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RESEARCH ARTICLE

A Small-Scale Medication of Leflunomide as a Treatment of COVID-19 in an Open-Label Blank-Controlled Clinical Trial

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Abstract

We recently reported that inhibitors against human dihydroorotate dehydrogenase (DHODH) have broad-spectrum antiviral activities including their inhibitory efficacies on SARS-CoV-2 replication in infected cells. However, there are limited data from clinical studies to prove the application of DHODH inhibitors in Coronavirus disease 2019 (COVID-19) patients. In the present study, we evaluated Leflunomide, an approved DHODH inhibitor widely used as a modest immune regulator to treat autoimmune diseases, in treating COVID-19 disease with a small-scale of patients. Cases of 10 laboratory-confirmed COVID-19 patients of moderate type with obvious opacity in the lung were included. Five of the patients were treated with Leflunomide, and another five were treated as blank controls without a placebo. All the patients accepted standard supportive treatment for COVID-19. The patients given Leflunomide had a shorter viral shedding time (median of 5 days) than the controls (median of 11 days, $P = 0.046$). The patients given Leflunomide also showed a significant reduction in C-reactive protein levels, indicating that immunopathological inflammation was well controlled. No obvious adverse effects were observed in Leflunomide-treated patients, and they all discharged from the hospital faster than controls. This preliminary study on a small-scale compassionate use of Leflunomide provides clues for further understanding of Leflunomide as a potential antiviral drug against COVID-19.

Keywords DHODH inhibitors · Leflunomide · Coronavirus disease 2019 (COVID-19) · Viral shedding time · Inflammation

Introduction

Though clinical trials of compassionate or off-label uses of several drugs were conducted in China and other countries, however, there is still no specific and effective drugs to

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cure COVID-19. The WHO is encouraging global solidarity trial mainly focusing on three kinds of drugs that may prove useful against the SARS-CoV-2, that are an anti-viral compound of Remdesivir previously developed as a treatment for Ebola, anti-HIV combination treatment of Lopinavir and Ritonavir in the present of interferon-beta or not, and anti-malaria compound chloroquine & hydroxychloroquine (Kupferschmidt and Cohen 2020). Partial clinical trial results of the above drugs had already gained in different countries, showing only modulate and inconsistent efficacies (Beigel *et al.* 2020; Cao *et al.* 2020; Geleris *et al.* 2020; Wang *et al.* 2020). It is therefore still mandatory to seek other safe and solid strategies to treat COVID-19 when facing the increasing number of patients worldwide.

Recently, we reported that acute replication of RNA virus including SARS-CoV-2 deeply relying on the intracellular pyrimidine resource, and inhibitors against DHODH, a rate-limiting enzyme in the fourth step of *de novo* pyrimidine biosynthesis pathway, can efficiently block viral genome replications in infected cells (Xiong *et al.* 2020). Furthermore, DHODH inhibitors can rescue mice from severe influenza-A-virus infection by the dual use of both anti-viral and immuno-repression of pathological inflammation. Leflunomide and its active metabolite Teriflunomide, as approved DHODH inhibitors in many countries, have been widely used in the clinic to treat autoimmune diseases for years (Fragoso and Brooks 2015). During this SARS-CoV-2 outbreak, we for the first time proved that Teriflunomide conferred a profound antiviral efficacy of EC50 = 6 μmol/L at MOI = 0.03 in SARS-CoV-2 infected cells. However, whether Leflunomide/Teriflunomide can be equivalently effective in COVID-19 patients is unknown. As COVID-19 patients also suffered from excessive inflammations similar to autoimmune patients (Huang *et al.* 2020), Leflunomide/Teriflunomide may benefit a large number of COVID-19 patients through a dual-use of both antiviral and anti-inflammation. We now present the initial clinical experience in using Leflunomide to treat five COVID-19 patients of moderate type, and another five blank-controlled patients treated under standard therapy without placebo are enrolled as controls. Clinical outcomes were compared before and after Leflunomide treatment with the control group.

Materials and Methods

Method

This study was an open-label, compassionate use of Leflunomide on limited COVID-19 patients approved by the Clinical Research Ethics Committee of Renmin Hospital of Wuhan University (WDRY2020-K047), and each patient signed written informed consent. Patients' enrollment began from February 20th and stopped on February 28th because of a recheck procedure on all registered COVID-19 trials in China by CDC (we additionally supplemented required documents and our registration got approval again on 18th March), and the patients were followed up to the clinical endpoint. The study was also registered at the Chinese Clinical Trial Registry (ChiCTR 2000030058).

Patients

The diagnosis guidelines of COVID-19 followed by the National Health Commission of China (NHC 2020). These guidelines classified SARS-CoV-2 infections into four groups (mild type, moderate type, severe type, and critical type). Laboratory confirmed moderate type (with fever respiratory symptoms, and lung imaging with visible lesions) of COVID-19 patients, diagnosed using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) (Shanghai GeneoDx Biotech Co., LTD, China) were eligible to receive Leflunomide treatment if they fulfilled the following criteria: (1) Men or women, 18–70 years old; (2) meet the diagnostic criteria of a confirmed case according to national diagnostic and treatment program; (3) within 10 days of onset; (4) subjects who can take oral medication or receive oral medication; (5) non-pregnant women, and effective contraception within 7 days of the last drug use to ensure that no pregnancy is taken. (6) Men agree not to have sex with women for 7 days after taking the last drug. Exclude criteria: (1) cases of severe vomiting that are difficult to take pills or cases that have difficulties in absolving the medicine after oral administration; (2) pregnant women and lactating women; (3) subjects received specific antiviral medications such as lopinavir/lidonavir, ribavirin, monoclonal antibodies, etc. within 1 week of admission; (4) breathing failure cases and mechanical ventilation; (5) liver dysfunction; (6) cases of shock; (7) combined with other organ failure requiring ICU monitoring; (8) cases where there is no survival, only hospice care, or cases of deep coma and no response to supportive treatment within 3 h of hospitalization. Clinical endpoint: the primary endpoint was the time from Leflunomide initiation to clinical improvement meeting the discharge criteria (set by the National Health Commission of China). The discharge criteria include: (1) the body temperature returns to normal for more than 3 days; (2) respiratory symptoms improve significantly; (3) lung imaging shows that acute ostonic lesions significantly improved; (4) two consecutive sputum, nasopharyngeal swabs and other respiratory samples nucleic-acid test are negative (sampling time at least 24 h interval). Other clinical outcomes included length of hospitalization, duration of viral shedding, and safety outcomes.

Study Medication

The 10 patients are randomly assigned to either treatment group or control groups. Both groups receive the equal standard supportive treatment of Arbidol and Lianhua Qingwen Capsule (A traditional Chinese medicine recommended by the 7th edition of diagnosis and treatment guidance of novel coronavirus infected pneumonia issued by National Health Commission of the People's Republic of China). Magnesium Isoglycyrrhizinate and Cefoperazone were also included in the supportive treatment.

Treatment group: Oral Leflunomide (10 mg per tablet), 50 mg, q12h (every 12 h), three consecutive times, after 20 mg, qd (every day), a total course of 10 days. Leflunomide tablets are produced by Suzhou Long March-Xinkai Pharmaceutical Co., Ltd, China. The supportive treatments (Arbidol, Lianhua Qingwen Capsule, Magnesium Isoglycyrrhizinate, and Cefoperazone) were accompanied to all the patients.

Control group: Blank control without a placebo but with the supportive treatment (Arbidol, Lianhua Qingwen Capsule, Magnesium Isoglycyrrhizinate, and Cefoperazone).

Clinical Information

Clinical information for the 10 patients before and after drug treatment was obtained from hospital documentation including demographic data, days of symptom onset, days of admission, and clinical symptoms; clinical measurements of body temperature were monitored daily; chest imaging studies and standard whole blood tests including white blood cell count, lymphocyte count, chemistry panels, inflammatory factors C-reactive protein (CRP) *et al.* were conducted before treatment and at day 7 after treatment respectively.

Measurement of Virus Shedding by Quantitative RT-PCR

The qRT-PCR for SARS-CoV-2 was conducted by collecting nasopharyngeal swab specimens during hospitalization through using a commercial kit specific for SARS-CoV-2 detection (Shanghai GeneoDx Biotech Co., LTD, China) approved by the China Food and Drug Administration with an interval of every 24–48 h. According to the illustration manual, a Ct value of 37 or lower was considered positive. Specimens with a Ct value higher than 37 were repeated to double confirmation. If the repeated Ct was still higher than 37 or undetectable, the specimen was considered negative. An endpoint of virus clearance was set as qRT-PCR with the first negative for two continuous samplings from the same patient.

Outcomes

We describe demographics, signs, and symptoms on admission, laboratory results, and chest CT imaging. All the patients survive and discharged from the hospital at the end of the study but with different duration days of hospitalization.

Statistical Analysis

Continuous data are presented as were presented as median (interquartile range 25–75 percentile), and differences between continuous data were analyzed using Mann-Whitney U-test. Categorical variables were expressed as number (n) and compared by the χ^2 test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Patients Clinical Characteristics

From February 20th to February 28th, ten patients with laboratory-confirmed COVID-19 were enrolled in this study to evaluate the efficacy of Leflunomide on SARS-CoV-2 infection. The ten patients randomly assigned into two groups (5 for each) and all received the standard supportive treatments (Arbidol, Lianhua Qingwen Capsule, Magnesium Isoglycyrrhizinate, and Cefoperazone). One group of five patients treated with Leflunomide with a similar dose as the rheumatoid arthritis (RA) treatment, another group of five patients were treated as blank controls without a placebo. There were no significant differences in age (51[49–63] vs 54 [50–59]) and gender (2:3 vs 1:4 [M: F]) between Leflunomide treatment group and blank group, and all the patients are categorized as moderate type according to COVID-19 diagnosis and treatment guide (7th version) issued by the Chinese National Health and Care Commission. All the patients had fevers and respiratory symptoms and clear inflammatory lesions in chest CT before treatment. The patients' information is summarized in Table 1. The Leflunomide treatment group took Leflunomide pills orally for 10 continuous days.

General Results from Blood Examination

The whole blood routine examination was conducted before and after treatment for all the patients. The data were summarized in Table 2. There were no differences between two groups or between pre- and post-treatment in

Table 1 Clinical characteristics of SARS-CoV-2-infected patients.

	Patient No.	Sex	Age, y	Onset days	Anamnesis
Patients treated with Leflunomide	1	M	65	10	None
	2	F	61	10	Hypertension
	3	M	47	9	None
	4	F	51	8	Hypertension, Hyperlipidemia
	5	F	51	10	Hypertension, Atherosclerosis
Patients treated with blank	1	F	54	9	Hypertension, Atherosclerosis, Hysteromyoma
	2	F	50	10	Atherosclerosis
	3	M	56	10	Hypertension
	4	F	63	8	COPD
	5	F	51	9	Hypertension
P value		1	0.91		

each group in either the counts of white blood cell (WBC), neutrophilia (N), lymphocytes (L), platelet, or the levels of alkaline, phosphatase, bilirubin, potassium, sodium, urea, creatinine and albumin ($P \geq 0.05$). A slight decrease of hemoglobin was observed after treatment in both the Leflunomide treatment group (from 140 to 115 g/L, $P > 0.05$) and the control group (from 135 to 112 g/L, $P < 0.05$). Nevertheless, it is considered to be no difference as hemoglobin levels can be affected by multiple factors beyond the drug intervention. The level of creatine kinase also decreased after treatment in both the Leflunomide treatment group (from 50 to 24 mg/L, $P > 0.05$) and in the control group (from 70 to 48 mg/L, $P < 0.05$). However, due to samples missing in after-treatment of the Leflunomide treatment group, there is no statistical significance in creatine kinase degrade between two groups ($P > 0.05$). Nevertheless, the changes in creatine kinase after treatment may suggest that heart muscle damage could occur in SARS-CoV-2 infections.

Drug Treatment Effects

There was a significant decrease in the level of the inflammatory biomarker C-reactive protein (CRP) in the Leflunomide treatment group (from 37.4 to 5 mg/L) when compared with the control group (from 5 to 5 mg/L) ($P = 0.047$) (Table 2), indicating that Leflunomide could repress harmful inflammations in COVID-19 as it does in RA treatment. However, due to patient recruitment difficulties, all five patients in the control group exhibited low levels of CRP before treatment as compared to the Leflunomide treatment group.

Considering the clinical features, although there were no differences in body maximum temperature (Tmax) and antipyretic time, the Leflunomide treatment group showed significantly shorter duration of viral shedding time in 3 patients (5 days [median]) as compared to the control group (n = 3) (11 days [median]) with $P = 0.046$ (Table 3). Two patients in each group (Patient No. 2 and 5 in the Leflunomide treatment group and Patient No. 2 and 3 in the control group) showed negative in viral shedding immediately on the day of drug administration but still with obvious lung inflammatory opacities. We, therefore, excluded these four patients in viral shedding time analysis but continued with treatment until they met the discharge criteria.

Because viral nucleic acid detection by RT-PCR kit may have false-negative results due to the limit of detection, the chest CT is more reliable for pneumonia diagnosis to visualize the lung damage lesions. The chest CT imaging from a representative patient in the Leflunomide treatment group showed patchy ground-glass opacity in both left and right lobes before treatment, which are the common CT features for COVID-19 patients (Fig. 1A). However, at 7 days after Leflunomide treatment, the areas of ground-glass opacity became much smaller and there was obvious absorption of lesions in the bilateral lung as compared to before-treatment (Fig. 1B). Moreover, this patient (No. 4) has a disease history of both hypertension and hyperlipidemia, but she responded positively to Leflunomide treatment by clearing the virus within 5 days after-treatment and showed obvious absorption of lung inflammatory opacities.

Adverse Effects

The levels of liver enzymes Alanine Aminotransferase (ALT) and Aspartate transaminase (AST) both increased in the Leflunomide treatment group as compared to the control group ($P = 0.049$ and $P = 0.176$ respectively) (Table 2). The elevations of these two enzymes reflected the commonly observed adverse effects of Leflunomide

Table 2 Clinical parameters between the Leflunomide group and the control group.

Parameters	Leflunomide, n = 5			Control, n = 5		
	Before treatment	After treatment	*P* value	Before treatment	After treatment	*P* value
WBC, × 10^9/L (3.5–9.5)	4.67 (3.91–7.61)	5.97 (4.34–9.69)	0.715	5.28 (3.95–6.97)	5.58 (3.27–7.47)	0.893
Differences before and after treatment	1.73 (0.63–5.59)		–	0.95 (0.68–2.27)		0.624
N, × 10^9/L (1.8–6.3)	2.80 (2.00–6.60)	4.56 (2.54–7.52)	0.715	2.78 (1.81–4.62)	2.89 (1.48–4.3)	0.893
Differences before and after treatment	2.04 (0.31–5.08)		–	0.49 (0.33–1.90)		0.327
L, × 10^9/L (1.1–3.2)	0.9 (0.58–1.58)	1.18 (0.95–1.41)	0.465	1.83 (1.13–2.02)	2.21 (1.30–2.36)	0.225
Differences before and after treatment	0.46 (0.30–0.55)		–	0.36 (0.17–0.68)		0.624
Platelet count, × 10^9/L (125–350)	217 (200.5–230.5)	195.5 (152.5–270)	0.465	258 (181–315)	231 (221.5–246.5)	0.893
Differences before and after treatment	40.5 (4.75–86)		–	43 (31.5–79.5)		0.624
Alkaline phosphatase, U/L (45–125)	74 (45.5–156)	76 (48–143.75)	0.273	76 (67–83)	73 (65–85.5)	0.893
Differences before and after treatment	15.5 (5.75–54.5)		–	4 (2–12)		0.11
Bilirubin, mmol/L (0–23)	15.4 (11.2–18.15)	13.45 (6.7–20.58)	1	8.5 (6.05–10.65)	6.8 (5.95–7.1)	0.225
Differences before and after treatment	4.2 (1.98–10.4)		–	1.8 (1.25–4.15)		0.176
Potassium, mmol/L (3.5–5.3)	3.79 (3.45–4.05)	3.75 (3.23–4.07)	0.715	3.9 (3.8–4.16)	4.26 (3.72–4.48)	0.345
Differences before and after treatment	0.19 (0.12–0.35)		–	0.57 (0.05–0.7)		0.624
Sodium, mmol/L (137–147)	132 (132–139)	139 (135.75–143.75)	0.068	137 (134.5–141.5)	143 (138.5–147.5)	0.136
Differences before and after treatment	5.5 (3.5–11.25)		–	3 (1.5–12.0)		0.387
Urea, mmol/L (2.17–7.14)	3.1 (2.25–4.63)	3.62 (2.1–5.56)	1	4.89 (4.30–5.58)	4.55 (3.66–4.67)	0.138
Differences before and after treatment	0.62 (0.16–2.28)		–	0.64 (0.34–1.48)		0.624
Creatinine, µmol/L (57–97)	51 (43.5–78.0)	63 (44.25–78)	0.285	55 (47.5–61.5)	52 (47.5–58)	0.581
Differences before and after treatment	3 (0.5–6.25)		–	4 (1.5–8.5)		0.707
Albumin, g/L (40–55)	40.1 (37.0–43.6)	31.7 (26.35–37.43)	0.068	41.8 (38.45–44.45)	40.8 (39.6–41.65)	0.686
Differences before and after treatment	6.35 (4.48–16.18)		–	3.8 (1.05–4.5)		0.05
Haemoglobin, g/L (130–175)	140 (117–157.5)	115 (101–134)	0.08	135 (125–178.5)	112 (108.5–127)	0.043
Differences before and after treatment	26 (4–38)		–	27 (8–58)		0.465
Creatine kinase, U/L (50–310)	50 (34.5–79.5)	24 (19– N.T.)	0.109	70 (41.5–96)	48 (30.5–63)	0.043
Differences before and after treatment	31 (25– N.T.)		–	11 (3.5–46)		0.368
CRP, mg/L (0–10)	37.4 (7.8–120.6)	5 (5–5)	0.109	5 (5–14.75)	5 (5–5.7)	0.18
Differences before and after treatment	32 (5.6–N.T.)		–	0 (0–9.05)		0.047
ALT, U/L (9–50)	26 (19.5–80.5)	123.5 (61.25–251.75)	0.068	18 (17–34.5)	23 (15–42)	0.684
Differences before and after treatment	58.5 (36.75–186)		–	8 (6.5–29.5)		0.049
AST, U/L (15–40)	23 (17.5–47.5)	83(37.25–96.5)	0.068	18 (17–23.5)	16 (14.5–35)	0.893
Differences before and after treatment	48.5 (13.5–55)		–	6 (4–20)		0.176

Table 2 (continued)

Parameters	Leflunomide, n = 5			Control, n = 5		
	Before treatment	After treatment	*P* value	Before treatment	After treatment	*P* value
Tmax (°C)	38 (38–38.85)			38 (37.95–38.65)		0.738
Antipyretic time (d)	2 (1–3)			3 (2.5–3.5)		0.131

The data are presented as the medians and interquartile ranges. *P* values comparing cases are from Mann-Whitney U-test.

WBC white blood cell, *N* neutrophil, *L* lymphocyte, *CRP* C-reactive protein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Tmax* maximum body temperature, *N.T.* not tested.

Table 3 Viral shedding time after drug treatment of indicated patients.

Patient No.	Patients treated with Leflunomide			Patients treated with blank			*P* value
	1	3	4	1	4	5	
Viral shedding time after treatment (d)	4	8	5	11	9	11	0.046

The data are presented as medians. *P* values comparing cases are from Mann-Whitney U-test.

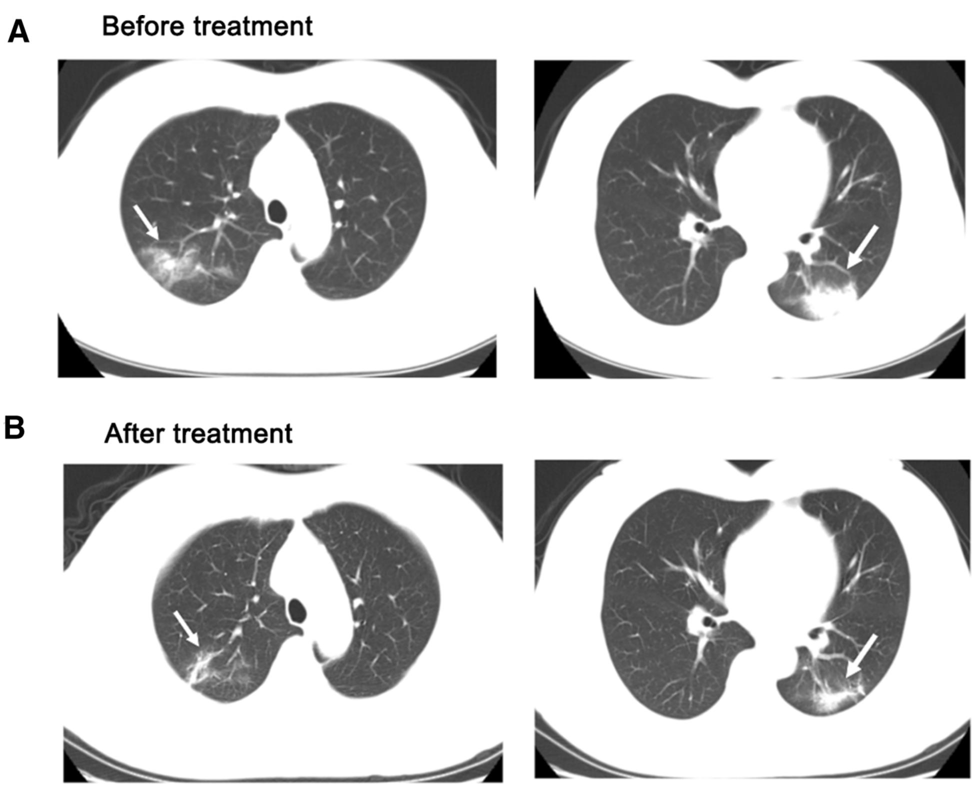

Fig. 1 CT image of a patient in the Leflunomide treatment group. A 51-year-old female was admitted to the hospital for 8 days due to fever, fatigue, and myalgia, with a maximum body temperature of 38 °C. A comparison of CT imaging was shown before and at day 7 after treatment with Leflunomide. Arrows show the ground-glass opacity in the lung.

and other drugs that need to be metabolized through the liver. However, such increases are normally reversible after stopping drug administration and can be cleaned by standard clearance protocols. Besides this, no other obvious adverse effects in the Leflunomide treatment group were observed.

Discussion

With the rapidly increasing number of COVID-19 patients globally, the SARS-CoV-2 pandemic is still on-going spreading worldwide to over 200 countries and infected more than 7.7 million people (up to June 14th, 2020). There is also a concern that SARS-CoV-2 might become seasonal and co-exist with the human for a long time (Kissler *et al.* 2020). However, with great efforts in drug development for this new coronavirus, only a few old drugs have been promoted to clinical trials with moderate efficacies. Therefore, searching for high effective antiviral drugs against SARS-CoV-2 is still mandatory.

The most hopeful drug so far was likely the anti-viral compound called Remdesivir, which is an adenosine nucleotide analog prodrug, previously designed to antagonize RNA-dependent RNA polymerase of Ebola and related virus (Warren *et al.* 2016; Siegel *et al.* 2017). A structured study showed Remdesivir also binds to RNA-dependent RNA polymerase of SARS-CoV-2 (Yin *et al.* 2020). The first clinical trial of Remdesivir showed mild improvement in severe COVID-19 patients but without controls (Grein *et al.* 2020). A randomized, double-blind, placebo-controlled, multicentre trial in China show no association with clinical improvement of Remdesivir, although early treatment within 10 days from symptom onset could help improve moderately (Wang *et al.* 2020). Another preliminary result by NIH indicated that patients who received Remdesivir had a 31% faster time to recovery than those who received a placebo (11 days *vs* 15 days, $P < 0.001$) (NIAID 2020). The FDA had approved Remdesivir for emergence use in COVID-19 treatment. Another nucleotide analog drug is Favipiravir previously approved to treat the influenza virus. A clinical trial in mild COVID-19 infections showed a shorter viral clearance time for Favipiravir treatment when compared with Lopinavir/ritonavir (which proved to have no therapeutic efficacy for COVID-19 (Cao *et al.* 2020) (Cai *et al.* 2020). Nevertheless, the dose used for Favipiravir (Day 1: 1600 mg twice daily; Days 2–14: 600 mg twice daily) is much higher than the effective dose of Leflunomide used here (Day 1–1.5: 50 mg every 12 h; Days 2–10: 20 mg daily).

Similar to nucleotide analogs such as Remdesivir and Favipiravir, Leflunomide is also capable to inhibit viral RNA genome replication (Xiong *et al.* 2020). But the action of Leflunomide and nucleotide analog are totally different. Nucleotide analog, which acts through replacing the functional nucleotide with nucleotide analog to disrupt viral RNA chain elongation, acts at the final steps of viral genome replication. In contrast, Leflunomide directly targets the host *de-novo* pyrimidine synthesis enzyme, DHODH, to cut off the intercellular pyrimidine resources at the starting step of building the viral RNA genome. Thus, Leflunomide may perform a more complete role to stop viral genome replication. Moreover, coronavirus encodes nsp14 to regulate replication fidelity with proof-reading activity (Denison *et al.* 2011), which may weaken drug efficacies for nucleotide analogs. However, the action of the host-targeting antivirals (HTA), such as Leflunomide, will not be affected by virus proof-reading or viral mutations (Xiong *et al.* 2020). Besides the differences in drug targets, DHODH inhibitors were also found able to rescue mice from advanced influenza infections suffering from cytokine storms at the late phase of infection (Xiong *et al.* 2020). On the contrary, nucleotide analog drug normally acts better in the early phase of infection before the virus load grows into exponential levels. Although the patients enrolled in this study were all from moderate type, it is predicted that Leflunomide might be equivalently effective in patients of severe type due to dual functional roles of Leflunomide in both anti-viral and immune regulation.

The second class of promising drug candidates chloroquine or hydroxychloroquine or combination treatment, also used to treat rheumatoid conditions such as arthritis and malaria treatment. Hydroxychloroquine is previously reported to have both antiviral activity and an ability to regulate the immune system (Devaux *et al.* 2020). However, the clinical trial results of these drugs are rather controversial with no efficacy in some studies, but some efficacy in other studies (Chowdhury *et al.* 2020; Geleris *et al.* 2020).

Similar to chloroquine/hydroxychloroquine, Leflunomide also has a dual mechanism of antiviral and immunoregulation and was also approved to treat arthritis for many years. However, the difference between Leflunomide and chloroquine/hydroxychloroquine is distinct that Leflunomide had a clear-cut drug target of DHODH and little off-target effects (Breedveld and Dayer 2000), whereas chloroquine/hydroxychloroquine are multi-targeted and with more severe adverse effected reported (Schrezenmeier and Dorner 2020). Furthermore, it is proved that DHODH inhibition mainly hinders the activated, fast proliferating/replication of immune cells/viruses that require *de-novo* synthesis of pyrimidine bases, whereas resting cells are less affected wherein pyrimidine bases can be recruited by the salvage pathway (Singh *et al.* 2017). Therefore, DHODH is an attractive drug target in acute and severe virus infection diseases accompanied by the hyper-inflammation response.

We observed side effects of Leflunomide by elevation of liver enzyme, ATL and AST. However, according to the FDA drug illustration of Leflunomide (commercial brand name ARAVA), elevations of ALT and AST after treatment were generally reversible. Most transaminase elevations were mild ($\leq$ twofold ULN, Upper limit of normal) and usually resolved while continuing treatment. Marked elevations ($>$ threefold ULN) occurred infrequently and reversed with dose reduction or discontinuation of treatment mostly within 0–3 months. Alternatively, there is also a clearance protocol to eliminate Leflunomide metabolite from the body by the administration of cholestyramine or activated charcoal (https://www.accessdatas.fda.gov/drugsatfda_docs/nda/98/20905_ARAVA_BIOPHARMR.PDF).

Due to patient management policy in Wuhan, we could not follow the patients' ATL/ASL levels after they discharged from our hospital. Nevertheless, we did not receive further side-effect reports from any of the patients.

Additionally, we also noticed that the baseline of CRP in two groups was different, with all patients in the control group being normal CRP level [5 (5–14.7)] before treatment, most patients in Leflunomide group being higher level [37.4 (7.8–120.6)] before treatment. This was due to limited patients we could enroll in the study time. Nevertheless, all the high levels of CRP dropped to normal level [5 (5–5)] after Leflunomide treatment indicating that the immune-repression effect of Leflunomide shown in RA disease may also be effective in infectious disease. Further study needs to be done in this aspect.

The limited sample size and no-placebo design in this study are shortness due to difficulties in patient recruitments in China. However, the patients treated with Leflunomide recovered faster and showed shorten virus clearance days than patients without Leflunomide treatment. Thus, this preliminary study still supports the potential effectiveness of Leflunomide to treat COVID infections with satisfied antiviral efficacy and drug safety.

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Author Contributions KL, KX, HL initiated the work and contributed the conception of the work. KL, KX, HL, and KH design the study. KH, MW, YZ, YZ, TW, ZZ, XL, SZ, DZ organized and performed the clinal study and acquire the clinical data; KH, MW, DZ, KX analyzed and interpreted the data. KL, KX, KH, and MW drafted and finalized the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

Animal and Human Right Statement Additional informed consent was obtained from all patients for which identifying information is included in this article.

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