucleic acid test result, faster resolution of fever, and faster imaging improvements, all of which contributed to a shorter period of hospitalization than that by other treatments.

3.3. Safety evaluation

No significant difference was observed in the occurrence of adverse events between the two groups (RDN: 0.0% vs. controls: 5.00%, \( P = 0.2065 \)), however, there were three deaths among the patients in the control group. None of the patients who received RDN experienced allergic reactions or suffered functional damage to the heart, liver, kidneys, or other organs.

3.4. Reductions in SARS-CoV-2-induced cytopathic effects and plaque formation in response to RDN treatment in vitro

The cytotoxicity of RDN in both of the cell lines in this study was evaluated using the MTT assay. The \( \text{TCD}_{50} \) value for RDN in Vero E6 cells was 168.2 mg/mL (Fig. 6A), and the \( \text{TCD}_{50} \) value for RDN in Huh-7 cells was 30.77 mg/mL (Fig. 6C). CPE inhibition assay revealed that RDN (65, 32.5 and 16.25 mg/mL) markedly reduced SARS-CoV-2 CPE in Vero E6 cells. The \( \text{IC}_{50} \) value and the selectivity index of RDN were 16.19 mg/mL and 10.39, respectively (Fig. 6B). The plaque formation assay revealed that the average size and number of plaques in the RDN group were significantly reduced in comparison to the control and that these reductions were dose-dependent (Fig. 6D). Taken together, the results of the CPE and plaque formation assays indicate that RDN inhibits SARS-CoV-2 proliferation in a dose-dependent manner.

3.5. RDN mediated inhibition of proinflammatory cytokines in vitro

It is well established that SARS-CoV-2 infection induces a strong inflammatory reaction. Thus, we examined the anti-inflammatory effects of RDN in infected cells by evaluating its effects on the expression of SARS-CoV-2-induced proinflammatory cytokines. RT-qPCR revealed that the mRNA expression levels of IL-2, IL-6, IFN-\( \gamma \), CCL-5, TNF-\( \alpha \), MCP-1, and IP-10 were significantly upregulated in the virus group (\( P < 0.01 \)) when compared to the no treatment control (Fig. 7). Treatment with different concentrations of RDN for 48 h significantly reduced the expression of IL-2, IL-6, IFN-\( \gamma \), IFN-\( \alpha \), TNF-\( \alpha \), IP-10, MCP-1, and CCL-5 mRNAs in a dose-dependent manner (\( P < 0.05 \)), indicating that RDN may function as an effective anti-inflammatory agent.

### Table 1

| Target Gene | Direction | Sequence (5′-3′) |
|-------------|-----------|-----------------|
| TNF-\( \alpha \) | Forward | AACATCCAACCTCCCAACAG |
| CCL-5 | Reverse | GACCTAAGCCGCCCCACCTTC |
| MCP-1 | Forward | CAGGAGTGGTCTGTTGTCACC |
| IP-10 | Reverse | GTTGGATTCCTACTCCCAAGT |
| IFN-\( \gamma \) | Forward | ATGAAGCCTGACACGTCATC |
| IFN-\( \alpha \) | Reverse | CATCCTTGGGACGCTC |
| IL-2 | Forward | TCTGTTTGGATGTCAGAAG |
| IL-6 | Reverse | CAGGAGAAGGAAAGAAAGCCTCA |
| GAPDH | Forward | GAAGGTGAAAGGTCGAGTC |
| GAPDH | Reverse | GAAGGTGATGGGATTTC |

### Table 2

Characteristics of the patients at baseline.

| Indicators | All (n = 50) | RDN group (n = 27) | Control group (n = 23) | P |
|------------|-------------|--------------------|-----------------------|---|
| Age (years) | 50.6 ± 15.8 | 49.7 ± 16.0 | 51.5 ± 15.9 | 0.6961 |
| >65 years old, n (%) | 8 (16.0) | 5 (18.52) | 3 (13.04) | 0.5987 |
| Male, n (%) | 28 (56.0) | 16 (59.26) | 12 (52.17) | 0.6149 |
| Temperature at onset (°C) | 37.37 ± 0.69 | 37.39 ± 0.82 | 37.35 ± 0.52 | 0.8511 |
| Disease grade, n (%) | 0.3512 |
| Mild | 49 (98.0) | 26 (96.30) | 23 (100.0) | 0.0001 |
| Severe | 1 (2.00) | 1 (3.70) | 0 (0.00) | |
| Symptoms, n (%) | |
| Fever | 38 (76.0) | 20 (74.07) | 18 (78.26) | 0.7297 |
| Fatigue | 17 (34.0) | 11 (40.74) | 6 (26.09) | 0.2756 |
| Cough | 25 (50.0) | 21 (77.78) | 14 (60.87) | 0.1935 |
| Wheeze | 2 (4.00) | 1 (3.70) | 1 (4.35) | 0.9078 |
| Sputum | 10 (20.0) | 7 (25.93) | 3 (13.04) | 0.2564 |

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**Fig. 2.** Generation curve of recovery time (hours) of the two groups of patients.
Fig. 3. Comparison of COVID-19 nucleic acid negative conversion time process between the two groups. (A) The survivor curve of nucleic acid negative conversion time process between the two groups. (B) Time of nucleic acid negative between the two groups of patients after treatment.

Fig. 4. The length of hospital stay (days) in the two groups. (A) The survivor curve of the length of hospital stay (days) in the two groups. (B) Time (days) of hospital stay between the two groups of patients after treatment.

Fig. 5. Time for defervescence and imaging improvement time (days) of the two groups of patients after treatment. (A) Time for defervescence of the two groups of patients after treatment. (B) Imaging improvement time (days) of the two groups of patients after treatment.
Fig. 6. The reduction of virus-induced CPE and plaque formation by different concentrations of RDN. (A) The cytotoxicity of RDN in Vero E6 cells was determined by using MTT assay. (B) The antiviral effects of RDN on virus in cells. (C) The cytotoxicity of RDN in Huh-7 cells was determined by using MTT assay. (D) The inhibitory effects of RDN on plaque formation of virus. Data were obtained from three separate experiments and presented as the mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001, compared with SARS-CoV-2-infected cells.

Fig. 7. The effects of RDN treatment on the mRNA expression levels of inflammatory factors (TNF-α (A), IP-10 (B), IL-6 (C), IFN-γ (D), CCL-5 (E), MCP-1 (F), IFN-α (G) and IL-2 (H)) in SARS-CoV-2-infected Huh-7 cells. Data were obtained from three separate experiments and presented as the mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001, compared with SARS-CoV-2-infected cells.
4. Discussion

The sudden outbreak of SARS-CoV-2, the third virulent coronavirus to emerge in recent history, has rapidly evolved into a global pandemic. Although our understanding of coronaviruses has been substantially enhanced in recent years, effective approaches for the treatment and epidemiological control of SARS-CoV-2 are lacking. Traditional Chinese medicine (TCM) has been used to treat viruses for many years and has been proven to have obvious therapeutic effects via the direct inhibition of viral proliferation and enhanced immune function. RDN, a TCM, recommended for the treatment of COVID-19, has multiple pharmacological activities, including antibacterial, antiviral, antipyretic, and anti-inflammatory effects (Zhang et al., 2013a,b; Chen et al., 2014; Tang et al., 2014a,b; Gao et al., 2013; Chang et al., 2014). However, its clinical efficacy in patients with COVID-19 is yet to be established. Further, to date, there has been no evidence to indicate that RDN is effective against SARS-CoV-2 in vitro. In this study, we sought to evaluate the clinical data of patients with COVID-19 treated with RDN and determined the antiviral and anti-inflammatory effects of RDN against SARS-CoV-2 in vitro. We clarified that RDN could be used to treat patients with COVID-19. Moreover, RDN was found to inhibit SARS-CoV-2 infection by downregulating the expression of various proinflammatory cytokines induced by SARS-CoV-2.

RDN has previously been demonstrated to have a degree of efficacy against several viral diseases, including H1N1 influenza A, hand-foot-mouth disease, and bronchiolitis (Zhang et al., 2014; Li et al., 2020; Zhao et al., 2010; Wang et al., 2010; Tang et al., 2014a,b). RDN is comprised of a mixture of three herbs (A. carvifolia, L. japonica, and G. jasminoides), and its main functions can be described as “clearing heat, relieving wind and detoxifying”. RDN is indicated for hyperpyrexia, mild nausea and wind cold, headache, pain, cough, yellow phlegm, upper respiratory tract infections, and acute bronchitis with the aforementioned symptoms (Zhang et al., 2013; Li et al., 2020; Zhao et al., 2010; Wang, 2010). In addition, RDN has been included in 12 guidelines from the Chinese Ministry of Health, including the “Diagnosis and Treatment Program for Influenza A H1N1 Influenza” in 2010, the “Chinese Medicine Guidelines for diagnosis and treatment of viral pneumonia in children” published in 2011, and the “Hospital Infection Prevention and Control Technical Guidelines for Middle East Respiratory Syndrome” published in 2015.

Based on its pharmacological effects and the findings of evidence-based medicine in critical fields such as influenza A H1N1 influenza, RDN could be used to treat patients with COVID-19. In this study, we found that the RDN treatment improved symptom resolution after 7 and 10 days of treatment when compared to that in the control group (96.30% vs. 39.13% at day 7 and 96.30% vs. 56.52% at day 10). Similarly, we detected an improvement in symptoms after 14 days of treatment, although no significant difference was found between the two groups at this time point (92.59% vs. 78.26%). Patients in the RDN group had a shorter median time to resolution of the clinical symptoms than patients in the control group (120 vs. 220 h), a shorter time to receiving a negative nucleic acid test result (215 vs. 310 h), a shorter period of hospitalization (14.8 vs. 18.5 days), and a shorter time to defervesce (46 vs. 93 h) than those in the control group. In addition, patients in the RDN group presented with a shorter time to improved imaging than those in the control group (6 vs.8.9 days). Collectively, these results indicate that treatment with RDN is associated with a shorter time to improved symptom resolution, a shorter time to receiving a negative nucleic acid test result, a faster resolution of fever, and a faster imaging improvement time, which contributed to a shorter period of hospitalization than that by the other treatment methods.

We also performed CPE and plaque formation assays to evaluate, the antiviral effects of RDN against SARS-CoV-2 in vitro (Fig. 2). These assays revealed that RDN could protect against cell death and reduce the average size and number of plaques in treated cells in a dose-dependent manner. Furthermore, we obtained a selectivity index IC₅₀ value of 10.39 against SARS-CoV-2 in Vero E6 cells.

Highly pathogenic coronaviruses, such as MERS-CoV and SARS-CoV, can cause fatal pneumonia, which is associated with rapid viral replication, massive inflammatory cell infiltration, and elevated inflammatory cytokine responses. Although our understanding of the fatal pneumonia caused by highly pathogenic coronaviruses is currently incomplete, recent studies have demonstrated the pivotal role played by the cytokine storm in inducing fatal pneumonia (Channappanavar et al., 2017). IL-6, MCP-1, and IP-10 were all shown to increase in the serum of MERS patients in response to an increase in the concentrations of proinflammatory cytokines including IFN-γ, TNF-α, IL-15, and IL-17 (Leong et al., 2006; Assiri et al., 2013). Further, the cytokine storm in novel coronavirus-infected pneumonia patients in ICUs was reported to be higher than that in non-ICU patients (Chaoxin Huang et al., 2020). Given this we went on to examine the anti-inflammatory effects of RDN on the mRNA expression levels of IL-2, IL-6, IFN-γ, IFN-α, TNF-α, IP-10, MCP-1, and CCL-5. RDN was found to significantly reduce the expression of IL-2, IL-6, IFN-γ, IFN-α, TNF-α, IP-10, MCP-1, and CCL-5 in a dose-dependent manner, thereby indicating that RDN may serve as an effective anti-inflammatory agent.

Based on the findings of a centralized monitoring study on the clinical safety of RDN at 40 medical institutions, involving a total of 30,860 patients treated with RDN, the incidence of stated adverse reactions was 0.65% (unpublished). Furthermore, the total incidence of adverse drug reactions following RDN was shown to be 0.38% in a centralized monitoring study on the clinical safety of RDN, involving a total of 12,427 observation forms from 46 hospitals in Jiangsu Province in China (Xu et al., 2009). Among these side effects, the main manifestations included rash, skin pruritus, diarrhea, and chest tightness. Nevertheless, all these adverse reactions were mild and were subsequently relieved or disappeared after drug withdrawal or symptomatic treatment. These findings indicate that RDN is likely to be safe under the conditions of reasonable clinical use (Zhang et al., 2013a,b). In the present study, we observed no allergic reactions or anaphylactic shock among the patients treated with RDN, thereby providing provisional evidence that this medication can be safely used to treat patients with SARS-CoV-2 infection.

In conclusion, our results show that RDN improves the clinical symptoms of patients with COVID-19. Further, RDN could markedly inhibit SARS-CoV-2 proliferation in vitro. The antiviral effects of this medication can be attributed to its inhibition of viral proliferation and its regulation of the expression of proinflammatory cytokines. Further evaluations are required to confirm that RDN can be used as a potential agent in the treatment of SARS-CoV-2 and provide a basis for more in-depth investigations of its underlying mechanisms.

Author contributions

Qinhai Ma, Qingquan Liu and Zifeng Yang designed the study. Qinhai Ma, Bin Liu, Yuqi Xie, Zhourang Wang, Yutao Wang and Biao Lei collected the samples. Qinhai Ma, Bin Liu, Yuqi Xie, Zhourang Wang, Biao Lei, Ruihan Chen and Haimeing Jiang performed the tests and analyzed the data. Qinhai Ma and Yuqi Xie wrote the manuscript. Zifeng Yang and Qingquan Liu revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing 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.

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