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

European Journal of Pharmaceutical Sciences 157 (2021) 105631

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Clinical Outcomes and Plasma Concentrations of Baloxavir Marboxil and Favipiravir in COVID-19 Patients: An Exploratory Randomized, Controlled Trial

Yan Lou [1], Lin Liu [1], Hangping Yao, Xingjiang Hu, Junwei Su, Kaijin Xu, Rui Luo, Xi Yang, Lingjuan He, Xiaoyang Lu, Qingwei Zhao, Tingbo Liang [2], Yunqing Qiu [2,*]

State Key Laboratory for diagnosis and treatment of infectious diseases, Key Laboratory for Drug Evaluation and Clinical Research of Zhejiang Province, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China

ARTICLE INFO

Keywords:
COVID-19
baloxavir marboxil
favipiravir
pharmacokinetics
the half-maximal effective concentration (EC_{50})

ABSTRACT

Background: Effective antiviral drugs for COVID-19 are still lacking. This study aims to evaluate the clinical outcomes and plasma concentrations of baloxavir acid and favipiravir in COVID-19 patients.

Methods: Favipiravir and baloxavir acid were evaluated for their antiviral activity against SARS-CoV-2 in vitro before the trial initiation. We conducted an exploratory trial with 3 arms involving hospitalized adult patients with COVID-19. Patients were randomized assigned in a 1:1:1 ratio into baloxavir marboxil group, favipiravir group, and control group. The primary outcome was the percentage of subjects with viral negative by Day 14 and the time from randomization to clinical improvement. Virus load reduction, blood drug concentration and clinical presentation were also observed. The trial was registered with Chinese Clinical Trial Registry (ChiCTR 2000029544).

Results: Baloxavir acid showed antiviral activity in vitro with the half-maximal effective concentration (EC_{50}) of 5.48 μM comparable to arbidol and lopinavir, but favipiravir didn't demonstrate significant antiviral activity up to 100 μM. Thirty patients were enrolled. The percentage of patients who turned viral negative after 14-day treatment was 70%, 77%, and 100% in the baloxavir marboxil, favipiravir, and control group respectively, with the medians of time from randomization to clinical improvement was 14, 14 and 15 days, respectively. One reason for the lack of virological effect and clinical benefits may be due to insufficient concentrations of these drugs relative to their antiviral activities. One of the limitations of this study is the time from symptom onset to randomization, especially in the baloxavir marboxil and control groups, which is higher than the favipiravir group.

Conclusions: Our findings could not prove a benefit of addition of either baloxavir marboxil or favipiravir under the trial dosages to the existing standard treatment.

Introduction

In December, 2019, several patients with pneumonia of unknown cause were confirmed to be infected with a novel coronavirus, initially named as 2019-nCoV and now named as SARS-CoV-2, in Wuhan, Hubei province, China. The pneumonia was later named as Coronavirus

Abbreviation: EC_{50}, the half-maximal effective concentration; COVID-19, coronavirus disease 2019; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; RT-PCR, real-time fluorescent quantitative PCR; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; C_{min}, the minimum plasma concentration; WBC, white blood cell; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; LDH, lactate dehydrogenase; CPK, creatine phosphohykinase; CRP, C-reactive protein; PCT, procalcitonin; ICU, intensive care unit.

* Correspondence to: State Key Laboratory for diagnosis and treatment of infectious diseases, Key Laboratory for Drug Evaluation and Clinical Research of Zhejiang Province, The First Affiliated Hospital, Zhejiang University School of Medicine, 79 Qingchun Road, Hangzhou 310003, China, Tel: +86 57187236626. Fax: +86 87236626.

E-mail address: qiuyq@zju.edu.cn (Y. Qiu).

[1] Yan Lou and Lin Liu contributed equally to this work.

[2] Yunqing Qiu and Tingbo Liang contributed equally to this work.

https://doi.org/10.1016/j.ejps.2020.105631
Received 14 July 2020; Received in revised form 21 October 2020; Accepted 23 October 2020
Available online 25 October 2020

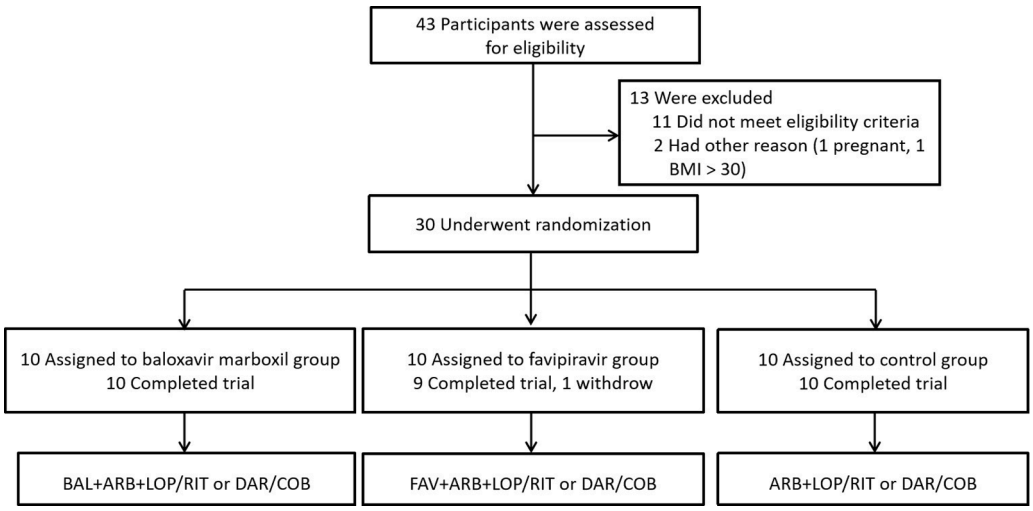

Figure 1. Overview of the Clinical Study. BAL: baloxavir marboxil; FAV: favipiravir; ARB: arbidol; LOP/RIT: lopinavir/ritonavir; DAR/COB: darunavir/cobicistat.

disease 2019 (COVID-19), and soon drew global attention because of the rapidly increasing patient numbers (Chan et al., 2020). As of 17:00 on April 29, 2020, over 3,080,000 cases confirmed in China and other 212 countries. Therefore, the situation is grim for the prevention and control of COVID-19.

Until our study is finished, there is still a lack of effective antiviral drugs for COVID-19. The treatment experience can only draw on the characteristics of other coronaviruses, such as highly pathogenic Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). The antiviral drugs that have been recommended by Diagnosis and treatment program of the novel coronavirus pneumonia (COVID-19) (Trial Sixth Edition,China) include broad-spectrum antiviral drugs (interferon-α, ribavirin), hemagglutinin inhibitors (arbidol), protease inhibitors (lopinavir/ritonavir), and chloroquine phosphate. The latest data shows that some antiviral drugs, including favipiravir, remdesivir, chloroquine phosphate, have inhibitory effect against SARS-CoV-2 in vitro (Wang et al., 2020). However, due to the limited clinical experience of using these drugs in COVID-19 patients, inadequate understanding of their mechanism of action against SARS-CoV-2, the antiviral drugs currently in use need more in-depth research basis in clinical application for COVID-19, and their potential efficacy against the SARS-CoV-2 virus has been controversial.

Baloxavir marboxil and favipiravir are novel inhibitors of the influenza RNA replication process by targeting different protein subunits of the influenza polymerase complex. Briefly, Baloxavir marboxil is prodrug and baloxavir acid is its active metabolite. Baloxavir acid inhibits cap-dependent endonuclease (Hayden et al., 2018), and favipiravir inhibits polymerase basic protein 1 (Hayden and Shindo, 2019). Since the SARS-CoV-2 and the influenza virus are both RNA viruses, baloxavir acid and favipiravir are considered to be potentially effective against SARS-CoV-2 by blocking its RNA synthesis. Meanwhile, we found that baloxavir acid and favipiravir have antiviral activity against SARS-CoV-2 in vitro. Moreover, favipiravir is a small purine analogue and converted into its active ribofuranosyl 5'-triphosphate metabolite in the cell, which can be incorporated in the growing RNA strand (Abdelnabi et al., 2017). The antiviral activity of favipiravir in vivo may be stronger than that in vitro. Based on the existing treatment experience and related theoretical basis, we decided to study their clinical efficacy in the treatment of COVID-19.

In this study, in vitro activities of antiviral drugs against SARS-CoV-2 were screened firstly. Then, an exploratory single center, open-label, randomized, controlled trial was conducted to evaluate the efficacy and safety of adding baloxavir marboxil or favipiravir to the current standard antiviral treatment in patients confirmed as COVID-19 who are still positive for the SARS-CoV-2 (ChiCTR2000029544). We also measured the plasma concentrations of these antiviral drugs, compared them to the half-maximal effective concentration (EC_{50}) values.

Methods

In Vitro Antiviral Assay Against SARS-CoV-2

The antiviral activity was evaluated by quantifying the virus yield in the supernatants of infected cell after treatment by qRT-PCR. For additional detailed operation steps, please refer to Supplementary Material (appendix p1).

Study Design

This trial was an exploratory single center, open-label, randomized, controlled trial to evaluate the efficacy and safety of adding baloxavir marboxil or favipiravir to the current standard antiviral treatment in patients confirmed as COVID-19 who are still positive for the SARS-CoV-2 (ChiCTR2000029544). Participants confirmed as COVID-19 infection were enrolled to the study after approval by the ethics committees. SAS software was used to generate the random number and the treatment group corresponding to the random number. After the subjects passed screening, the researchers assigned the random number according to the order of enrollment, removed the random envelope according to the random number, and treated the subjects according to the random envelope group and treatment plan. Because the epidemic situation of COVID-19 was serious, in order to promote the research, it was too late to make double-blind and double simulation preparations. Therefore, the blind method was not used in this clinical trial.

The blind method is not suitable for this trial. Figure 1 shows an overview of this trial. The trial was initiated on February 3, 2020, and the data were collected in The First Affiliated Hospital, Zhejiang University School of Medicine. This study was approved by the Ethics Committee of the First Afliated Hospital, Zhejiang University School of Medicine (2020 llT-7). Specific inclusion and exclusion criteria are listed in Supplementary Material (appendix p3).

Drug Administration

All patients start the recommended antiviral treatment (lopinavir/ritonavir, darunavir/cobicistat or arbidol, described below) immediately after the admission diagnosis and received standard care. The trial treatment scheme started as soon as consent was obtained (Day 1). The specific administration is as follows: (1) Baloxavir marboxil group: baloxavir marboxil is used in combination with the existing antiviral

treatment (described below). The dose was 80 mg once a day orally on Day 1 and Day 4; for patients who are still positive in virological test, they can be given again on Day 7; (2) Favipiravir group: favipiravir was used in combination with the existing antiviral treatment. The first dose was 1600 mg or 2200mg orally, followed by 600 mg each time, three times a day, and the duration of administration was not more than 14 days; (3) Control group: Continue the existing antiviral treatment.

The existing antiviral treatment included lopinavir/ritonavir (400 mg/100 mg, bid, po.) or darunavir/cobicistat (800 mg/150 mg, qd, po.) and arbidol (200 mg, tid, po.). All of them were used in combination with interferon-α inhalation (100,000 iu, tid or qid).

Outcomes

Primary and Secondary Outcomes

The primary efficacy endpoint was the percentage of subjects with viral negative by Day 14 and the time from randomization to clinical improvement, defined as the time from randomization to an improvement of two points (from the status at randomization) on a seven-category ordinal scale or live discharge from the hospital, whichever came first. The ordinal scale, which is referred to National Early Warning Score 2 (NEWS2), have been used as endpoints in clinical trials in patients hospitalized with COVID-19 (Cao et al., 2020). Secondary clinical endpoints included the percentage of subjects with viral negative by Day 7, the incidence of mechanical ventilation by Day 14, ICU admission by Day 14, and all-cause mortality by Day14.

Viral Negativity

SARS-CoV-2 (molecular viral load) was immediately assessed at the hospital laboratory using a semi-quantitative RT-PCR assay (Light Cycler 480 real-time fluorescent quantitative PCR, Roche). The results are expressed in terms of Ct, whose value is inversely proportional to viral load.

Drug Concentration Measurement

Blood samples were collected less than one hour after the first dose of favipiravir or baloxavir marboxil on Day 1, then just before dosing on the Day 4 and the Day 7. All samples were immediately shipped to the biosafety level 3 laboratory in our hospital. In this laboratory, the blood samples were heated at 60℃ for one hour to inactivate SARS-CoV-2, which did not significantly affect the quantification of these six compounds in plasma (appendix p3). Plasma samples were prepared, frozen at -20℃ and transferred to another laboratory in our hospital for drug concentration measurement. Plasma concentrations of baloxavir acid, arbidol, lopinavir, ritonavir and darunavir were determined simultaneously using a validated liquid chromatography-tandem mass spectrometry method which was firstly established in this study (appendix p5). Favipiravir plasma concentrations were analyzed using the liquid chromatography (appendix p12).

Statistical Analysis

Cox proportional hazards model was used to investigate the effect of covariates at baseline (age, days from system onset to randomization, CRP and viral load) on the primary outcome.

Results

In Vitro Antiviral Activity Against SARS-CoV-2

The activity against SARS-CoV-2 was tested in vitro for the antiviral drugs used in this trial, including arbidol, ritonavir, lopinavir, darunavir, baloxavir acid, and favipiravir. Among the six tested drugs, three drugs showed measurable activity against SARS-CoV-2. Based on the results of non-linear regression fitting, EC_{50} against SARS-CoV-2 was estimated to be 3.32, 5.48, and 10.4 μM, for arbidol, baloxavir acid, and lopinavir, respectively (Table S1). The antiviral activity of favipiravir was not as effective as observed in a previous study in which an EC_{50} value of 61.88 μM was reported (Wang et al., 2020), and SARS-CoV-2

Table 1
Baseline characteristics of patients with 2019-nCoV infection (COVID-19)

Characteristic	Baloxavir marboxil Group	Favipiravir Group	Control Group	Total
N	10	9	10	29
Age (year) -mean (SD)	53.5 (12.5)	58.0 (8.1)	46.6 (14.1)	52.5 (12.5)
Male sex - no. (%)	7 (70)	7 (77)	7 (70)	21 (72.4)
Days from symptom onset to randomization-mean (SD)	12.7 (3.5)	8.5 (3.7)	13.6 (4.6)	11.7 (4.4)
Comorbidity- no. (%)				
Diabetes	0	2(22)	0	2 (6.9)
Hypertension	2 (20)	1 (11)	3(30)	6 (20.7)
Hyperlipidemia	0	0	1 (10)	1 (3.4)
Cardiovascular disease	3 (30)	1(11)	0	4 (13.8)
NEWS2 score- median (IQR)	4 (4-5)	4 (4-5)	4 (4-5)	4 (4-5)
Ct value- median (IQR)	28.2 (22.1-36.8)	25.4 (19.8-36.0)	29.6 (19.8-37.1)	28.2 (19.8-37.1)
Body temperature (°C) -median (IQR)	36.9 (36.2-38.4)	36.9 (36.3-39.6)	36.9 (36.0-37.9)	36.9 (36.0-39.6)
Fever - no. (%)	1 (10)	3 (33)	2 (20)	6 (20.7)
Respiratory rate >24/min - no. (%)	0	1(11)	0	1 (3.4)
Serum biochemistry - median (IQR)				
Haemoglobin (g/dl)	133 (118-153)	141 (127-156)	133 (89-155)	138 (89-156)
Total peripheral WBC count (*10E9/l)	8.3 (3.3-27.9)	7.8 (3.9-14.1)	6.3 (2.9-19.4)	7.5 (2.9-27.9)
Lymphocyte count (*10E9/l)	0.6 (0.2-2.1)	0.9 (0.3-1.6)	0.8 (0.3-1.6)	0.7 (0.2-2.1)
Platelet count (*10E9/l)	174 (108-459)	206 (82-281)	199 (97-347)	(82-459)
ALT (U/l)	22 (10-72)	21 (8-148)	27 (12-76)	22 (8-148)
AST (U/l)	19 (9-44)	17 (12-77)	16 (12-44)	17 (9-77)
Albumin (g/l)	34.8 (28.8-43.6)	37.8 (29.7-43.0)	34.9 (31.1-44.6)	36.8 (28.8-4.6)
Creatinine (μmol/l)	67 (54-83)	76 (65-104)	82 (54-91)	76 (54-104)
LDH (U/l)	265 (219-370)	252 (174-323)	307 (142-358)	249 (142-370)
CPK (U/l)	63 (20-223)	50 (34-117)	43 (22-186)	53 (20-223)
CRP (mg/l)*	14.1 (0.65-50.9)	27.3 (0.32-79.9)	2.1 (0.32-26.4)	10.6 (0.32-79.9)
PCT (ng/ml) ,†	0.06 (0.03-0.3)	0.07 (0.02-0.1)	0.05 (0.02-0.1)	0.06 (0.02-0.3)

WBC = white blood cell; ALT = alanine aminotransferase; AST= aspartate aminotransferase; LDH = lactate dehydrogenase; CPK = creatine phosphohykinase; CRP = C-reactive protein; PCT = procalcitonin

* Four missing values (two in Baloxavir marboxil group, two in Favipiravir group).

† Five missing values (two in Baloxavir marboxil group, two in Favipiravir group, one in Control group).

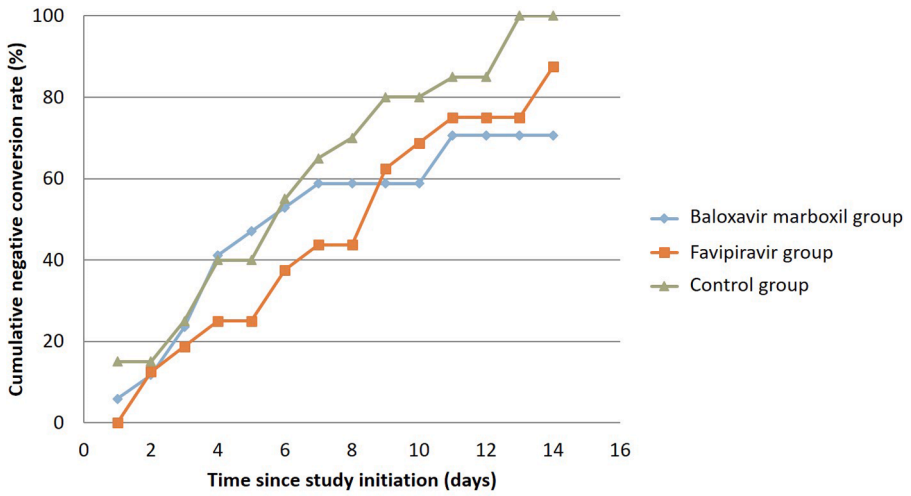

Figure 2. Cumulative negative conversion rates of subjects by Day 14. The x-axis represents the time (days) since trial initiation. The y-axis represents cumulative negative conversion rat.

was inhibited by less than 50% at concentrations up to 100 μM, the highest concentration tested in antiviral assay. Similarly, ritonavir and darunavir did not show antiviral activity against SARS-CoV-2. Furthermore, all the tested drugs had a toxic effect on cells at high concentration but did not show cytotoxicity at the effective concentrations. Together, these results indicate that arbidol, baloxavir acid, and lopinavir could be potential candidates for clinical treatment against COVID-19.

Baseline Characteristics of the Patients

Thirty patients were enrolled in the trial and were randomized into baloxavir marboxil group, favipiravir group, and a control group (Figure 1). One patient in the favipiravir group was subsequently excluded from the final analysis because of his personal refusal to continue to use favipiravir after Day 1. The remaining 29 patients were included in the analysis. The baseline characteristics are presented in Table 1. All these patients had no previous history of malignant, chronic obstructive pulmonary disease (COPD), renal insufficiency, and hepatic insufficiency, and there were no deaths during the trial.

The demographic characteristics, Ct value, and initial serum biochemistry were balanced in the three groups. Figure 1 shows that although the COVID-19 patients were treated with various existing standard antiviral treatments following the recommendation by the National Health Commission of People's Republic of China (Trial Sixth Edition), in this trial, the three groups of patients received similar antiviral treatment.

Clinical Outcomes

Primary Outcome

A total of 24 (82.8%) patients turned viral negative (defined as two consecutive tests with viral RNA undetectable results) within 14 days after the initiation of the trial. The percentage of patients who turned viral negative after 14-day treatment was 70%, 77%, and 100% in the baloxavir marboxil, favipiravir, and control group respectively, of which the control group was higher than that of the other two treatment groups (Figure 2). The medians of time from randomization to clinical improvement were 14, 14 and 15 days in the baloxavir marboxil, favipiravir, and control group, respectively (Table 2).

Cox proportional hazard model was used. Age, days from systemic onset to randomization, CRP and viral load at baseline were used as covariates, and virus negative was used as the endpoint. Firstly, the schoenfeld residual method was used to analyze whether the covariates satisfied the proportional hazard assumption. The results showed that the days from system onset to randomization didn't meet the proportional hazards assumption. Thus Time-Dependent Cox Proportional Hazards model was conducted. The results showed that all covariates were not associated with the time for the virus to turn negative (the p values of CRP, age, days from system onset to randomization and Ct value were 0.369, 0.657, 0.854, 0.175, respectively) (Table S2).

Secondary Outcomes

A total of 15 (51.7%) patients turned viral negative within 7 days after the initiation of the trial (60%, 44% and 50% in the baloxavir marboxil, favipiravir, and control group, respectively). One patient in the baloxavir marboxil group, and two patients in the favipiravir group were transferred to ICU within seven days after trial initiation. The reason for these patients transferred to ICU is that the oxygenation index continues to decline under the condition of high-throughput oxygen inhalation (<100 mmhg), or the transverse chest computed tomograms showed that the disease progressed rapidly (bilateral ground glass opacity and consolidation aggravated). The patient in baloxavir marboxil group was treated with ECMO ten days after trial initiation. There was no death. Clinical outcomes were showed in Table 2. Together, these results suggest that the addition of either baloxavir marboxil or favipiravir under the current dosage to the exiting standard treatment did not provide additional benefits to the clinical outcome in this study.

Viral Negativity

Throughout the trial, viral load was monitored everyday for each patient. Figure 3 shows the kinetics of the viral load in the three groups of patients. These results indicate that the addition of either baloxavir marboxil or favipiravir didn't appear to improve the median T1/2 time for patients to achieve undetectable viral RNA compared to the control group.

Drug Concentrations in COVID-19 Patients

To determine whether the apparent lack of benefits by the addition of either baloxavir marboxil or favipiravir is related to their pharmacological exposure in the COVID-19 patients, drug concentrations were measured in the patients. Overall, 70 plasma samples were collected, 19 samples on Day-1, 28 samples on Day-4, and 23 samples on Day-7. Among these samples, 28 contained baloxavir acid, 20 contained favipiravir, and other samples contained arbidol, lopinavir, ritonavir, and darunavir were detected by UPLC-MS/MS. Administered orally, favipiravir is rapidly absorbed with a t_{max} ranging from 0.5 to 1 hour (Nguyen et al., 2017). Favipiravir total concentration was measured at Day-2 and Day-4 from plasma samples collected before the first

Table 2
Clinical Outcomes of patients with 2019-nCoV infection (COVID-19)*

	Baloxavir marboxil Group (n=10)	Favipiravir Group (n=9)	Control Group (n=10)	Total (n=29)
Primary Outcome				
Viral negative in Day 14 - no. (%)	7 (70)	7 (77)	10 (100)	24 (83)
Time to clinical improvement - median no. of days (IQR)	14 (6-49)	14 (6-38)	15 (6-24)	14 (6-49)
Secondary Outcomes				
Viral negative in Day 7 - no. (%)	6 (60)	4 (44)	5 (50)	15 (52)
Incidence of mechanical ventilation - no. (%)	1 (10)	0	0	1 (3)
Transfer to ICU in Day 14 - no. (%)	1 (10)	2 (22)	0	3 (10)
Other Clinical Outcomes				
Time to viral negative-median no. of days (IQR)	6 (1-46)	9 (2-34)	9 (1-13)	7 (1-46)
Clinical improvement - no. (%)				
Day 14	6 (60)	5 (55)	5 (50)	16 (55)
Day 7	1 (10)	2 (22)	1 (10)	4 (14)
Oxygen support - days (IQR)	13 (3-41)	13 (3-37)	12 (5-23)	12 (3-41)
Score on seven-category scale at day 14 - no.of patients (%)				
2: Not hospitalized, but unable to resume normal activities	6 (60)	4 (44)	4 (40)	14 (48)
3: Hospitalization, not requiring supplemental oxygen	0	2 (22%)	2 (20)	4 (14)
4: Hospitalization, requiring supplemental oxygen	3 (30)	3 (33)	4 (40)	10 (34)
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation	0	0	0	0
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both	1(10)	0	0	0
Changes of Ct value compared with Day1- median (IQR)				
Day14†	6.1 (-5.0-8.1)	9.2 (2.0-13.9)	11.4 (0.9-13.6)	7.1 (-5.0-13.9)
Day7	4.7 (-13.8-13.0)	6.7 (-3.4-13.9)	5.5 (-9.7-15.1)	5.6 (-13.8-15.1)

* Clinical improvement was defined as a decline of two categories on the modified seven-category ordinal scale of clinical status, or hospital discharge. The seven-category ordinal scale consisted of the following categories: 1, not hospitalized with resumption of normal activities; 2, not hospitalized, but unable to resume normal activities; 3, hospitalized, not requiring supplemental oxygen; 4, hospitalized, requiring supplemental oxygen; 5, hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 6, hospitalized, requiring ECMO, invasive mechanical ventilation, or both; and 7, death.

† Patients who have turned negative on Day 7 were not recalculated.ICU= intensive care unit.There was no death.

favipiravir intake of the day in Ebola-infected patients (Nguyen et al., 2017). As a result, the sampling time was 1-2 hour before dose administration for the Day-4 and Day-7 samples. The plasma concentrations in Day-4, and Day-7 samples are listed in Table S11. The total plasma concentration of favipiravir was 11.8 ± 11.6 μg/mL in Day-4 samples, and 21.8 ± 28.7 μg/mL in Day-7 samples (Table S11). With a plasma protein binding of 54%, the calculated free favipiravir trough concentrations on Day-7 was close to the in vitro EC_{50} value of 9.7 μg/mL reported in G.X et al. (Wang et al., 2020), but lower than the EC_{50} value (>15.7 μg/mL) determined in our study.

Following absorption, baloxavir marboxil is almost entirely converted to its active metabolite, baloxavir acid. The observed concentrations of baloshavir at Day-4 and Day-7 were 0.024 ± 0.019 μg/mL and 0.020 ± 0.013 μg/mL, respectively. The concentrations of baloxavir acid were much lower than its EC_{50} value of 2.65 μg/mL (5.48 μM), which is consistent with the lack of viral inhibition by this compound.

The plasma concentrations of arbidol, lopinavir, ritonavir and darunavir were shown in Table S11.

Adverse Events

Respiratory failure occurred in 14 patients (Table 3). Other adverse events were generally mild or moderate among the three Groups. The most frequent adverse events occurring in the study population were similar among all groups, including elevation of triglyceride (20 events, Figure S4 in appendix p19), liver function abnormality (18 events, Figure S5 in appendix p20), rash (7 events), and diarrhea (4 events) (Table 3). No abnormal serum creatinine was found in all patients.

Discussion

In this study, we assessed the efficacy and safety of baloxavir marboxil and favipiravir in 29 COVID-19 patients who were still virus positive under the current antiviral treatment according to the recommendation of Diagnosis and treatment program of novel coronavirus pneumonia (COVID-19) (Trial Sixth Edition). The results of viral negativity, clinical symptoms, and laboratory tests indicated adding either baloxavir marboxil or favipiravir to the current standard treatment did not provide additional benefits to the clinical outcome in this clinical study. Adverse events were generally mild or moderate with no differences in frequency or severity among the three groups. No moderate or severe drug-related unsolicited adverse events were reported during the trial.

Furthermore, in order to provide the pharmacological rationale of drug antiviral activities in vivo, the drug exposure of baloxavir acid, favipiravir, arbidol, lopinavir, ritonavir, and darunavir were also measured. The drug concentrations of favipiravir in our study were slightly lower than that of Ebola patients (Nguyen et al., 2017), which may be caused by different dosage. The exposure of baloxavir acid and lopinavir/ritonavir in these COVID-19 patients were similar as that in influenza (Abraham et al., 2020) or HIV-infected patients (Moltó et al., 2007) which reported in the previous studies. However, the pre-dose drug concentrations of abidol in this study were 2-3 times higher than the steady-state concentrations of $C_{min,ss}$ (0.17 μg/mL) in healthy Chinese volunteers (Sun et al., 2013), which may be due to the drug-drug interaction between abidol and ritonavir or cobicistat based on CYP3A4 (Deng et al., 2013). Similarly, comparing the minimum plasma concentration (C_{min},1.3 μg/mL) of darunavir in HIV-1-infected adults (Tashima et al., 2014), the plasma concentration in this study were much higher. Although, the results from this trial showed that the calculated free drug concentrations of these five antiviral drugs were generally lower than their respective EC_{50} values. The latest published clinical trial report that patients with severe COVID-19 had no benefit from lopinavir-ritonavir treatment (Cao et al., 2020), which to some extent confirms our view.

Our findings do not support adding either baloxavir marboxil or favipiravir under the current dosage as antiviral agents to the existing standard treatment in COVID-19 patients. However, this conclusion should be taken with caution for several reasons. In this study, the analysis relied only on plasma concentrations and in vitro antiviral activity against SARS-CoV-2, while intracellular concentrations of the active phosphorylated moiety were not available. For favipiravir, the intracellular concentrations of the active metabolite has been shown to be associated with antiviral efficacy, instead of the plasma concentrations of the parent molecule (Bazzoli et al., 2010). There are many

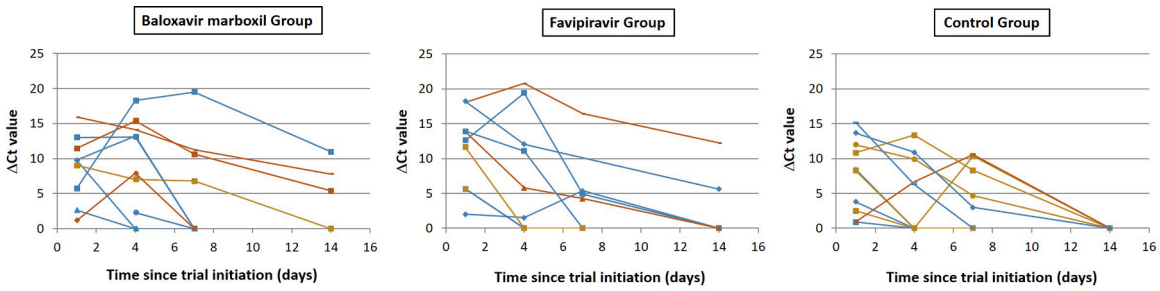

Figure 3. Changes in Ct values since trial initiation. The x-axis represents the time (days) since trial initiation. The y-axis represents ΔCt value (38 minus the RT-PCR Ct value, with 38 being the Ct cutoff value for positivity). Each line represents one patient. Patients are coded according to clinical classification of COVID-19, with red line representing critical cases, blue line representing severe cases, and gold line representing moderate cases. Clinical classifications were made from discharge diagnosis.

Table 3
Summary of Adverse Events in the Safety Population

	Baloxavir marboxil Group (n=10) number (percent)	Favipiravir Group (n=9)	Control Group (n=10)
Any adverse event			
Respiratory failure or ARDS	6 (60)	4 (44)	4 (40)
Lymphopenia	8 (80)	7 (77)	7 (70)
Leukopenia	2 (20)	1 (11)	1 (10)
Decreased hemoglobin	8 (80)	7 (77)	6 (60)
Increased aspartate aminotransferase	3 (30)	1 (11)	3 (30)
Increased alanine aminotransferase	7 (70)	4 (44)	6 (60)
Increased total bilirubin	2 (20)	1 (11)	1 (10)
Decreased albumin	10 (100)	8 (88)	9 (90)
Increased creatine phosphohykinase	0	1 (11)	0
Increased lactate dehydrogenase	7 (70)	5 (55)	8 (80)
Increased triglyceride	6 (60)	6 (66)	8 (80)
Increased D-dimmer	7 (70)	5 (55)	5 (50)
Diarrhea	1 (10)	2 (22)	1 (10)
Rash	1 (10)	1 (11)	5 (50)
Nausea	0	1 (11)	0
Anemia	1 (10)	0	0

Adverse events that occurred in more than 1 patient after randomization through day 14 are shown. Some patients had more than one adverse event. There was no death in the trial. ARDS indicates acute respiratory distress syndrome.

reasons for the failure of viral load reduction, some of which may be related to the pharmacology of drugs. We are not sure if these drugs will appear in respiratory secretions, or if they are not so effective against SARS-CoV-2.

According to the data obtained from the previous and current clinical trial, the safety of fapiravir was relatively reliable, but its exact efficacy for COVID-19 has not been confirmed. It was showed significantly better treatment effects on COVID-19 in terms of disease progression and viral clearance versus lopinavir/ritonavir in an open-label nonrandomized control study in China (Cai et al., 2020). However, in a prospective, randomized, controlled by arbidol, open label multicenter trial in China (Chen et al., 2020), among the 240 patients with COVID-19, favipiravir did not significantly improve the clinically recovery rate at Day 7 compared to Arbidol. Similar negative results are also reflected in a clinical trial for critical ill patients with COVID-19 in Japan (Irie et al., 2020), fapiravir concentrations in critically ill patients were much lower than that in healthy volunteers, and the targeted favipiravir concentration was not reached (Irie et al., 2020). These results are consistent with our findings in this study.

There are some limitations to the current study. Firstly, the subjects were all under treatment with other medication. The treatment scheme and medication time before the initiation of the trial were different among the patients, which makes their progression of the disease at the beginning of the trial quite different. There was therefore a risk of influencing the results and conclusions. During a rapidly evolving COVID-19 situation, it was difficult to obtain large number of newly detected cases without previous treatment. Second, the poor correlation could be due to the delay between infection and treatment initiation. Viral dynamic modelling shows that a drug affecting viral replication, will only have a limited impact on viraemia if treatment is initiated after the viraemia peak, regardless of drug efficacy (Madelain et al., 2015). Since the subjects of this trial were those who were still positive after the treatment of the recommended scheme, the optimum time to start antiviral therapy may have been missed. The time from symptom onset to randomization was long in these patients, especially in the baloxavir marboxil (12.7 ± 3.5 d) and control groups (13.6 ± 4.6 d), which is later than the favipiravir group (8.5 ± 3.7 d). Third, patients in favipiravir group showed oldest average age and shortest time from symptom onset to randomization, even though, the clinical performance of favipiravir group was not inferior to the other two groups. However, we are not sure if the efficacy of favipiravir under current dosage is underestimated because its drug exposure does not reach the EC_{50} value. Fourth, the relatively small sample size of our study poses an additional limitation. Nevertheless, it was conclusive that the calculated free plasma concentrations of these antiviral drugs did not reach their respective EC_{50} values, which can be almost certainly that the drugs have no effect against SARS-CoV-2 at the dose as mentioned above. Our research cannot be simply regarded as negative results, as it is very meaningful for clinical treatment of COVID-19 in global outbreak. In addition, our exploratory research provides useful information for further studies to find the best strategy for application of these drugs.

Conclusion

This exploratory trial does not prove a benefit of addition of baloxavir marboxil in COVID-19 patients, because the calculated free drug concentration of baloxavir acid is far below than its EC_{50} values (more than 100 times). Under the current dosage, the insufficient exposure of favipiravir also resulted in no additional antiviral benefit by adding favipiravir to the existing standard treatment. Administration of favipiravir at different dosage or at different stages of COVID-19 (for example, early stage) deserves further study. Additional studies are needed to confirm the no clinical benefit from the current standard treatment drugs.

Supplementary Data: Supplementary materials are available online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

CRediT authorship contribution statement

Yan Lou: Writing - original draft. **Lin Liu:** Writing - original draft. **Hangping Yao:** Visualization, Investigation. **Xingjiang Hu:** Methodology, Data curation. **Junwei Su:** Visualization, Investigation. **Kaijin Xu:** Visualization, Investigation. **Rui Luo:** Visualization, Investigation. **Xi Yang:** Methodology, Data curation. **Lingjuan He:** Software, Validation. **Xiaoyang Lu:** Software, Validation. **Qingwei Zhao:** Supervision. **Tingbo Liang:** Writing - review & editing. **Yunqing Qiu:** Conceptualization.

Declaration of Competing Interest

We declare no competing interests.

Acknowledgments

This article was supported by Zhejiang Provincial Science and technology department key R & D plan emergency project (No. 2020c03123-8). We acknowledge Dr. Chengyong Yang and Dr. Guofeng Cheng for their technical support to this study. Dr. Yang and Dr. Cheng were given the opportunity to review the manuscript for medical and scientific accuracy. This manuscript has been released as a pre-print at medRxiv (Lou et al., 2020).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105631.

References

Chan, J.F., Yuan, S., Kok, K.H., et al., 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395, 514–523. https://doi.org/10.1016/S0140-6736(20)30154-9.

Wang, M., Cao, R., Zhang, L., et al., 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 30, 269–271. https://doi.org/10.1038/s41422-020-0282-0.

Hayden, F.G., Sugaya, N., Hirotsu, N., et al., 2018. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. N Engl J Med 379, 913–923. https://doi.org/10.1056/NEJMoa1716197.

Hayden, F.G., Shindo, N., 2019. Influenza virus polymerase inhibitors in clinical development. Curr Opin Infect Dis 32, 176–186. https://doi.org/10.1097/QCO.0000000000000532.

Abdelnabi, R., Morais, ATS., Leyssen, P., et al., 2017. Understanding the Mechanism of the Broad-Spectrum Antiviral Activity of Favipiravir (T-705): Key Role of the F1 Motif of the Viral Polymerase. J Virol 91 (12). https://doi.org/10.1128/jvi.00487-17.

Nguyen, T.H., Guedj, J., Anglaret, X., et al., 2017. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted. PLoS Negl Trop Dis 11 (2), e0005389. https://doi.org/10.1371/journal.pntd.0005389.

Abraham, G.M., Morton, J.B., Saravolatz, L.D., 2020. Baloxavir: A Novel Antiviral Agent in the Treatment of Influenza. Clin Infect Dis. https://doi.org/10.1093/cid/ciaa107.

Moltó, J., Valle, M., Blanco, A., et al., 2007. Lopinavir/ritonavir pharmacokinetics in HIV and hepatitis C virus co-infected patients without liver function impairment: influence of liver fibrosis. Clin Pharmacokinet 46, 85–92. https://doi.org/10.2165/00003088-200746010-00005.

Sun, Y., He, X., Qiu, F., et al., 2013. Pharmacokinetics of single and multiple oral doses of arbidol in healthy Chinese volunteers. Int J Clin Pharmacol Ther 51, 423–432. https://doi.org/10.5414/CP201843.

Deng, P., Zhong, D., Yu, K., et al., 2013. Pharmacokinetics, metabolism, and excretion of the antiviral drug arbidol in humans. Antimicrob Agents Chemother 57, 1743–1755. https://doi.org/10.1128/AAC.02282-12.

Tashima, K., Crofoot, G., Tomaka, F.L., et al., 2014. Cobicistat-boosted darunavir in HIV-1-infected adults: week 48 results of a Phase IIIb, open-label single-arm trial. AIDS Res Ther 11, 39. https://doi.org/10.1186/1742-6405-11-39.

Cao, B., Wang, Y., Wen, D., et al., 2020. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. https://doi.org/10.1056/NEJMoa2001282.

Bazzoli, C., Jullien, V., Le Tiec, C., 2010. Intracellular Pharmacokinetics of Antiretroviral Drugs in HIV-Infected Patients, and their Correlation with Drug Action. Clin Pharmacokinet 49, 17–45. https://doi.org/10.2165/11318110-000000000-00000.

Madelain, V., Oestereich, L., Graw, F., et al., 2015. Ebola virus dynamics in mice treated with favipiravir. Antiviral Res 123, 70–77. https://doi.org/10.1016/j.antiviral.2015.08.015.

Cai, Q., Yang, M., Liu, D., et al., 2020. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering (Beijing). https://doi.org/10.1016/j.eng.2020.03.007.

Chen, C., Huang, J.Y., Yin, P., 2020. Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. medrxiv. 10.1101/2020.03.17.20037432.

Irie, K., Nakagawa, A., Fujita, H., et al., 2020. Pharmacokinetics of Favipiravir in Critically ill Patients with COVID-19. Clin Transl Sci. https://doi.org/10.1111/cts.12827.

Lou, Y., Liu, L., Yao, H.P., et al. 2020. Clinical Outcomes and Plasma Concentrations of Baloxavir Marboxil and Favipiravir in COVID-19 Patients: An Exploratory Randomized, Controlled Trial. medrxiv. 10.1101/2020.04.29.20085761.