Efficacy of Chloroquine versus Lopinavir/Ritonavir in mild/general COVID-19: a prospective, open-label, multicenter randomized controlled clinical study

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Study protocol

Abstract

Background The outbreak of novel coronavirus pneumonia is very serious, and no effective antiviral treatment has been confirmed. The fresh drug research and development cycle is too long to meet clinical emergency needs, and "old drugs and brand new applications" have a huge therapeutic potential. During our previous treatment, we found that the lopinavir/ritonavir treatment recommended in the Fifth edition of the treatment plan had little effect. Earlier studies have shown that chloroquine can inhibit coronavirus replication through multiple mechanisms. Our previous use of chloroquine to treat patients with SARS-CoV-2(novel coronavirus)-infected pneumonia has a higher negative rate of nucleic acid in throat swabs within 5 days after administration than that using lopinavir/ritonavir. However, the half-life and side effects of chloroquine vary greatly among individuals.

Methods/design We plan to conduct a prospective, open-label, multicenter randomized controlled, comprehensive treatment clinical study. The study consisted of three phases: a screening period of 1-110 days, a treatment period of no more than 28 days, and a follow-up period of 1 month. Participants will be assessed at baseline and on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 21, and 28 after the intervention begins. In this study, chloroquine and lopinavir/ritonavir tablets were used to treat patients with eligible novel coronavirus pneumonia diagnosed at various centers between February 12, 2020 and May 31, 2020. The efficacy and safety of chloroquine and lopinavir/ritonavir are to be evaluated. At the same time, explore the correlation between patient genetic polymorphisms and chloroquine steady-state concentration, therapeutic effects and adverse reactions in the body. It is an anti-virus for pneumonitis caused by novel coronavirus. The optimization and update of the antiviral treatment plan provides evidence-based evidence.

Disscussion Our study is a prospective, open-label, multicenter randomized controlled, comprehensive treatment clinical study to evaluate the efficacy and safety of chloroquine phosphate and lopinavir/ritonavir in patients with mild/general COVID-2019. The results of this study will provide valuable clinical evidence for the treatment of novel coronavirus pneumonia.

Background

In December 2019, patients with unexplained pneumonia appeared in some medical institutions in Wuhan, China, which were subsequently identified as a novel type of coronavirus. On January 30, 2020, the virus was named by the World Health Organization as "COVID-19 (Corona Virus Disease-2019, caused by SARS-CoV-2))". At present, the COVID-19 epidemic is spreading in every provinces and cities in China. As of February 17, 2020, 72,436 confirmed cases, 6,242 suspected cases, and 1,868 deaths have been reported.

Coronavirus is a single-stranded positive-strand RNA(Ribonucleic Acid) virus with an envelope[1]. There are six HCoVs (Human Corona Viruses), except SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus-2), are known to cause respiratory infections, of which SARS-CoV in 2002 and MERS-CoV (Middle East Respiratory Syndrome Corona Virus) are highly pathogenic viruses that have caused outbreaks worldwide or in some regions. The other four human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) are common pathogens that cause human upper respiratory tract infections, accounting for about 15-30% of all isolated pathogens.

There are currently no clinically specific drugs for these 7 HCoVs. An effective treatment is urgently needed to aid in control of the COVID-19 epidemic. The National Health Commission of China announced a new coronavirus-infected pneumonia diagnosis and treatment program (the Fifth edition, URL: http://www.nhc.gov.cn/yzygj/s7653p/202002/3b09b894ac9b4204a79db5b8912d4440/files/7260301a393845fc87fcf6dd52965ecb.pdf), which proposed the trial of lopinavir/ritonavir.. Lopinavir/ritonavir has a certain anti-SARS-Cov-2 effect[2 3 17]. However, our previous use found that the effcet of lopinavir/ritonavir on COVID-19 was unsatisfactory, for itcan only help improve minority throat swab nucleic acid results (3/15) .

Chloroquine is a known 4-aminoquinoline that has been used clinically since 1944. In addition to being used as an antimalarial drug, chloroquine is also used for the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, etc. due to its immunomodulatory activity[4]. In terms of physical and chemical properties, chloroquine dissolves in water and is weakly alkaline. After entering the cell, it can accumulate in cytoplasmic acidic organelles such as lysosomes and the reverse Golgi networks through protonation, increase its pH(hydrogen ion concentration) value, destroy the structure and function of organelles. Taking typical lysosomes of acidic organelles as an example, chloroquine mediates the increase of lysosome pH in vivo, to attenuate the release of iron ions, decrease the iron ion content in cells, and then interfere with intracellular DNA(Deoxyribonucleic Acid) replication and gene expression in cells[5].

Because chloroquine can change the pH value of endosomes, it has a significant inhibitory effect on viral infections that invade cells through the endosome pathway, such as Borna Disease Virus[6], avian Leukemia Virus[7], and Sika Virus[8]. At the same time, chloroquine can

affect viral replication by inhibiting viral gene expression. In vitro and in vivo experiments have shown that chloroquine can change the glycosylation pattern of HIV gp120 envelope and inhibit the replication of HIV(Human Immunodeficiency Virus) in CD4+ T cells[9]. In addition, chloroquine also acts as a good autophagy inhibitor and interferes with virus infection and replication by affecting autophagy. Animal tests have shown that the application of chloroquine can effectively inhibit autophagy in the lungs of avian influenza H5N1 mice and reduce alveolar epithelial damage[10]. Recently, it has been reported that chloroquine can block Sika Virus-induced autophagy, thereby inhibiting virus replication, and it has been shown in mouse experiments that chloroquine can cut off vertical infection of Sika Virus from the maternal-fetal pathway[11].Two independent research teams found that chloroquine has anti-SARS-CoV activity at the cellular level. Chloroquine phosphate can inhibit virus replication in Vero E6 cell line induced by SARS-CoV, with a 50% inhibitory concentration (IC50) of 8.8 ± 1.2 μm, which is close to the chloroquine plasma concentration achieved during the treatment of acute malaria[12]. It is significantly lower than the 50% cytostatic concentration(CC50 = 261.3 ± 14.5 μm), suggesting the safety of chloroquine for this cell line. At the same time, the antiviral activity of chloroquine can be extended to 5 hours after infection without a significant decrease. A study by the United States Centers for Disease Control and Prevention further clarified that chloroquine inhibits virus replication by reducing terminal glycosylation of angiotensin-converting enzyme 2(ACE2) receptors on Vero E6 cells and interfering with the binding of SARS-CoV and ACE2 receptors[13]. HCoV-229E and SARS-CoV both belong to the α group HCoVs. Chloroquine could inhibit the replication of HCoV-229E on the L132 human embryonic lung cell line by inhibiting p38 mitogen-activated protein kinase (MAPK) activation[14] The latest study found that the S(spike) protein of SARS-CoV-2 is similar in structure with that of SARS-CoV[15], and can also bind to the ACE2 receptor on the surface of host cell through the S protein, thereby infecting the epithelial cells of the host. At the cellular level, remdesivir (GS-5734) and Chloroquine (Sigma-C6628) can be effective inhibit the infection of SARS-CoV-2 in vitro[16].

Based on the above evidence, we proposeda prospective, open-label, multicenter randomized controlled clinical study to evaluate the efficacy and safety of anti-viral treatment of chloroquine phosphate compared with lopinavir/ritonavir in patients diagnosed with mild/general type SARS-CoV-2 infection. Previous studies have shown that chloroquine phosphate has a good antiviral effect in the clinic (Data was not shown), and has been highly valued by Guangdong Province and even the National Health Commission. It has been included in the National Health Commission novel coronavirus pneumonia diagnosis and treatment plan (trial version 6,

URL: http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f922bf6b817.pdf) on February 19, 2020. However, the effectiveness and safety of chloroquine (phosphate) require more evidence-based medical evidence.

Our objective is to initiate antiviral therapy to prevent deterioration to more severe COVID-19, to evaluate the safety of the drug, and to explore the correlation between drug concentration and treatment effect and adverse reactions by measuring the blood concentration of chloroquine phosphate.

Methods/design

Background and design: This study is a prospective, open-label, multicenter randomized controlled, comprehensive treatment clinical study. Eligible participants are randomized assigned to the experimental group (chloroquine phosphate group) or the control group (lopinavir/ritonavir), with 56 patients in each group. Participants arerecruited from 4 hospitals, including the Fifth Hospital Affiliated of Sun Yat-sen University, the Ninth People's Hospital of Dongguan, Zhongshan Second People's Hospital, and the Jiangmen Central Hospital. The study consists of three phases: a screening period of 1-110 days from February 12, 2020 to May 31, 2020, a treatment period of no more than 28 days(outcomes will be evaluated over a period of 28 days from the time of enrolment.), and a follow-up period of 1 month for each participant. Participants are assessed at baseline on day 0, recorded adverse reactions on each day and clinical and laboratory data on days required after beginning the treatment. This study investigates the use of chloroquine or lopinavir/ritonavir tablets in patients diagnosed with novel coronavirus pneumonia (in line with the inclusion and exclusion criteria) between February 12, 2020 and May 31, 2020, to explore the safety of chloroquine and lopinavir/ritonavir tablets in the treatment of patients with COVID-19, the appropriation of antiviral treatment, the correlation between steady-state concentration of chloroquine phosphate and treatment effects and adverse reactions. The flow chart of the research process is shown in Figure 1. The schedule of treatment visits and data collection (also known as Clinical Research Flowchart) is shown in Table 1.

Participants:

Inclusion criteria

The study inclusion criteria are as follows:(All the following criteria are met before being selected)

(1) Age ≥18 years;

(2) Meet all the following criteria (refer to confirmed cases in the Diagnosis and Treatment of pneumonitis caused by novel coronavirus (trial version7, URL:
http://202.116.81.74/cache/4/03/www.nhc.gov.cn/5d9aa1423a8a577e1cc197a0d3c434d8/ce3e6945832a438eaae415350a8ce964.pdf):

⬜ Epidemiological history

1. Within 14 days before the onset of illness, a history of travel or residence in Wuhan and surrounding areas, or other communities with reported cases;
2. Within 14 days before the onset of illness, exposure to a person with COVID-19 (positive nucleic acid test);
3. Exposure to patients with fever or respiratory symptoms from Wuhan and surrounding areas, or communities with case reports within 14 days before onset;
4. Aggregated disease : 2 or more cases of fever and/or respiratory symptoms happen in a small area such as home, office, school class, etc. within 2 weeks.);

⬜ Clinical manifestations:

1. Fever or respiratory tract symptoms: cough, nasal stuffiness, nasal discharge, etc.;
2. Normal or decreased white blood cell counts in the early stages of disease, normal or decreased lymphocyte counts;
3. Multiple small patchy shadows and interstitial changes in the early stage of chest imaging, which are evident with the extrapulmonary zones⬜ it develops multiple ground glass infiltrations and infiltrates throughout both lungs. In severe cases, pulmonary consolidation may occur, and pleural effusion is rare.

⬜ Confirmed: The suspected case⬜Diagnosis of a suspected case requires the fulfillment of any one epidemiological history and any 2 clinical manifestations; it is must to satisfy three clinical manifestations if no epidemiological history.⬜ has one of the following etiological or serological evidence:

1. Real-time fluorescent RT-PCR(Reverse Transcription PCR) detects positive novel coronavirus nucleic acid;
2. The gene sequencing results of patients' specimens(blood⬜stool, etc.) are highly homologous to that of known novel coronavirus;
3. Serum SARS-CoV-2 specific antibodies IgM and IgG are positive, Serum SARS-CoV-2 specific antibodies IgG change from negative to positive or the quantity of IgG in the recovery phase is at least 4 times higher than in the acute phase(3-5 days after onset).

⬜ mild or general patients

1. mild: Mild clinical symptoms (only manifested as low-grade fever, minimal fatigue, etc.), and no pneumonia manifestations in imaging;
2. general: with fever, dry cough and other respiratory tract symptoms, visible pneumonia imaging.);

⬜ Those who have not used antiviral drugs.

Exclusion criteria

The exclusion criteria are as follows:

 (If the subject meets any of the following conditions, they cannot enter the study)

1. Patients with a history of allergy to chloroquine phosphate, lopinavir, and ritonavir;
2. Patients with hematological diseases;
3. Patients who have failure in liver and kidney ;
4. Patients with arrhythmia and chronic heart disease;
5. Patients known to have retinal disease, hearing loss;
6. Patients known to have mental illness;
7. Patients who must use digitalis because of the original underlying disease;
8. Pancreatitis;

9. Hemophilia;

10.Favism ;

11. Female patients during pregnancy.

Outcomes:

Primary outcome

The clinical recovery time (not more than 28 days), that is, the time (in hours) from the start of study drug intervention to normalization of body temperature, respiratory symptoms, respiratory frequency, and blood oxygen saturation. Specifically meet the following criteria at the same time:

No fever, Axillary body temperature ≤37.2 ℃;

Relief of respiratory symptoms (72 consecutive hours);

Respiration rate ≤24 / minute (resting state);

Fingertip blood oxygen> 94%;

Secondary outcome(not more than 28 days)

Respiratory tract sample SARS-CoV-2 RT-PCR negative for two consecutive times (calculated based on the first time);

Respiratory tract, blood, feces or all other samples to SARS-CoV-2 RT-PCR were negative twice in a row (both calculated at the first time);

Death from all causes(both the outcome of death and the time to death included);

Time for body temperature to return to normal (calculated from the onset of illness);

The time of mild cough or no cough (cough is severe or moderate when enrollment);

Time of progress to severity, according to the Diagnosis and Treatment of pneumonitis caused by novel coronavirus (trial version 5), the definition of severity is to meet any of the following: respiratory frequency greater than 30 times/minute, or fingertip blood oxygen ≤94% or PaO_2(Partial Pressure of Oxygen)/FiO_2(Fraction of inspiration O_2) <300mmHg;

The time for the improvement of chest imaging (chest CT), the improvement of imaging is determined by the professional doctor of radiology based on the reduction of the scope of the lesion and the decrease in density;

Frequency of serious adverse events.

Sample size

The main therapeutic index of this study is the clinical recovery time (not more than 28 days), which is from the beginning of the study drug intervention treatment to the normalization of body temperature, respiratory symptoms, respiratory rate and blood oxygen saturation,

In the later analysis, the Logrank method is used to compare the differences in clinical recovery time between the two groups of patients. The sample size of this study is calculated based on the Logrank method by using the Logrank Tests (Lakatos) (Median Survival Time) module in the PASS11.0 statistical software.

Based on clinical experience[17 18], the median clinical recovery time of the patients in the control group is expected to be 8 days, and the median clinical recovery time of patients in the experimental group can be shortened to 4 days (corresponding HR = 2.0) 112 patients (56 in each group) will be required to detect this difference with a significant level of α = 0.05 (both sides) with 85% confidence.

The trial is planned to be enrolled for 90 days, followed up for 28 days, and final analysis is performed after 78 clinical recovery events occurr. It is estimated the drop-out rate of the experimental group and the control group is 5%.

Recruitment

Participants will be recruited from SARS-CoV-2 infected inpatients according to the Inclusion criteria and the Exclusion criteria. The potential study candidate will be screened to determine if they meet the basic criteria. Once volunteered participants have been included or excluded from the criteria assessment, researchers will explain the research procedures in detail and require them to sign a written informed consent form (written informed consent form signed by the subject or his legal representative, these are available from the corresponding author on request) . All participants can withdraw their consent at any time during the trial.

Randomization allocation and blinding

Grouping is carried outusing a central stratification, randomization block method. Before the experiment, a statistical expert uses SAS software to set the number of centers to be 4, the length of the block to be 4, the number of blocks to be 28, a 1: 1 ratio between the test group and the control group, to generate 112 random numbers and corresponding grouping information. According to the haphazard allocation table in advance, the statistical expert gives random numbers (1-112) in ascending order. Each random number and grouping information corresponds to an envelope. The envelope is sealed and given to the researchers responsible for screening.Qualified subjects are selected, and the envelopes are received in the order of enrollment. After the envelopes are opened, the random number and grouping information is taken out, so that the subjects will be randomly assigned to the experimental group or the control group, and the corresponding treatment and observation were performed. Each subject's random number is unique and is the same throughout the trial.

Interventions

The study subjects were divided into experimental group and control group for corresponding treatment regimens.

1. Experimental group (chloroquine phosphate): Chloroquine phosphate tablets were administered twice a day, 0.5 g each time (equivalent to 0.3 g of chloroquine). Novel coronavirus nucleic acids in respiratory tract samples continue to be negative for twice before discontinuation of the drug, the total course of treatment does not exceed 10 days.

Relevant concomitant interventions prohibited during the trial:

Contraindications: Digitaloid drugs, Amiodarone, Domperidone, Droperidol, Haloperidol, Clarithromycin, Methadone, Procainamide, Hydrochlorothiazide, Cisapride, Indapamide, Quinolones.

Avoid combination: Phenylbutazone, Chlorpromazine, Streptomycin, Heparin, Penicillamine, Ammonium Chloride, Monoamine Oxidase Inhibitors, Triamcinolone.

2. Control group (lopinavir/ritonavir): lopinavir/ritonavir is administered twice a day, two tablets each time (equivalent to 400mg of lopinavir / 100mg of ritonavir). Novel coronavirus nucleic acids in respiratory tract samples continue to be negative for twice before discontinuation of the drug, the total course of treatment does not exceed 10 days.

Itis a CYP3A inhibitor and is also metabolized by CYP3A. It cannot be combined with drugs that mainly rely on CYP3A for clearance and high blood concentrations that can cause serious or fatal adverse events.

Relevant concomitant interventions prohibited during the trial:

Contraindications: Lovastatin, Simvastatin, Cisapride, Quetiapine, Dronedarone, Colchicine, Rifampicin, Rifapentine, Bromocriptine, Ranolazine, Midazolam (oral), Triazolam, Elbasvir, Grazoprevir, Pibrentasvir.

Avoid combination: Atorvastatin, Rosuvastatin, Domperidone, Amiodarone, Disopyramide, Quinidine, Voriconazole, Clarithromycin, Alprazolam, Diazepam, Clonazepam, Niratinib, Abemaciclib.

Researchers fill out the inpatient medical records at the same time when the subjects are being treated, ensuring that the data records are timely, complete, accurate and true. At the same time, the researcher fills out the case report form after the diagnosis and treatment of the subjects in time to ensure that the content of the case report form is consistent with the content on the outpatient or inpatient medical records., the researcher should fill out the relevant data on the case report form in time, and submit it to the main researcher at the center for review and signature confirmation.

Safety assessment and adverse events

Office of Clinical Research Center and the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University monitorthe safety throughout the test. Typical laboratory safety tests include routine tests for blood, urine, liver function, eg: ALT(Alanine

aminotransferase) and AST(Aspartate aminotransferase), and renal function, eg: blood urea nitrogen(BUN) and creatinine(Cr) will be performed during the treatment period. Along with treatment, safety will also be evaluated by monitoring adverse events(AEs) and vital signs.

1.Adverse events (AE) refer to adverse medical events that occur after a patient or clinical trial subject receives a drug, but they are not necessarily causally related to treatment.

2 Severe adverse events (SAE) are adverse events that occur during medication and obvious abnormalities in hematology or other laboratory tests, which need to take targeted medical measures to return to normal.

3 Serious Adverse Event (SAE) refers to events that require hospitalization, prolonged hospital stay, disability, affect working ability, life-threatening or fatal, congenital malformation and other events that occurred during the clinical trial.

Investigators should evaluate the possible associations between adverse events and chloroquine phosphate or lopinavir/ritonavir according to the five-level classification of "definitely related, possibly related, possibly irrelevant, definitely unrelated, and undecidable."

Treatment of adverse events in hospitalized subjects

1 If the subject has an adverse event during hospitalization, the doctor in charge or the doctor/nurse on duty should inform the researcher in time, and if necessary, treat the symptom first.

2 The researcher assesses the grade of the adverse event and its relevance to the test drug, and gives further treatment opinions:

(1) General adverse events: You can closely observe the outcome of the event or carry out corresponding symptomatic treatment according to the trial plan.

(2) Significant adverse events: The researcher should notify the principal investigator in time, and suspend treatment, adjust the dosage of the drug, and give targeted treatment according to the requirements of the protocol.

(3) Severe adverse events: The researcher implements treatment according to the condition. If the subject 's damage exceeds the research department 's ability to rescue, notify the emergency team to initiate the "Prevention and Treatment of Subject Damage and Emergencies in Clinical Trials" and report according to the severe adverse event reporting process.

Record of adverse events:

The researcher should strictly follow protocol requirements, and record the adverse events/ serious adverse events in the original medical record and case report form(CRF) in a true, accurate, complete, timely and legal manner, sign and date them.

The record includes at least the name of the adverse event and a description of all symptoms, the start time, the end time, the severity and the frequency of attacks, the relevance to the test drug, the examination due to the adverse event, the treatment measures and the outcome.

Data collection and verification

In our research, professional researchers specialize in recording. At the same time, we appoint qualified supervisors to conduct on-site comprehensive inspection visits to the research center. In order to ensure the accuracy of numerical data, Epidata 3.1 is used for data double-checking. The data is entered and proofread. For the questions in the case report form, the data administrator will fill them in the Data Rating Questionnaire(DRQ) and send an inquiry to the researcher through the clinical monitor. The data administrator will modify the data according to the researcher's response, confirm and enter, and issue a DRQ again if necessary. The verification of data will be divided into manual verification and systematic verification. Data can be locked when the following whole conditions are met: all data have been entered into the database and double-checked; all questions have been resolved; The analysis of the crowd has been defined and judged. After the data is locked, submit the database to the statistical analyst for statistical analysis according to the requirements of the statistical plan, and complete the statistical analysis report. These measures can help improve the reliability and generality of the assessment results.The responsibility of data monitoring and trial guidance is jointly undertaken by the Office of Clinical Research Center of the Fifth Affiliated Hospital of Sun Yat-sen University and the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University, independent of the Department of Infectious Diseases, and have no conflicts of interest.The datasets analysed during the current study are available from the corresponding author on reasonable request.

Test termination criteria:

(1) Due to lack of efficacy, no improvement in results or worsening of symptoms, the investigator believes it is not suitable to continue the trial.

(2) The subject have an adverse event, and after taking appropriate treatment measures, the investigators consider it inappropriate to continue the trial.

(3) Other than the above reasons, those who cannot follow the plan.

(4) After using the test drugs, it is found that the subjects did not meet the inclusion criteria.

(5) Subjects or family members request to withdraw from the trial.

(6) Other reasons that the researcher thinks it is necessary to terminate the experiment.

Quality control

This study will be conducted in four hospitals to ensure its rigor and quality.

All observations and findings in clinical research should be verified to ensure the reliability of the data and to ensure that the conclusions in the clinical research are derived from the original data. Quality control must be used at each stage of data processing to ensure that all data is reliable and processed correctly.

Before the formal start of clinical research, the head of the research centertrain the researchers on the research plan, to help them to unify the knowledge, be familiar with the collection methods and procedures, and understand the special requirements of the research project, to improve the internal observational consistency and inter-observer consistency of clinical research data collectors, to ensure the reliability of clinical research conclusions.

Investigators conscientiously implement an informed consent so that subjects fully understand the research requirements and cooperate with the research.

A qualified auditor is appointed, and regular on-site inspections of the research center are conducted to ensure that all the contents and requirements of the research plan are strictly observed, and the original data is checked to ensure consistency in the content on the CRF(Clinical Research Flowchart).

The clinical research management department and the project responsible unit may entrust the inspectors to conduct a systematic inspection of the clinical research to determine whether the research execution is consistent with the research plan, and whether the reported data is consistent with the records of the clinical participating units, that is, whether the data recorded in the case report form is the same as that of the medical record or other original records.

Statistical analyses

1. Statistical analysis

(1) Full analysis set (FAS)

According to the principle of intention-to-treat (ITT), all the cases that are randomized and use the study drug at least once and have post-medication evaluation data constitute the FAS of this study. The missing data of the relevant part of the efficacy in FAS will be supplemented by the method of the last observation carryied forward.

(2) Per-protocol set (PPS)

The standards of the PPS set and its population will be finalized during the blind data verification, including at least the following standards:

Meet the inclusion criteria specified in the test plan;

Complete all planned visits;

No drugs or treatments that may affect the evaluation of efficacy are used and received during the trial.

(3) Safety analysis data set (safety set, SS)

All cases that are randomized into groups and have used the study drug at least once and have post-medication safety evaluation data constitute the safety population in this study.

2.Effectiveness analysis

The comparison of the main efficacy indicators' clinical recovery time is a log-rank test. Cox proportional hazard model can be used to provide hazard ratios and 95% confidence intervals(CIs). For the comparison of other efficacy indicators, t-test or Wilcoxon rank sum test was used for comparison of measurement data, and test or Fisher's exact probability method was used to test differences in a binary outcome and provide 95% CI of relevant indicators

Take the viral nucleic acid negative or not and the occurrence of adverse events as the dependent variables on the 7th, 14th, ,21st and 28th days in the two groups. Its steady-state valley concentration is taken as the independent variable, Logistic regression analysis is used to investigate the correlation between blood concentration and clinical efficacy and adverse reactions.

3.The safety analysis

Use the test or Fisher's exact probability method to compare the incidence of adverse events in each group, and list the adverse events occurred in this study; the normal/abnormal changes in laboratory test results before and after the trialand the relationship with the test drug when abnormal changes occur.

Clinical trial registration

The trial was registered under the registration number ChiCTR2000029741(http://www.chictr.org.cn/showproj.aspx?proj=49263) on 11 Feb-ruary 2020. On February 10, 2020, this research was approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University, ZDWY[2020] Lunzi No. (K15-1).

Discussion

To the 10 April 2020, the total number of COVID-19 diagnosed in the world is more than 1.3 million , with more than 80,000 deaths. (https://www.who.int/dg/speeches/detail/the-cooperation-council-of-the-turkic-speaking-states--10-april-2020) No curative treatments for COVID-19 with sufficient effectiveness, neither novel treatments nor vaccines. Cao observed that no benefit with lopinavir/ritonavir treatment in severe COVID-19 patients[19]. Lim obversed that beta-coronavirus viral loads significantly decreased and no or little coronavirus titers after administering lopinavir/ritonavir[2]. Gautret P[20] concluded that chloroquine is significantly associated with viral load reduction/disappearance in COVID-19 patients.

In the seventh edition of the Chinese version of the COVID-19 Diagnosis and Treatment Plan (http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf), the recommended antiviral drugs are lopinavir/ritonavir, chloroquine phosphate and other drugs. The Chinese have decreased the current epidemic situation in China by using recommended drugs. The relief of the epidemic in most provinces of China has at least confirmed the effectiveness of the treatment to a certain extent, but further a strong and effective evidence-based evidence is needed.

The trial will be conducted in a clinical outpatient and inpatient setting by experienced clinicians, and participants will be recruited from the patients base of the other three hospitals participating in the trial. The purpose of this prospective, open-label, multicenter randomized controlled, comprehensive clinical study is to evaluate the efficacy and safety of chloroquine phosphate and lopinavir/ritonavir in patients with mild/general COVID-19. The results of this study will provide meaningful information and evidence for clinical practice and will help design a proven and reasonable RCT(Randomized Controlled Trial) soon.

Limitations

Randomized controlled studies still have some design limitations. First, the sample size is relatively small and the 28-day treatment period is shorter. We will not be able to estimate the possible relapse of pneumonia after long-term treatment. Second, the pathophysiology of novel coronavirus pneumonia has not been elucidated. Only clinician assessment (including lung CT results and viral accounting load), there is no objective indicator to judge the effect of treatment on COVID-19. Finally, the follow-up period in this study was relatively short. In

light of these limitations, we will develop a more reasonable treatment cycle and follow-up period to explore the efficacy of chloroquine in patients with COVID-19. We also know there will be many biases in the open trial, and have taken a number of measures to control the possible bias in the trial, as follows:

(1) Strict exclusion criteria are formulated to effectively control other confounding factors that may affect the efficacy;

(2) The trial uses random grouping to ensure that the two groups of patients are comparable;

(3) Before the patient signs the informed consent form, the researchers and the patients make full communication to ensure the patients understand the entire trial content, and try to eliminate the impact of the patient's psychological state on the trial effect.

(4) The main and secondary indicators for evaluating the efficacy are objective indicators to avoid the influence of subjective factors

(5) Before the start of the trial, the researchers conduct unified system training to ensure the uniformity and correctness of data collection and index evaluation.

Trial status The trial was registered under the registration number ChiCTR2000029741(http://www.chictr.org.cn/showproj.aspx?proj=49263) on 11 February 2020. On February 10, 2020, this study was approved by the Medic-al Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen Unive-rsity in Zhuhai, ZDWY[2020] Lunzi No. (K15-1). Unique Protocol ID: ZDWY.GRBK.011. Protocol version date: February 7, 2020. The first particip-ant was randomized on February 2020, and recruitment is ongoing. It is estimated that the recruitment will be completed on May 31, 2020. The f-inal results will be reported next year.

Abbreviations

COVID-19: Corona Virus Disease-2019; nCoV: novel Corona Virus; RNA: Ribonucleic Acid; HCoVs: Human Corona Viruses; SARS-CoV: Severe Acute Respiratory Syndrome Corona Virus; MERS-CoV: Middle East Respiratory Syndrome Corona Virus; IC50: 50% inhibitory concentration; CC50: 50% cytostatic concentration; ACE2: angiotensin-converting enzyme 2; MAPK: mitogen-activated protein kinase; S protein: spike protein; RT-PCR: Reverse Transcription PCR; PaO_2: Partial Pressure of Oxygen; FiO_2: Fraction of inspiration O_2; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood Urea Nitrogen; Cr: creatinine; AEs: adverse events; AE: adverse event; SAE: Severe Adverse Event; SOPs: standard operation procedures; CRF: Clinical Research Flowchart; FAS: full analysis set; ROC: receiver operating characteristic; DNA: Deoxyribonucleic Acid; pH: hydrogen ion concentration; HIV: Human Immunodeficiency Virus; DRQ: Data Rating Questionnaire.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

JX, Professor carried out the design of the study. MH and ZH, carried out the design of the study. XL, participated in the study design and drafted the manuscript. HC and YS, participated in the study design and drafted the manuscript. HZ and GC helped to draft the manuscript. YC, SL and YZ follow the research and help to collect data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed in the current study are available from the corr-esponding anthor on reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University in Zhuhai on February 10, 2020, with file number ZDWY[2020] Lunzi No. (K15-1). This study is designed in accordance with the principles of the Declaration of Helsinki. All participants will provide written informed consent before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

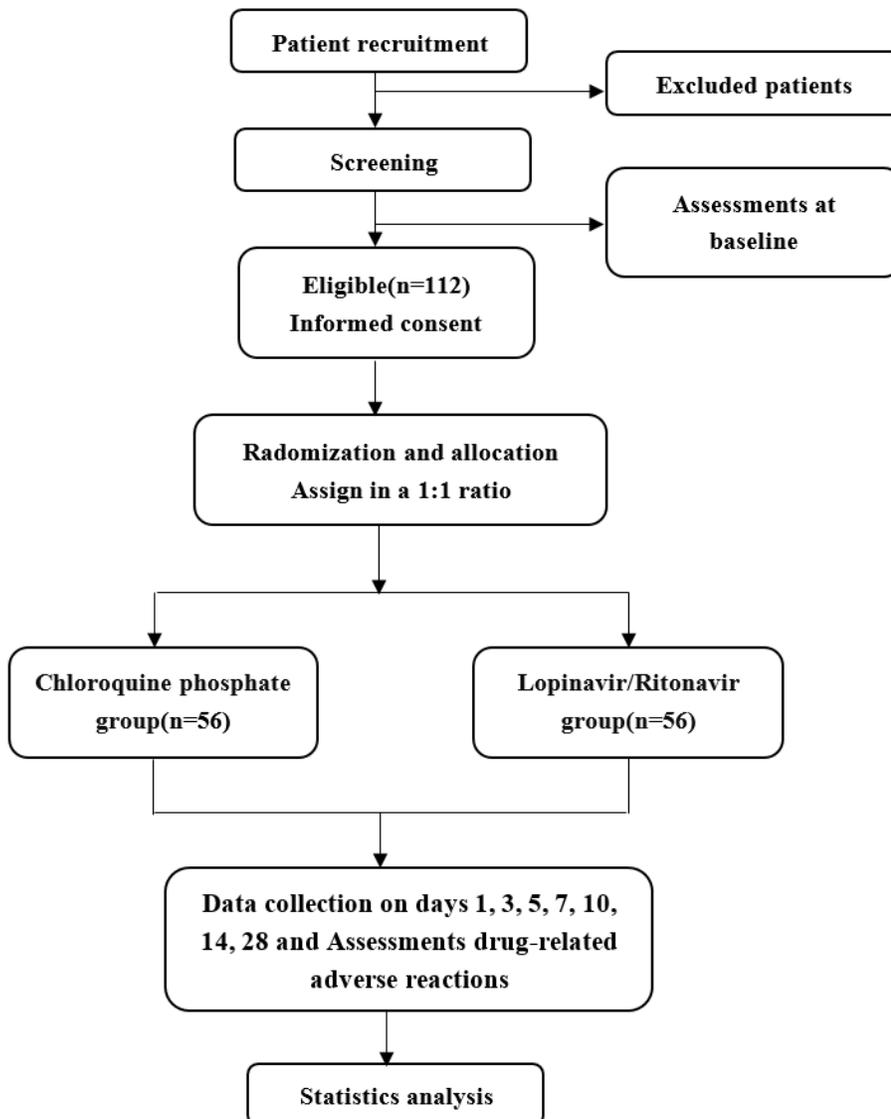[Please see the supplementary files section to view the table.]

Figures

Figure 1

The flow chart of the research process

Supplementary Files

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