REVIEW

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Progresses in clinical studies on antiviral therapies for COVID-19 — Experience and lessons in design of clinical trials

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ABSTRACT

Antiviral therapy with antiviral agents is a very important component of treatment for the 2019 novel coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It is important to clarify how to evaluate efficacy and safety of antiviral agents in treatment of COVID-19 during the pandemic of this disease. We need to answer the following questions: do we still need to use rigorously designed randomized controlled clinical trials (RCTs)? Or, will it be enough if we use loosened criteria, observational studies or even retrospective case series and case reports? The answer is "No, we still need to use the strictly designed preferably blinded multicenter RCTs to evaluate the antiviral agents." In this article, we reviewed almost all the RCT reports on monotherapies and combined therapies with antiviral agents for COVID-19, and found that among the reports on monotherapies, only remdesivir, and among combined antiviral agents, only the combined regimen with interferon-β1b, lopinavir-ritonavir and ribavirin were effective and safe based on evidences from RCTs. The results of five RCTs for chloroquine or hydroxychloroquine consistently showed that they were ineffective and unsafe in the treatment of COVID-19, especially at larger doses. Many aspects in the design of the clinical trials may be related to success or failure of a trial and the relevant factors need to be analyzed, discussed and emphasized from the specific requirements and considerations of antiviral therapies. We hope such discussions be of certain use in designing clinical trials for pediatric antiviral therapies.

KEYWORDS

2019 novel coronavirus disease (COVID-19), Antiviral agents, Coronavirus, Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), Remdesivir

Introduction

The outbreak and global pandemic of 2019 novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) formed unprecedented threatening and challenges to the global populations. According to the World Health Organization's statistics, as of November 10, 2020, the number of confirmed cases of the COVID-19 was 50 459 886 and the number of deaths from this disease reached 1 257 523 and the current situation of the disease is still extremely worrisome and fearful in some countries and regions since the pandemic has a tendency of surging again.[1] The treatment, control and prevention of such

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an extremely hazardous pandemic of infectious disease became the most prioritized tasks of medical care givers, including medical researchers. Unlike the previous outbreaks and epidemics of severe acute respiratory syndrome (SARS) in 2003 (during the whole process and thereafter), there was almost no randomized controlled well-designed clinical trials provided evidences for any therapeutic approach.[2] During this pandemic of COVID-19, at least one RCT presented evidences demonstrating that remdesivir was safe and effective against COVID-19. Based on the evidences, the Food and Drug Administration (FDA) of the United States issued and publicized an Emergency-Use Authorization for the treatment of adults and children patients with severe COVID-19 disease.[3]

Antiviral treatment is an extremely important component of the treatment, control and prevention of COVID-19, especially during the early stage of the disease. However, dozens of drugs, including chloroquine, hydroxychloroquine, azithromycin and even famotidine were claimed to have antiviral effects against SARS-CoV-2. Of the total number of articles, up to 23 949, that can be retrieved at PubMed published since December 1, 2019, 2836 articles are related to or on antiviral therapies. We need to consider, which drugs are truly antiviral agents, which are not, and we should clarify how to evaluate antiviral agents, and reach a clear conclusion about which drug(s) are effective and safe in treatment and prevention of this pandemic disease and worthy of recommending for clinical application. In addition, it is also important for pediatricians to learn from experience and absorb lessons from the clinical trials that are already reported although most or all the clinical trials for antiviral treatments were conducted in adults, and make good use of them for future pediatric clinical studies for COVID-19 as well as for any other viral diseases in children.

What is antiviral therapy? Definition and requirements.

In this review article we discuss issues concerning antiviral therapies for COVID-19. However, we need to recall the following issues closely related to antiviral drugs/therapies. What is antiviral therapy? Are azithromycin, chloroquine and hydroxychloroquine antiviral drugs?

We could not find a definition for antiviral drugs/therapies. But we found requirements for antiviral agents,[4] they must have inhibitory effects on virus-specific events, including attachment to the cell or fusing with the cell membrane, uncoating of the genome of the virus, assembly of progeny virions, or on virus-directed synthesis of the viral nucleic acids and/or proteins. These characteristics are usually confirmed via laboratory experiments in cell culture of the viruses or *in vivo* in animals during the preclinical development stages. If drugs that have never been tested in laboratory for antiviral effects, in principle, those drugs should not be regarded as antivirals. Therefore, drugs such as azithromycin and chloroquine/hydroxychloroquine (unless they were studied for *in vitro* antiviral effects) should not be listed among antiviral agents.

How to evaluate the efficacy and safety of antiviral agents in the setting of a pandemic?

All the medical care givers follow guidelines in their normal daily practice. There are more than 6000 clinical practice guideline documents from more than 70 countries,[5] and most of the guidelines are evidence-based. The high quality of evidences are from multiple RCTs or meta-analyses and single RCTs, and large scale well designed RCTs are believed to provide high quality evidences.[6]

Currently, the whole world is in an extremely unusual situation, in a rare global emergency health crisis. During such a pandemic, should we give up the routine ways of evaluating treatments with RCTs? Do we need to "loosen" or lower our criteria for the evidences, and to use case series, uncontrolled or not randomized trials? The answers are "No!" although there are many challenges and difficulties. We need to overcome the challenges and difficulties and to use rigorously designed, sufficiently large-scale RCTs. Designing and conducting RCTs are possible and feasible during the pandemic. The fact is that the total number of published articles on "COVID-19 treatment" searched at PubMed only was as high as 24 082, which included 122 clinical trials, and 67 RCTs, and for "antiviral treatment for COVID-19", the number of articles was 2853, which included 33 clinical trials and 19 RCT reports. RCT reports were also published during the pandemic of influenza in 2009.[7] These facts indicate that it is possible to conduct well-designed clinical trials, RCTs during a sudden public health crisis like SARS and COVID-19 pandemics. The numbers of published articles, ongoing and planned clinical trials are increasing rapidly, which clearly reflect the need for high-quality evidences for treatments of COVID-19.[8]

As a panel of 29 pediatric experts of the US pointed out: "Antivirals should be tested in clinical trials, as this is the only way to establish efficacy and safety of these therapies for COVID-19."[9] The clinical trial is regarded as the most definitive tool for evaluation of the applicability of clinical research. Clinical trial has been called "the gold standard" on many occasions for measuring and evaluating all the clinical research.[10]

For designing RCTs, there should be no many difficulties, however, there are challenges in conducting the trials and collecting data. In some studies, the sample size could not be fulfilled because in the setting of study the disease was soon controlled well and subsequent enrollment was

impossible.[11]

To obtain high quality evidences for antiviral treatments of COVID-19, it is important to rigorously design the studies. Specific considerations for clinical trials of antiviral agents should include the following. 1) Early beginning of antiviral medication. 2) There must be primary or secondary end points that reflect virological status, e.g., viral load in nasopharyngeal secretion. 3) Specimens for viral nucleic acid determination should be taken from each patient at least once a day or even more frequently, so that the virological parameters can be analyzed and compared accurately. 4) Stratification of the participants for various factors, including severity of diseases, age, and time between onset of symptoms and randomization, etc. Only one RCT had started antiviral therapy within 5 days after onset of symptoms, and collected specimens for virological testing at least once a day.[12]

Efficacy and safety of major single antiviral agents

Remdesivir

Remdesivir is an RNA-dependent RNA polymerase inhibitor, a nucleoside (adenosine) analog which was originally developed for treatment of Ebola virus infection. The mechanism of action is similar to that of the other well-established nucleoside/nucleotide analog antivirals. Its use 24 hours before an animal model infected with Middle East respiratory syndrome (MERS) coronavirus (CoV) showed that it could completely prevent development of symptoms caused by the virus, strong inhibition of viral replication in the respiratory tract and it prevented the formation of pulmonary lesions. However, a phase 2 clinical trial showed that the group treated with remdesivir could not lower the fatality rate.[13,14] Recently, *in vitro* and animal model studies demonstrated that remdesivir had potent activity against SARS-CoV-2 and might become a more promising treatment for COVID-19.[15]

For evaluation of efficacy and safety of antiviral drugs, we will focus mainly on RCTs.

Before WHO SOLIDARITY trial interim report became available on October 15, 2020, only three RCTs on remdesivir were published (Table 1). Of them, only the ACTT-1 study[16] provided confirmed evidence (in shortening the time to recovery) to support the efficacy of remdesivir in treatment of patients with COVID-19 whose disease was moderate in severity. The study was a well-designed randomized, double-blind placebo-controlled phase 3 trial, which was conducted at 60 sites and 13 subsites in 10 countries. The results of the study showed that the time to recovery (primary outcome) in patients in remdesivir group was 4 days (in the final report that was published on October 8,[17] 5 days) shorter than that of the placebo group ($P < 0.001$). The mortality by day 14 on the Kaplan-Meier estimate was 7.1% vs. 11.9% (in the final report, 6.7% vs. 11.9%); while the rate of serious adverse events was similar. These results are the evidences, although not very strong, that support the establishment of efficacy and safety of remdesivir in treatment of COVID-19 adult cases. Just because of the results described in the preliminary report, the US FDA had issued an emergency-use authorization of remdesivir for severe COVID-19 adult and children patients. While the other two RCTs failed to provide evidences due to different reasons (See below).

An important concern about this study is that the primary outcome (time to recovery) was not directly related to the virus. Previous studies on other viral diseases and COVID-19 have used outcomes like time to first negative viral testing,[18] viral load reflected by viral nucleic acid determination, and daily changes of viral load etc.[12] Despite the challenges from different aspects, some studies applied virological parameters as primary outcomes and obtained significant results.[12] The importance of virological outcomes should be emphasized for clinical studies evaluating the efficacy and safety of an antiviral agent. [19]

Another issue we must consider is the importance of early starting of antiviral therapy. If the therapy starts too late, the viral infection may have caused serious or irreversible lesions and therefore antiviral treatment will not help much for recovery.

The other 2 RCTs[20,21] failed to provide any evidence for the efficacy and safety of remdesivir in treatment of COVID-19. The reasons are different. The major reason of Wang et al's study was obviously because the sample size could not reach the planned target, in fact the sample was almost only the half of what was planned. On the other hand, there seemed to be two major limitations in that study.

1) The patients were allowed to enter in the study as late as on the 12th day after the onset of the symptoms (in fact in some other studies, treatment with antiviral agents started as late as 17th days after the onset of the symptoms). Starting the antiviral treatment too late may affect the results of treatment very much. In the RCT by Hung et al,[12] the antiviral therapy was started within 5 days after the onset of symptoms, which is a very good practice.

2) No virological outcome was used in that study. Setting up of proper main outcome or and secondary outcomes are very important for a clinical trial. For clinical trials for antiviral agents, there should be virological or virus-related outcome or end point, so that the researchers can understand the effect of the antiviral therapy on the virus status.

In addition, the patients in either group were allowed to use other drugs that might have antiviral effect against SARS-CoV-2, such as interferon, lopinavir-

ritonavir, Arbidol etc., although none of these agents as a monotherapy has been demonstrated by RCTs to have antiviral effects against the SARS-CoV-2. If any of them really has an antiviral effect against the virus, and if the proportion of patients who were treated with such "other drugs", and the dosage applied are not balanced between groups and the length of time of the use of those drugs were different, bias may occur unless there is predefined stratification and well-planned subgroup analyses.

The RCT reported by Spinner et al[21] experienced certain major amendments on their protocol during the process of the study. One of the notable amendments is the change of the lower limit of the participants' age: from 18 to 12 years. If the randomization is not stratified against the age, and if distribution of children between the ages of 12 and 18 years is not balanced between the two groups, since there is a tendency that children have less severe disease, the result may produce bias.

Mainly based on the preliminary report of the ACTT-1 study and meta-analyses, Rochwerg et al (an international guideline panel composed of 27 persons) issued and published a clinical practice guideline for use of remdesivir for severe COVID-19.[22] The US National Institute of Health and Australian National Guideline made weak recommendation in favor of remdesivir for severe COVID-19.

Important issues related to the use of remdesivir include the following: optimal time to administer the drug, the length of course of treatment, the possible problems with concomitant use of other medicines, contraindicated in patients with liver and renal dysfunction. The uncertainties in effects on mortality, length of hospitalization, and long-term safety and complications, and generalized use in other regions and populations will also need to be studied.

In contrast to the RCT of Beigel et al[16,17] that provided evidences for efficacy and safety of remdesivir in treatment of COVID-19, the interim report of WHO's SOLIDARITY trial[23] declared that remdesivir as well as three other monotherapies for COVID-19 were ineffective in this largest scale rigorously designed multinational RCT. However, this large-scale well designed clinical trial should not be regarded as the final trial, since some arguments concerning the study design should be considered, and further clinical studies that are ongoing and being planned may provide different results and evidences.

1) Inspiration from natural history of COVID-19. In the WHO trial, the design of the study seemed not to have considered and clearly defined early initiation of antiviral treatment. As an acute viral infection, the overall natural history of COVID-19 is about 3 to 4 weeks for most of the patients. The course of the disease can be divided into three stages as depicted by Dos Santos et al[24]: stage 1 is early infection; stage 2 is pulmonary phase; and stage 3 is hyperinflammatory phase, during which pneumonia may deteriorate, sepsis, respiratory failure, cytokine storm and even death may occur. Of the three stages, only the stage 1 and 2 are the best period for application of antiviral agents to stop replication of the virus or even clearing the virus from the body. On the other hand, regardless what the outcome of a patient is in this stage, which may not reflect the effectiveness of an antiviral agent. Therefore, from the point of antiviral therapy, early beginning of antiviral therapy is critically important.

If we make a very preliminary rough estimation based on reports including that of Rees et al,[25] phase 1 may be around 5–7 days, phase 2 around 7–10 days and phase 3 about 3–8 days. A few previously reported RCTs enrolled patients as late as a mean of 16.6 days or 12 days after the onset of symptoms, that means that many of their patients have already missed the best time for antiviral therapy. Starting the antiviral therapy within 5 days after the onset of symptoms will be ideal.

2) Importance of virological outcomes. We have discussed about this earlier.

3) Importance of stratification of participants while they are randomized. Certain characteristics of participants are extraordinarily important for antiviral therapies, these include a) How long after onset of symptoms the antiviral therapy is started; if the study group and control group are not balanced in this regard, bias will occur in results; b) The severity of disease is another important factor. Imbalance in the severity of disease may also produce bias in the results. It will be better if participants are stratified against at least these two characteristics.

4) Choosing proper intervention for the control group. For antiviral and other drugs for treatment of human diseases, initial in-human trials ideally should use randomized placebo-controlled trials to confirm the efficacy of the drug. However, in such a pandemic of acute life-threatening infectious disease, use of placebo is ethically not allowed and also not practical. Therefore, clinical trials have chosen standard of care, or another antiviral drug against COVID-19. Using standard care regimens for patients in each group, and administering the antiviral agent for patients in the test drug group generally is a good practice. However, the problem is that many of the standard of care protocols contain certain antiviral or potential antiviral agents, such as ribavirin, interferon, etc. It should be OK since there is a control group where no test antiviral is used. However, if the proportions of patients treated with the potential antiviral agents contained in standard of care are not balanced among the groups, which may still cause certain bias in the results. How to solve such problems? The only way is not to recommend listing potential antiviral medicines into standard regimens since the efficacy of them has not been

established.

The use of another antiviral medicine as control, although has been applied in clinical trials and reported in literature, it may raise the questions of uncertainty. For example, if the results show that there are no significant differences in the main and major secondary outcomes between the test drug group and control group, it will be hard to conclude if the test drug is effective or not since so far, except for remdesivir, none of the monotherapy with supposed antiviral drugs is confirmed to have antiviral effect on SARS-CoV-2. Therefore, for clinical trials for evaluation of antiviral drugs against SARS-CoV-2, the control group should not be treated with potential antiviral agent.

In summary, based on the above-mentioned arguments, the WHO SOLIDARITY trial may not rule out the possibility that the tested drugs, especially remdesivir, may have antiviral effects on COVID-19.

Lopinavir-ritonavir

There has been only one RCT report on the efficacy and safety of monotherapy with lopinavir-ritonavir.[26] This study could not obtain any evidence for benefit. However, in the modified intention to treat analysis, after excluding 3 cases who died in the early stage of the disease, the median time to clinical improvement was 15 days vs. 16 days, and this difference was significant according to the authors. An important fact is that the patient population had relatively more severe disease with overall rate of death of this study was 22.1%, while in initial descriptive studies the death rates were between 11% and 14.5%.

This study has a remarkable strength in design, which is virus-related outcome measures included in the secondary outcomes, one was viral RNA titer area-under-the-curve (AUC) (which may represent viral load) and proportions of patients positive for the viral RNA over time. Such outcome measures are essential for clinical trials evaluating efficacy of an antiviral agent, although more resources are required. The authors had to obtain the specimens from all the patients for detection of viral RNA on days 1, 5, 10, 14, 21, and 28. The viral RNA loads and the proportions of patients positive for the viral RNA were similar between groups.

Use of lopinavir-ritonavir was judged by the researchers to be associated with gastrointestinal adverse and serious adverse events, no death was judged to be associated with the test drug. Respiratory failure, acute renal injury and secondary infection were more common in the control group.

This RCT could not provide evidence for shortening the time to clinical improvement, and clearly showed that lopinavir-ritonavir had no antiviral efficacy against the SARS-CoV-2.

Arbidol

Arbidol (umifenovir) was originally developed as an antiviral agent for treatment of influenza and was approved for treatment of influenza, SARS and Lassa viruses in Russia and China.[27] Its mechanism of action was presumed by Vankadari based on analyses on molecular dynamics and structure that SARS-CoV-2 spike glycoprotein is the target of the drug, and the drug can effectively block the trimerization of the SARS-CoV-2 spike glycoprotein, and thereby to prevent adherence to cell and entering into cell.[28] On the other hand, Arbidol efficiently inhibited virus infection *in vitro* in Vero E6 cell culture.[29]

Literature search retrieved 4 original articles on treatment of COVID-19 with Arbidol, none of them was RCT, however. Two of the studies were retrospective case series and cohort study for treatment of COVID-19.[27,30] In Zhu et al's study 50 patients were enrolled totally, and Arbidol group was better in viral load and in duration of positive viral RNA ($P < 0.01$). No apparent side effects were found in any group.[27] The other study enrolled 141 patients; those in the test group were treated with combined Arbidol and interferon (IFN)-α2b and the other group with the IFN alone.[30] Only one outcome, the absorption of pneumonia on CT was in favor of the test group.

The other two studies were retrospective cohort studies and the authors concluded that Arbidol was effective in post-exposure prophylaxis of COVID-19.[31,32] There was no evidence from RCT for Arbidol in either treatment or prophylaxis of COVID-19.

Chloroquine and/or hydroxychloroquine

Both chloroquine (CQ) and hydroxychloroquine (HCQ) are antimalarial and antirheumatic drugs; they had not been regarded as antiviral medicines before the outbreak of the COVID-19. Interestingly, however, as early as 40 years ago, there were studies showing that these drugs had some antiviral activity *in vitro* via different mechanisms, and acting on as many as 12 stages of viral life cycle and related processes although most of the mechanisms were presumed, not confirmed.[33] CQ analogues have been reported to have an *in vitro* nonspecific antiviral effect at high concentration against a number of viruses, and recently against SARS-CoV-2.[34,35]

Since the outbreak of the COVID-19 in late 2019, more than 1700 articles were published on CQ, HCQ and "COVID-19" (PubMed, from December 1, 2019 to October 18, 2020), among which 21 were reports of clinical trials and 15 were RCTs. However, only 5 were RCTs for evaluation of CQ or HCQ in treatment or prevention of COVID-19, and most of the remaining were study protocols.

TABLE 1 Overview of the RCTs for evaluation of efficacy and safety of remdesivir in patients with COVID-19

References	Design and sample size	R dose and course	Primary end point, result	Secondary end points, results	Major adverse events	Limitations
Beigel et al[16,17]	RCT, double blind, P- controlled, multicenter *n* = 1062 (R group 541 vs. P group 521)	200 mg loading on day 1, then 100 mg/d for 9 days	Time to recovery 10 days in R group vs. 15 days in P group (rate ratio for recovery, 1.29; 95% *CI*, 1.12 to 1.49; $P < 0.001$)	Clinical status at day 15 Odds of improvement were higher in R group than in P group (*OR* for improvement, 1.5; 95% *CI*, 1.2 to 1.9); mortality by day 15, 6.7% in R group vs 11.9% in P group (NS)	Adverse events including severe adverse events were similar in 2 groups	No virological outcome was applied; the primary outcome was not mortality or survival
Wang et al[11]	RCT, double blind, P- controlled, multicenter *n* = 237 (R group 158 vs. P group 79)	200 mg on day 1, 100 mg/d on days 2–10 (permitted concomitant use of lopinavir-ritonavir, IFN)	Clinical improvement up to day 28 R use not associated with a difference in time to clinical improvement (hazard ratio 1.23 [95% *CI,* 0.87–1.75])	Clinical improvement was faster in R group whose symptom duration was 10 day or less (NS)	Adverse events were similar between 2 groups	Sample size much smaller than planned; treatment started 12 days after onset of symptom
Spinner et al[21]	Open label, randomized controlled *n* = 596 (10-day R group 197 vs. 5-day R group 199 vs. standard care group 200)	200 mg on day 1, followed by 100 mg/d, for 4, 9 days, or standard care	Clinical status on day 11 Higher odds of a better clinical status in 5-day R group (*OR*,1.65; 95% *CI*, 1.09–2.48; $P = 0.02$); no significant difference in 10-day R group	Death rate was similar among 3 groups	Hypokalemia and headache were more frequent in R group	Changed primary end point; open label; viral load not used; other lab parameter not collected; ordinal scale not ideal

RCTs, randomized controlled trials; COVID-19, 2019 novel coronavirus disease; R, remdesivir; P, placebo; NS, not significant; IFN, interferon; *OR*, odds ratio, *CI*, confidence interval.

TABLE 2 Comparison of the 5 RCTs on chloroquine (CQ) or hydroxychloroquine (HCQ) in treatment of COVID-19

References	Design and sample size	Drug & dose	Main outcome	Efficacy	Safety	Conclusion	Limitations
Borba et al[36]	RCT *n* = 81 (planned 440)	CQ HD: 1200 mg/d, 10 days; LD: 900 mg/d, 1 day, then 450 mg/d, 4 days	Reduction of lethality of HD by at least 50% as compared with LD	Lethality until day 13 was 39.0% in HD group and 15.0% in LD group	QTc prolong: HD (18.9%) vs. LD (11.1%)	HD ineffective, not safe	No placebo or SC control
Cavalcanti et al[37]	RCT *n* = 504	HCQ: 800 mg/d; Azi, 500 mg/d; 7 days	Clinical status at 15 days	No effect on clinical recovery, mean survival similar	QTc prolong and elevated liver enzyme often seen	HCQ or HCQ+Azi did not improve clinical status	AE: HCQ+Azi 39.3%, HCQ 33.7%, control 22.6%
Chen et al[40]	RCT *n* = 30	HCQ 400 mg/d, 5 days	Viral nucleic acid negative conversion rate on days 7 after randomization	Virus cleared in 13 cases in HCQ group vs. 14 cases in control group ($P > 0.05$)	AE did not differ	Conclusion not based on results	Conclusion was not based on results; SC including interferon, Arbidol, lopinavir-ritonavir
Tang et al[38]	RCT, multi-center *n* =150	HCQ 1200 mg/d, 3 days, followed by 800 mg/d, 2–3 weeks	Viral nucleic acid negative conversion rate by 28 days	Similar negative conversion rate in HCQ group vs. SC group (85.4% vs. 81.3%, $P = 0.34$)	AE: HCQ (30%) vs. SC (9%) , $P < 0.001$	No effect on virus clearance, poor safety	16.6 day from onset to enrollment was allowed; AE was high
Boulware et al[39]	RCT, PEP *n* = 821	HCQ 600 mg/d, 4 days	Incidence of lab-confirmed COVID-19 within 14 days	HCQ (11.8%) vs. placebo (14.3%), $P = 0.35$	SE were more common with HCQ group; no SAE recorded	Use of HCQ within 4 days after exposure not effective	Majority of participants were unable to access testing

RCTs, randomized controlled trials; COVID-19, 2019 novel coronavirus disease; CQ, chloroquine; HCQ, hydroxychloroquine; HD, high dose; LD, low dose; Bid, twice daily; QTc, corrected QT interval; Azi, azithromycin; SC, standard care; AE, adverse event; SAE, severe adverse event; PEP, post exposure prophylaxis.

Efficacy

None of the five RCTs and a few retrospective cohort and case series studies could provide evidences for antiviral effects against COVID-19, instead, the studies showed severe adverse events, especially at higher dose and when they were used in combination with azithromycin or oseltamivir. Moreover, use of HCQ was even associated with more deaths of patients (Table 2).[36-39] With regard to antiviral effects on COVID-19 in relation to design, only two out of the 5 RCTs had virological end point. These two studies had a very clear result that the viral RNA negative rates had no significant difference between the test group and control group on day 7[40] and day 28[38], respectively. The primary end point of the study reported by Borba et al[36] was mortality on day 13, which was a good choice, but the mortality on day 13 in high dose group was more than double of the low dose group. However, it is impossible to evaluate if the low dose therapy with CQ was effective or not because the study did not include a control group that was not treated with CQ. This lesson emphasizes the importance of a proper control group. The remaining two RCTs were well-designed, one was for treatment and the other was for post-exposure-prophylaxis, and both were ineffective.

Based on the results of the 5 RCTs in terms of main outcome, it is clear that neither CQ nor HCQ was effective in either treatment or prophylaxis of COVID-19. We should also need to look at the secondary outcomes (clinical status, laboratory examinations, electrocardiogram, imaging, etc.) for efficacy and safety.

In the study of Cavalcanti et al,[37] on day 15, clinical status was not improved either in HCQ (400 mg twice daily) + azithromycin (500 mg/d) or in HCQ alone group. There was no significant differences in any of the secondary outcomes. The primary and secondary outcomes were all similar between the 2 groups in the study of Tang et al.[38]

Safety

In the study of Borba et al,[36] both groups had prolonged QTc interval (18.9% and 11.1% of cases). Creatine kinase increase was seen more frequently in high dose group (50% vs. 31.6%). Two of 37 (2.7%) in the high dose group had ventricular tachycardia before death without torsade de pointes. Overall death rate was 27.2%. Lethality was 39.0% (16/41) in high dose group and 15.0% (6/40) in low dose group. The authors say "No apparent differences despite more deaths in the high dosage group (log-rank, −2.183, $P = 0.03$). (It was very clearly written at the end of "Statistical Analysis" that: "… and a 2-tailed $P < 0.05$ was considered significant".) Side effects were more frequently seen in HCQ group.[37-39]

Strength

The design of the 5 RCTs was generally good, since some were double masked, all were randomized, some were multicenter, and sample size was based on calculation. Two of the RCTs had virological outcome. Primary outcome included virus negative conversion and clinical improvement, and secondary outcome included negative conversion of the virus at days 4, 7, 10, 14, or 21.

Major limitations in methodology

a) The umber of enrolled cases was much smaller than calculated sample size (81 vs 440). b) There was no control group that was treated with a drug other than CQ, i.e., there was neither placebo nor standard care control, therefore, it is impossible to judge if the low dose chloroquine was effective or not. c) There was no virological outcome measure.[36] d) Single center. About 3/4 patients were virologically confirmed COVID-19 cases. e) Standard care included use of IFN, Arbidol, and or lopinavir-ritonavir,[38,40] which probably explains why most patients of both groups became viral RNA negative within 7 days. But it was not mentioned if the use of those was balanced. f) Mean duration from symptom onset to beginning of antiviral therapy was 16.6 days, too long.

Ribavirin

Ribavirin is an old antiviral drug, it is an analog of nucleoside (guanosine), and its main mechanism of antiviral action is similar to that of the other nucleosides/nucleotides. Unlike remdesivir, there are no *in vitro* evidence showing that ribavirin has antiviral effects against the SARS-CoV-2, and its *in vitro* activity against the other SARS-CoV was limited and was observed to have dose-dependent hematologic toxicity, mainly hemolysis, which was seen in over 60% of patients and elevated liver transaminase was seen in 75% of patients. Combined treatment with ribavirin at lower doses and IFN-α2a, or -α2b seemed to be effective with less severe adverse events.[12,41,42] No evidences for the efficacy and safety of ribavirin monotherapy for COVID-19 from RCT or controlled clinical trials are available. Use of lopinavir-ritonavir with ribavirin was not recommended by a panel of experts.[9]

Interferons

Interferons (IFNs) are well-known for their antiviral effects. The mechanisms of antiviral action of IFNs is believed to involve activation of interferon-stimulated genes.[43] Since the outbreak of COVID-19, IFNs have been used in combination with a few other antiviral agents in treatment and clinical trials. However, there is only one randomized controlled clinical trial on evaluation of IFN-β1a monotherapy reported by researchers from Iran.[44] This was an open label RCT on 91 cases. Dose of the IFN was 44 mg subcutaneously injected 3 times a week for 2 weeks. No virologic outcome was applied.

The primary outcome (time to clinical response) was not significantly different between the groups. However, significantly higher discharge rate and lower mortality were seen in IFN group. This study demonstrated that earlier start (before day 10 after symptom onset) of IFN therapy could lower the mortality as compared with later start. For antiviral drugs, the earlier the treatment starts, the less the virus causes lesions and severe lesions in the host. In an uncontrolled case series of 77 patients,[45] use of IFN-α2b with or without Arbidol significantly shortened the duration of virus persistence in the upper respiratory tract and reduced duration of elevated blood levels of inflammatory markers.

Azithromycin

Azithromycin, one of the macrolide antibiotics, should not be considered as an antiviral agent according to the requirements for antivirals mentioned earlier in this review, although it is believed to have certain immunomodulatory effects.[46,47] There are no many clinical reports on the efficacy and safety of azithromycin as a monotherapy for COVID-19. Only 2 RCT reports are available, one was from Brazil;[48] 397 patients constituted the intention-to-treat population; the primary outcome (clinical status at day 15 after randomization, assessed by an independent adjudication committee masked to treatment allocation) and the rate of adverse events were not significantly different between the two groups. This RCT demonstrated that azithromycin (combined in fact with HCQ) could not improve clinical outcome of patients with severe COVID-19. The other report was from Iran;[49] 111 patients with COVID-19 were enrolled and the test group (n = 56) received azithromycin (oral, 500 mg/d) in addition to HCQ. Among the 7 components of the main outcomes, only the SpO_2, respiratory rate and the "duration of admission" (hospitalization) were significantly better in azithromycin group. The mortality on day 15 was not significantly different. The patients received azithromycin had a better general status. Small case series studies showed opposite results for the combined use of HCQ and azithromycin.[50,51] Therefore, there have been no strong evidences from RCTs to support the efficacy of either combined or monotherapy with azithromycin for treatment of COVID-19.

Other potential candidate drugs

Other drugs that are suggested by articles might have potential antiviral effects against SARS-CoV-2 include famotidine, chlorpromazine, and others.

Rosa et al[52] pointed out that repositioning clinical trials possibly represent an attractive strategy because they facilitate the discovery of new classes of medicines at much lower costs and take less time to reach the market; and there are existing pharmaceutical supply chains for formulation and distribution, although such attempts may have certain limitations, and most importantly, these advantages can be utilized only if any of the drug(s) for repurposing is (are) confirmed to have solid clinical efficacy and safety.

Computational analyses performed by Ortega et al[53] showed that famotidine should have inhibitory effect on the protease of SARS-CoV-2, but its affinity to the proteases is low, therefore, famotidine should be tested in combination with some other antiviral agent.

According to a hypothesis based on recent *in vitro* studies on anti-MERS-CoV and anti-SARS-CoV activity (possibly via inhibition of clathrin-mediated endocytosis) of chloropromazine, an old psychotropic drug, and some clinical observation, a group of French researchers have planned to conduct a phase III randomized controlled therapeutic pilot clinical trial to evaluate repurposing for this drug.[54]

There are many other potential candidate drugs and regimens that are either ongoing or have been planned for clinical studies. We wish the studies would be able to provide high quality strong evidences for their antiviral effects.

Efficacy and safety of combined antiviral agents

For combined antiviral therapies against SARS-CoV-2 infection, there have been 3 RCT reports available at PubMed (as of November 15, 2020). The first is a phase 2 clinical trial conducted by Hung et al.[12] All the other available reports in this regard are on retrospective cohort study, case series or case reports. Table 3 shows the overview of the RCT and the retrospective cohort studies. The study reported by Hung et al provides the high quality evidence from the RCT that combination of IFN-β1b, lopinavir-ritonavir and ribavirin was effective in clearance of the viral RNA, clinical improvement and shortening hospital stay of patients with COVID-19.

The other two RCTs are on sofosbuvir and daclatasvir compared with standard care[55] and sofosbuvir, daclatasvir and ribavirin compared with standard of care,[56] and both were conducted in Iran. In both trials the primary endpoints failed to show significant difference between the test group and the control group. However, some secondary endpoints provided evidences to support the efficacy, e.g. Sodeghi et al[55] showed hospital stay was significantly shorter and the rate of discharge was significantly higher in the combined therapy group, and Kasgari et al[56] showed the clinical recovery status was significantly better in the combined treatment group. Addition of ribavirin onto the sofosbuvir and daclatasvir seemed not to have shown any evidences suggesting increased benefit. The limitations of these two trials include: small sample size, no blinding was applied, and from the points of antiviral therapy,

TABLE 3 Comparison of the 5 clinical studies on combined antiviral therapies against COVID-19

References	Design and sample size	Drugs combined	Dose	Main outcome	Efficacy	Safety	Conclusion	Limitations
Hung et al[12]	RCT, open label, mild to moderate cases *n* = 127	INF-β1b + Lop/rit + ribavirin vs. Lop/rit only (control)	INF-β1b: 8 mililion IU/2d, 3 doses; Lop/rit: 400/100 mg/12h; ribavirin 400 mg/12h	Time to negative viral nucleic acid	Significantly shorter time to viral clearance (7 vs. 12 d, $P = 0.001$)	Similar between groups	Safe and superior to control	Open label, without a placebo group, omitted IFN in a subgroup
Sodeghi et al[55]	RCT, open label, multicenter, moderate to severe cases *n* = 66	Sofosbuvir-daclatasvir + santandard care vs. standard care	Sofosbuvir-daclatasvir : 200/60 mg, single daily oral tablet	Clinical recovery within 14 days	88% vs. 67% ($P = 0.07$), shorter hospital stay ($P = 0.027$), higher discharge rate ($P = 0.041$)	3 vs. 5 cases died; no SAE reported	Significantly reduced hospital stay, large study needed	Not blinded, imbalanced rates of use of other antivirals
Kasgari et al[56]	RCT, single center, moderate cases *n* = 48	Sofosbuvir-daclatasvir + ribavirin vs. standard care	Sofosbuvir-daclatasvir: 400/60 mg/d; ribavirin (600 mg, Bid)	Length of hospital stay	Length of hospitalization same; ICU admission and death similar; incidence of recovery higher in combined treatment group ($P = 0.033$)	Not mentioned	There were trends in favor of combined therapy, large RCT needed	Sample size too small; imbalance in baseline features
Deng et al[57]	Retrospective cohort *n* = 33	Arbidol + Lop/rit (both oral) vs. Lop/rit only	Arbidol: 200 mg/8h; Lop/rit: 400/100 mg/12h	Viral negative conversion rate on day 7/14 and day 7 chest CT	Viral RNA negative conversion 12/16 (75%) vs. 6/17 (35%); CT significant better at day 7 after treat ($P < 0.05$ for all)	Good in both groups	Combined therapy was better	Sample size too small, not randomized, retrospective
Xu et al[30]	Retrospective cohort, multicenter *n* = 141	Arbidol + IFN-α2b vs. IFN-α2b only	IFN-α2b: 5×10^5 IU Bid; Arbidol: 200 mg, Tid	Duration of virus RNA	Viral RNA duration similar; CT absorption faster in combined group	Not mentioned	Combined therapy was effective	Retrospective non-randomized control, helpless with virus clearance

RCT, randomized controlled trial; COVID-19, 2019 novel coronavirus disease; IFN, interferon; Lop/rit, lopinavir/ritonavir; IU, international unit; CT, computed tomography; Bid, twice daily; Tid, three times per day; ICU, intensive care unit.

no virological outcomes were applied, no early beginning of antiviral treatment was specifically considered. For the particular combined regimens, we still need large scale, rigorously designed RCTs to confirm their efficacy and safety.

Of the two retrospective cohort studies, Deng et al[57] evaluated combination treatment with Arbidol and lopinavir-ritonavir, and 2 outcomes supported the efficacy of the combined treatment (viral negative conversion at day 7 and day 14, chest CT improvement); safety profile was good in both groups. However, in the study of Xu et al,[30] the main outcome (viral RNA positive duration) failed to show significant difference between the groups, although CT images showed that the absorption of lung lesions was faster in the combined treatment group.

After reading these articles on combined antiviral therapies against SARS-CoV-2, we have a general impression that combined antiviral therapies seem to be more effective as compared to the antiviral monotherapies, although such conclusions must be confirmed only based on high quality evidences from well-designed RCTs.

Summary and conclusions

In summary, so far remdesivir is the only effective and safe antiviral monotherapy for COVID-19 based on evidences from RCT, and combined therapy with IFN-β1b, lopinavir-ritonavir and ribavirin was effective and safe, and weak evidences support efficacy of combined therapy with sofosbuvir and daclatasvir. Five RCTs demonstrated that neither chloroquine nor hydroxychloroquine was effective in treatment of COVID-19, and higher doses showed poor safety. The strengths of the RCTs in design should be applied and the limitations should be taken as lessons for pediatric clinical trials.

CONFLICT OF INTEREST

None of the authors has any conflict of interest concerning this manuscript.

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