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.

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Multicenter, single-blind, randomized controlled study of the efficacy and safety of favipiravir and nafamostat mesilate in patients with COVID-19 pneumonia

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ABSTRACT

Objectives: To evaluate the efficacy and safety of nafamostat combined with favipiravir for the treatment of COVID-19.

Methods: We conducted a multicenter, randomized, single-blind, placebo-controlled, parallel assignment study in hospitalized patients with mild-to-moderate COVID-19 pneumonia. Patients were randomly assigned to receive favipiravir alone (n = 24) or nafamostat with favipiravir (n = 21). The outcomes included changes in the World Health Organization clinical progression scale score, time to improvement in body temperature, and improvement in oxygen saturation (SpO_2).

Results: There was no significant difference in the changes in the clinical progression scale between nafamostat with favipiravir and favipiravir alone groups (median, -0.444 vs -0.150, respectively; least-squares mean difference, -0.294; $P = 0.364$). The time to improvement in body temperature was significantly shorter in the combination group (5.0 days; 95% confidence interval, 4.0-7.0) than in the favipiravir group (9.0 days; 95% confidence interval, 7.0-18.0; $P = 0.009$). The changes in SpO_2 were greater in the combination group than in the favipiravir group (0.526% vs -1.304%, respectively; least-squares mean difference, 1.831; $P = 0.022$). No serious adverse events or deaths were reported, but phlebitis occurred in 57.1% of the patients in the combination group.

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Conclusion: Although our study showed no differences in clinical progression, earlier defervescence, and recovery of SpO_2 were observed in the combination group.

Introduction

The outbreak of COVID-19 caused by the SARS-CoV-2 first occurred in late 2019, and the struggle against the COVID-19 pandemic is still ongoing. Although an entry mechanism of SARS-CoV-2, such as binding to angiotensin-converting enzyme 2 at the first step and process cell fusion by transmembrane protease serine 2 (TMPRSS2), was known [1], no established treatment exists before this study was conducted. To date, four antiviral drugs, remidesivir, molnupiravir, ensitrelvir, and nirumatrelvir/ritonavir, have been approved for COVID-19 therapy in Japan; however, only remdesivir has shown clinical benefits for hospitalized patients [2].

Nafamostat, a serine protease inhibitor, specifically acts on TMPRSS2 and inhibits entry of the Middle East respiratory syndrome coronavirus and SARS-CoV-2 [3–6]. According to an *in vivo* study, the half maximal effective concentration of nafamostat on human lung epithelium-derived Calu-3 cells was approximately 10 nM [6], which is lower than the plasma concentration achieved with an approved dose of the drug.

Favipiravir, a selective inhibitor of viral RNA-dependent RNA polymerase, has been approved in Japan for the treatment of emerging and re-emerging influenza virus infections. Favipiravir is a broad-spectrum antiviral that has been shown to be effective against RNA viruses, such as the Ebola virus and influenza virus, *in vitro* and *in vivo* [7–9]. It also exhibits antiviral activity against SARS-CoV-2 [10,11]. A clinical trial of favipiravir was conducted in Japan before our study.

The previously mentioned results suggest that favipiravir and nafamostat inhibit SARS-CoV-2 replication through different mechanisms, and combination therapy is expected to show an additional antiviral effect against SARS-CoV-2. To the best of our knowledge, no clinical trials on the use of combination drugs—a cell-entry inhibitor and an RNA polymerase inhibitor—has, however, been reported currently. In the early phase of the COVID-19 pandemic, we treated 11 patients hospitalized with severe COVID-19 with favipiravir and nafamostat on a compassionate-use basis, which demonstrated a favorable clinical outcome [12].

Consequently, we designed a multicenter, single-blind, randomized controlled study to evaluate the efficacy and safety of nafamostat mesilate combined with favipiravir in hospitalized patients with mild-to-moderate COVID-19 pneumonia.

Methods

Study design

This study was a multicenter, randomized, single-blind, placebo-controlled, parallel assignment design and was conducted at 16 sites in Japan. The enrollment for the trial began on May 1, 2020 and ended on December 3, 2021. Eligible participants were randomly assigned in a 1: 1 ratio to receive favipiravir and standard of care (SOC), either with or without nafamostat.

This trial was registered in the Japan Registry of Clinical Trials (jRCT; trial registration identifier: jRCTs031200026) and the World Health Organization (WHO)'s International Clinical Trial Registry Platform (identifier: JPRN-jRCTs031200026). All participants provided written informed consent to participate in the study.

Participants

Hospitalized patients aged 20-74 years with COVID-19 infection confirmed by a SARS-CoV-2 antigen or nucleic acid amplification test were eligible for enrollment. Further inclusion criteria were confirmed pneumonia on chest imaging, a fever of >37.5°C, and a negative pregnancy test for premenopausal women. The exclusion criteria were patients with fever of >37.5°C for more than 10 days and an oxygen saturation (SpO_2) level of $<93\%$ without oxygen administration. Further eligibility criteria are described in the study protocol (Supplementary file: protocol).

Randomization and masking

Eligible participants were randomly assigned in a 1: 1 ratio using stratified permutated block randomization to the nafamostat and favipiravir combination group or favipiravir monotherapy group. Randomization was performed by stratifying by age and comorbidity. To allocate participants, doctors input patient information into the electronic data capture system cubeCDMS (version:1.1, www.cubecdms.com). The allocation results were visible to doctors on the electronic data capture system but not to participants due to the single-blind nature of the study. Both groups were administered nafamostat or 5% dextrose using labels with the names of the participants but not the names of the drugs.

Procedures

All participants received favipiravir (FUJIFILM Toyama Chemical, Tokyo, Japan) 1800 mg twice a day on day 1, then 800 mg twice a day with standard care on the subsequent days. Participants randomized to the combination group were intravenously administered nafamostat (Nichi-iko Pharmaceutical Co., Ltd., Toyama, Japan) as a continuous infusion at a dose of 0.2 mg/kg/h. A 1-day dose of nafamostat was added to 1000 ml of 5% dextrose. Favipiravir, with or without nafamostat, was administered for 7 days or until clinical improvement was achieved for up to 14 days.

Clinical improvement was defined as the persistence of both defervescence without the use of an antipyretic 4 hours before measurement of axillary temperature and SpO_2 of $>96\%$ without oxygen therapy for >48 hours.

A quantitative virology assay was performed at LSI Medience (Tokyo, Japan). The data are presented as the number of copies of SARS-CoV-2 per milliliter of saliva. Samples were collected starting on October 8, 2020.

Outcomes

The primary outcome was the change in patient condition according to the WHO clinical progression scale [13] (a 10-point ordinal scale) from baseline to day 7.

The secondary outcomes included the following: (i) change in patient condition on a 10-point scale from baseline to days 4 and 8; (ii) change in SARS-CoV-2 viral load on days 0, 3, and 5; (iii) time to improvement in clinical symptoms, body temperature, and SpO_2 from randomization (improvement in body temperature was defined as a "body temperature of ≤ 37.4°C for at least 24 hours

without the use of an antipyretic 4 hours before measurement of axillary temperature" and improvement in SpO_2 was defined as "SpO_2 of 96% for at least 24 hours without the use of oxygen therapy"); (iv) change in clinical manifestations; (v) change in vital signs; (vi) change in the national early warning score (NEWS); (vii) remission of chest image findings, requirement and duration of oxygen administration; (viii) requirement and duration of mechanical ventilation; and (ix) changes in laboratory testing values.

The safety outcomes included the incidence of adverse events (AEs), including all undesirable changes in vital signs, symptoms, laboratory test values, and electrocardiogram.

Statistical analysis

All the efficacy analyses were conducted on the modified intention-to-treat population, which included all participants who were enrolled in this study and received at least one dose of treatment. Intergroup comparisons of efficacy were performed between the allocated treatment groups, irrespective of whether they actually received the allocated treatment. However, safety outcomes were assessed in all participants who started study treatment and were compared between groups according to their actual treatment.

The participants' characteristics were compared between groups using Fisher's exact test or Pearson's chi-square test for categorical variables and Student's *t*-test or the Wilcoxon rank-sum test for continuous variables.

The primary outcome was compared between groups by analysis of covariance, with the baseline value of the WHO 10-point ordinal scale as a covariate. The primary end point was analyzed using a one-tailed test at a significance level of 0.1.

For secondary outcomes, time-to-event end points were compared between groups using the log-rank test, and survival curves were created for each group using the Kaplan–Meier method. Participants who discontinued the study after day 28 were censored on day 28. The median survival days were calculated and their confidence intervals (CIs) were constructed using the Brookmeyer-Crowley method. Hazard ratios were computed using the Cox proportional hazards model, which included only the treatment group as a covariate. Continuous outcomes at day 7, including SpO_2, were analyzed as change from baseline using mixed models for repeated measures with fixed effects, including group, time, and group-by-time interactions, and an unstructured assumption about correlation. The change in logarithm of viral load from baseline to day 5 was analyzed using a linear mixed-effects model without intercept, in which the fixed effects were continuous time and the time-by-treatment interaction and the random effects for each participant were continuous time. Secondary outcomes were analyzed using a two-tailed test at a significance level of 0.05 and 95% CI. AEs are summarized as counts and proportions. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for analyses, tabulations, and chart outputs.

The primary outcome and definition of clinical improvement were changed in September 2020. The initial primary outcome was time to clinical improvement. The initial definition of clinical improvement was maintenance for more than 24 hours of three findings, including defervescence, >96% SpO_2, and improvement of chest imaging, followed by two negative results of reverse transcriptase-polymerase chain reaction (PCR) testing for SARS-CoV-2 24 hours apart. In early 2020, the discharge criteria for COVID-19 in Japan were an improvement in both respiratory function and defervescence for more than 48 hours, followed by two consecutive negative results of reverse transcriptase-PCR testing for SARS-CoV-2. However, the discharge criteria were changed in June 2020 to more than 72 hours of improvement in both respiratory status and defervescence and more than 10 days after the appearance of symptoms. After the change, the study enrollment was delayed because the participants avoided staying in a hospital until clinical improvement was achieved, even after the national discharge criteria were achieved.

The required sample size was initially calculated as 160, with 80% power for detection of intergroup differences of 0.4 or more in changes in the WHO 10-point ordinal scale from baseline with a common SD of 0.9, using a two-tailed *t*-test, at a significance level of 0.05. However, due to the difficulty of completing patient enrollment, the sample size was changed to 45 patients on November 22, 2021, with 75% power for the detection of intergroup differences of 0.6 with an SD of 1.0, using a one-tailed test at a significance level of 0.1.

Results

Subjects

A total of 45 patients were enrolled between May 1, 2020 and December 15, 2021; none were excluded from the study. After randomization, 21 patients were allocated to the combination group with nafamostat and favipiravir, and 24 patients to the favipiravir monotherapy group. No patient died by day 28. Four patients in the combination therapy group and five patients in the monotherapy group discontinued the intervention. Among these, three in the combination therapy group and four in the monotherapy group discontinued the intervention before day 7. Two patients in the monotherapy group were administered tocilizumab, one of whom was also administered nafamostat as a secondary rescue therapy because of clinical determination. In the monotherapy group, two patients discontinued the study after the intervention. Overall, 21 patients in the combination therapy group and 24 patients in the monotherapy group were included in the modified intention-to-treat population and safety analysis set (Figure 1).

The baseline characteristics and vital signs were similar between the combination therapy and monotherapy groups (Table 1, Supplementary Table 1). All patients scored a four on the 10-point ordinal patient scale. Symptoms, except myalgia and joint pain, were similar between the two groups. The degree of myalgia and joint pain was more severe in the monotherapy group. Clinical laboratory testing showed that several parameters (uric acid, creatinine kinase, blood glucose, and prothrombin time and international normalized ratio) were higher in the monotherapy group.

The median duration of nafamostat administration was 8.0 days (range, 2-14 days) in the combination group and 0.0 days in the monotherapy group; although, one patient in this group received it for 6 days for primary rescue therapy. The median duration of favipiravir administration was 8.0 days (2-14 days) and 9.5 days (4-14 days) in the combination and monotherapy groups, respectively.

Primary outcome

There was no significant difference in the changes in patient score on day 7 between the two groups (least-squares mean -0.444, combination group vs -0.150, monotherapy group; least-squares mean difference [LSMD], -0.294 [95% CI, -0.944 to -0.355]; $P = 0.364$; Table 2). In the subgroup analysis, no specific population was found to have a significantly different patient score (data not shown).

Secondary outcomes

The median time to improvement in body temperature was significantly shorter in the combination group than that in the monotherapy group (5.0 days vs 9.0 days, respectively; 95% CI, 4.0-7.0 and 7.0-18.0, respectively; hazard ratio, 2.42; 95% CI, 1.26-4.63;

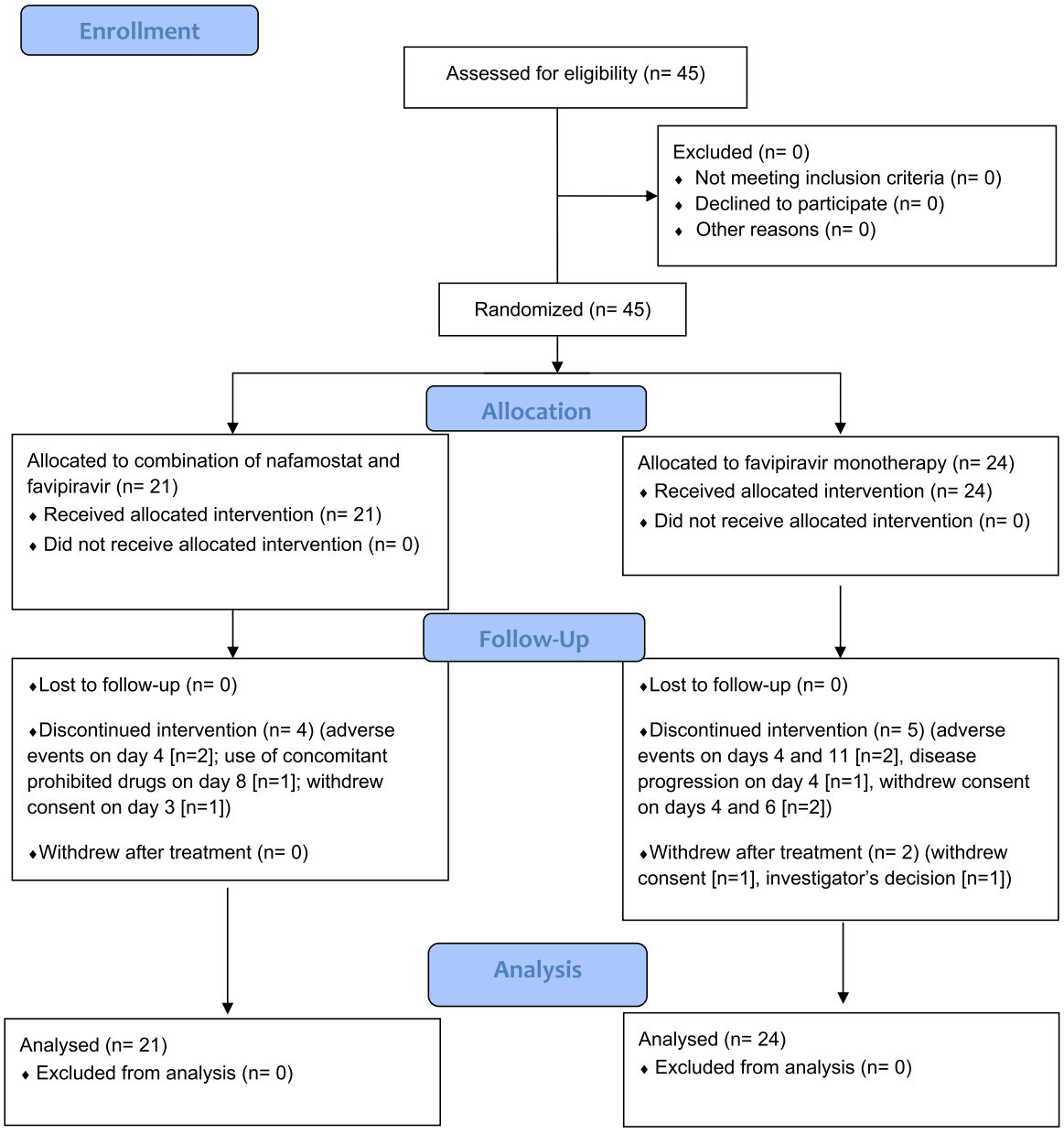

Figure 1. Flow diagram of patient enrollment.

$P = 0.009$; Table 2, Figure 2). The rate of improvement in body temperature on day 7 was 80.0% in the combination group and 29.2% in the monotherapy group. The median time to improvement in SpO_2 tended to be shorter in the combination group than in the monotherapy group; although, the difference was not significant (5.0 days vs 7.0 days, respectively; hazard ratio, 1.78; 95% CI, 0.88-3.63; $P = 0.097$) (Supplementary Table 2). The changes in SpO_2 on day 7 compared with preintervention were also greater in the combination group than those in the monotherapy group (0.526% vs -1.304%, respectively; LSMD, 1.831; 95% CI, 0.279-3.382; $P = 0.022$). Systolic blood pressure had decreased to a greater extent on day 7 in the combination group (-18.265 mm Hg vs -8.634 mm Hg, respectively; LSMD, -9.631; 95% CI, -18.660 to -0.602; $P = 0.037$). Other vital signs were similar between the two groups. The NEWS trend and 10-point ordinal scale score on days 4 and 28 did not differ between the two groups. Although the viral load in saliva on days 0, 3, and 5 was not measured in all patients, there were no significant differences between the groups based on those measured. There was a significant difference between the combination group and monotherapy group in the values of the following laboratory tests on day 7: hemoglobin (0.108 g/dl vs -0.830 g/dl, respectively, LSMD, 0.938; 95% CI, 0.470-1.405; $P < 0.001$), creatinine (0.087 mg/dl vs -0.122 mg/dl, respectively, LSMD, 0.209; 95% CI, 0.135-0.282; $P < 0.001$), total bilirubin (0.116 mg/dl vs -0.094 mg/dl, respectively, LSMD, 0.210; 95% CI, 0.092-0.328; $P < 0.001$), and prothrombin time and international normalized ratio (0.085 vs -0.022, respectively, LSMD, 0.107; 95% CI, 0.066-0.148; $P < 0.001$).

Safety

In total, 99 AEs were reported among 19 patients (90.5%) in the combination group and 92 AEs were reported among 20 patients (83.3%) in the monotherapy group (Table 3). The rate of AEs was similar between the two groups. No serious AEs or deaths were reported. Two patients from each group discontinued the study ow-

Table 1
Clinical characteristics of the patients at baseline.

	Nafamostat and favipiravir (N = 21)	Favipiravir (N = 24)	*P*-value
Age, median (range)	50.0 (25-68)	54.0 (39-72)	0.069
Gender			0.950
Male	12 (57%)	15 (63%)	
Female	9 (43%)	9 (37%)	
Ethnic origin			
Asian	21 (100%)	24 (100%)	
Body mass index (kg/m^2), median (range)	22.84 (20.83-35.99)	23.69 (19.13-34.60)	0.896
Comorbidity, no. (%)			
Diabetes mellitus	4 (19.0%)	5 (20.8%)	1.000
Cardiovascular disease	0	0	-
Chronic pulmonary disease	1 (4.8%)	1 (4.2%)	1.000
Chronic obstructive pulmonary disease due to smoking	0 (0%)	1 (4.2%)	1.000
Others	13 (61.9%)	14 (58.3%)	1.000
Drug allergy, no.	0	0	
Other allergy, no. (%)	2 (9.5%)	3 (12.5%)	1.000
SARS-CoV-2 viral load (Log scale)	N = 9 3.724 (3.10-6.30)	N = 13 5.763 (3.10-8.41)	0.082
Vital signs			
Body temperature (°C)	37.80 (35.7-41.2)	37.90 (36.1-40.3)	0.996
Systolic blood pressure (mmHg)	N = 21 122.0 (90-151)	N = 23 117.0 (96-169)	0.749
Diastolic blood pressure (mmHg)	N = 21 70.0 (55-99)	N = 23 79.0 (42-100)	0.272
Pulse rate (times/min)	82.0 (57-109)	89.5 (62-113)	0.069
Respiratory rate (times/min)	N = 20 19.0 (14-26)	N = 21 18.0 (12-30)	0.895
Oxygen saturation (%)	96.0 (94-98)	96.0 (88-99)	0.734
National Early Warning Score	N = 20 3.0 (0-7)	N = 21 2.0 (0-8)	0.981
10-point ordinal score of patients			
Score 4	21 (100%)	24 (100%)	
Oxygen therapy	3 (14.3%)	0 (0%)	0.187

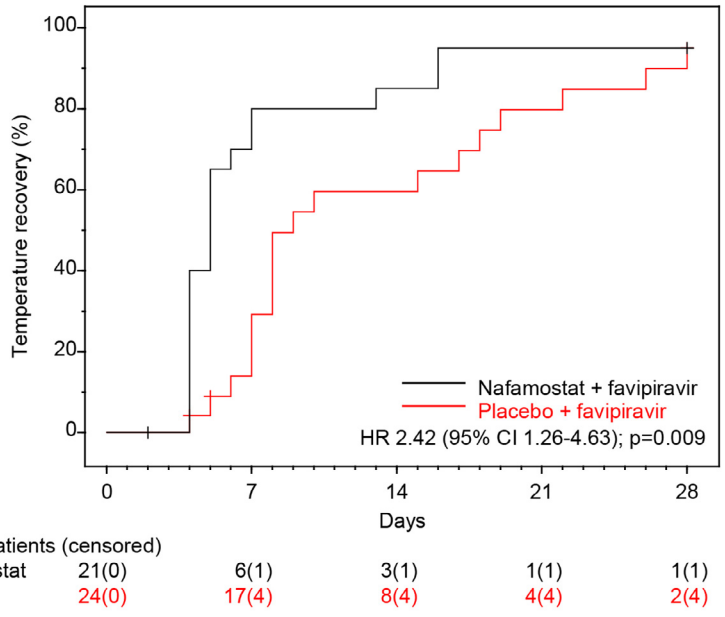

Figure 2. Proportion of temperature recovery in the combination group and monotherapy group.
The Kaplan-Meier estimates show that the rate of recovery for temperature on day 7 was higher in the combination group than that in the monotherapy group.
CI, confidence interval; HR, hazard ratio.

Table 2
Results of the primary outcome and key secondary outcomes.

Primary outcome	Nafamostat and favipiravir (N=21)				Favipiravir (N=24)				LSM difference	LSM difference 80% CI		LSM difference 95% CI		P value
	N	LSM	LSM 95% CI		N	LSM	LSM 95% CI							
Change in value of WHO 10-point ordinal scale on day 7 from baseline	18	−0.444	−0.916	0.027	20	−0.150	−0.597	0.297	−0.294	−0.713	0.124	−0.944	0.355	0.364
Key secondary outcomes	Nafamostat and favipiravir (N=21)				Favipiravir (N=24)				LSM difference			LSM difference 95% CI		P value
	N		95% CI		N		95% CI							
Rate of defervescence on day 7 (%) *	21	80.0	60.7	93.8	24	29.2	14.2	53.9						
Duration of fever (days)	21	5.0	4.0	7.0	24	9.0	7.0	18.0						0.009
	N	LSM	LSM 95% CI		N	LSM	LSM 95% CI		LSM difference			LSM difference 95% CI		P value
Change in value of SpO_2 on day 7 from baseline (%)	21	0.526	−0.607	1.659	24	−1.304	−2.364	−0.244	1.831			0.279	3.382	0.022
Change in value of systolic blood pressure on day 7 from baseline (mmHg)	21	−18.265	−24.815	−11.715	24	−8.634	−14.849	−2.419	−9.631			−18.660	−0.602	0.037
Change in value of hemoglobin on day 7 from baseline (g/dL)	21	0.108	−0.239	0.455	24	−0.830	−1.143	−0.517	0.938			0.470	1.405	<0.001
Change in value of creatinine on day 7 from baseline (mg/dL)	21	0.087	0.032	0.141	24	−0.122	−0.171	−0.073	0.209			0.135	0.282	<0.001
Change in value of total bilirubin on day 7 from baseline (mg/dL)	21	0.116	0.028	0.204	24	−0.094	−0.173	−0.016	0.210			0.092	0.328	<0.001
Change in value of PT-INR on day 7 from baseline	21	0.085	0.054	0.116	24	−0.022	−0.050	0.006	0.107			0.066	0.148	<0.001

LSM, least-squares mean; PT-INR, prothrombin time international normalized ratio; SpO2, oxygen saturation; WHO, World Health Organization.
* Proportion and its confidence interval were estimated by Kaplan-Meier method.

Table 3
Summary of AEs in the safety population.

Event N (%)	Nafamostat and favipiravir (N = 21)	Favipiravir (N = 24)
Any AE	19 (90.5)	20 (83.3)
Phlebitis	12 (57.1)	2 (8.3)
Elevated liver enzymes	7 (33.3)	9 (29.2)
Hyperuricemia	14 (66.7)	13 (54.2)
Hyponatremia	3 (14.3)	3 (12.5)
Hyperkalemia	2 (9.5)	1 (4.2)
Increased creatinine	2 (9.5)	0 (0)
Serious AE	0 (0)	0 (0)
Death	0 (0)	0 (0)

AE, adverse event

ing to AEs. Both agents were discontinued in the two patients from the combination group: one because of extravasation on day 4 and the other because of elevation of liver enzymes, uric acid, and creatinine, with the emergence of urinary protein and occult blood. The most common AE was hyperuricemia (66.7% in the combination group and 54.2% in the monotherapy group). Phlebitis was reported at a higher rate in the combination group than in the monotherapy group (57.1% and 8.3%, respectively) and was considered to be a nafamostat-related AE by the investigators. Hyponatremia and hyperkalemia were observed in 14.3% and 9.5% of patients in the combination group, respectively.

Discussion

In this study, nafamostat plus favipiravir therapy did not significantly improve the score on the 10-point patient ordinal scale on day 7 compared with favipiravir monotherapy. However, the duration of the fever was shortened and a higher SpO_2 on day 7 was achieved. Nafamostat administration did not cause severe AEs; although, phlebitis occurred in 57.1% of patients in the combination group.

To date, two clinical trials of nafamostat have been reported: a randomized phase II clinical trial in Russia and a randomized controlled phase Ib/IIa experimental study in the UK. Although nafamostat is a promising antiviral drug for SARS-CoV-2, both the UK study and our study failed to demonstrate that it reduced viral load, and the Russian study also showed that the time to virus elimination did not differ between the nafamostat group and the standard care group based on a qualitative PCR test. Notably, the viral load discussed previously does not indicate the quantity of live, infective virus particles. A possible reason for the lack of an effect on viral load despite the administration of a sufficient concentration of nafamostat is the duration from symptom onset to the administration of nafamostat. The viral load in patients with COVID-19 reaches a peak early in the course of symptoms [14]. This study enrolled patients who developed fever within 10 days, and the UK and Russian studies also enrolled patients 8 and 10 days [15] after the occurrence of symptoms, respectively. A mouse model study showed that administration of nafamostat immediately before infection protects respiratory tract cells [16]. Thus, earlier administration of nafamostat may have an antiviral effect. Another possibility is that favipiravir may mask the potential effect of nafamostat because favipiravir showed a higher rate of viral clearance and shorter clinical improvement in hospitalized patients with COVID-19 in a meta-analysis [17]. Nafamostat may have no additional effect when used with favipiravir to treat SARS-CoV-2.

This study did not achieve statistical significance in the analysis of the primary outcome using the patient ordinal scale. A UK study on patients with COVID-19 pneumonitis reported that the duration of hospital stay and time without oxygen therapy tended to be longer in the nafamostat therapy group than in the SOC group [15]. In contrast, the Russian study reported that among patients with NEWS >7, the time to clinical improvement, measured by the 7-category ordinal scale, was shorter in the nafamostat group than in the SOC group; although, the difference between the two groups was not significant in all patients [18]. Thus, nafamostat may have a more beneficial effect on patients with severe COVID-19 than on those in our study.

Regarding the secondary outcomes, combination therapy resulted in a shortened duration of fever and improvement in SpO_2 on day 7; although, the oxygen administration rate and duration did not differ between the monotherapy and combination therapy groups. A recent preprint of a clinical trial of camostat, an oral serine protease inhibitor similar to nafamostat, showed a similar beneficial effect in that the clinical scores, including fever and upper respiratory symptoms, were improved in the camostat group; although, there was no significant difference in viral load compared with the placebo group [19]. The mechanism underlying the improvement in the clinical course of COVID-19 by nafamostat is unknown beyond its antiviral effect. Nafamostat has been reported to have immunomodulatory and anticoagulant effects [20–22]. Clinical trials of nafamostat [15] and camostat [23] for the treatment of COVID-19 have reported the dynamics of cytokines in the blood; however, significant changes were not observed. The viral entry of SARS-CoV-2 occurs through a cell surface route through TMPRSS2 or an endosomal route [24]. Toll-like receptor (TLR) 7, which recognizes viral single-stranded RNA viruses, such as SARS-CoV-2, is expressed in the endosome, and nafamostat can inhibit peripheral inflammation, such as liver inflammation, triggered by stimulating TLR7 [25]. The difference in cytokine dynamics in the blood or tissue may be determined according to the severity and phase of COVID-19.

AEs associated with nafamostat were not serious, but phlebitis was observed in 57.1% of patients in the combination group in this study and in 13.5% of patients in a previous study [18]. Other common AEs were elevated liver enzymes (33.3% in the combination group and 29.2% in the monotherapy group) and hyperuricemia (66.7% in the combination group and 54.2% in the monotherapy group). A previous study comparing favipiravir and placebo therapy reported these events in 5-10% and approximately 40% of patients in the placebo and favipiravir groups, respectively [26]. Serious AEs due to nafamostat reported in a previous study [15], including elevation of creatinine and hyperkalemia, were uncommon (both 9.5%) and not observed in this study. The incidence of AEs must have decreased with the necessary and sufficient administration of nafamostat. One problem is that continuous infusion is required to maintain the blood concentration of nafamostat because it decreases rapidly after administration [27,28]. A study is currently ongoing in Japan to investigate the virus inhibitory effect, safety, and optimal dose of nafamostat mesilate in patients with early mild COVID-19 (jRCTs031210183). Further improvements in nafamostat delivery are needed to maintain patients' adherence.

This study had some limitations. First, this was a single-blind trial, and the number of patients was small. However, this study

enrolled patients with mild-to-moderate COVID-19, which represented the majority of cases before the Omicron variant. Therefore, the results of this study are generalizable outside of the study population. Second, the primary outcome changed 4 months after the start of the study. Two patients were enrolled in the combination group and four in the monotherapy group before the primary outcome was changed; however, these patients could be included in the study assessment because their scores were obtained retrospectively. Third, all patients received favipiravir because it had already been used off-label for patients with COVID-19 pneumonia in Japan during the planning of this study. At the start of this study, the use of nafamostat without favipiravir might have been unacceptable to participants and physicians. Fourth, the history of COVID-19 vaccination was not collected, and the sequencing of viral genomes was not performed in this study. However, 70% of the patients in this study were enrolled before priority vaccination started in Japan, and all participants were enrolled before the Omicron variant emerged.

In conclusion, this study showed earlier defervescence and SpO_2 recovery in patients with mild-to-moderate COVID-19 in the nafamostat and favipiravir combination group than those in the favipiravir monotherapy group but without any additional viral reduction. This result suggests that the combination therapy with favipiravir and nafamostat may be considered as a therapeutic option for hospitalized patients with COVID-19. Further studies are required to evaluate the clinical efficacy of the favipiravir and nafamostat therapy with expanded patient populations and reduce nafamostat-related AEs. Furthermore, nafamostat treatment in combination with other antivirals must be studied to prepare for the emergence of a new virulent variant.

Declaration of competing interest

JI, YS, and KM are co-inventors on patent applications of nafamostat as an antiviral agent (PCT/JP2021/9968, patent applicant: the University of Tokyo). All other authors declare no competing interests. FUJIFILM Toyama Chemical and Nichi-Iko supplied the favipiravir and nafamostat mesilate, respectively.

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Ethical approval

This study was designed and conducted in accordance with the tenets of the Declaration of Helsinki and Clinical Trials Act enforcement regulations. Previous ethical approval for the protocol was obtained from the University of Tokyo Clinical Research Review Board (CRB; approval number 3180024), and each participating hospital investigator received permission from their administrator to conduct the study. All participants provided written informed consent to participate in the study.

Authors' contributions

MI, S Okugawa, TM, and KM designed the study. JI, YS, and KM supervised the conduct of this study. MI, S Okugawa, YK, DJ, HH, KO, KT, YU, TM, HI, TT, HA, K Yatera, YY, Y Nakamura, AO, MY, YW, TS, MA, TO, AK, SH, YT, Y Nakano, YM, SS, MS, S Ogura, K Yamasaki, HK, and OH contributed to the collection of data for this study. KK performed statistical analyses. MI, S Okugawa, and KM interpreted the results. MI wrote the first draft of the report. S Okugawa, KK, and KM contributed substantially to the critical revision of early drafts of the manuscript. All authors had full access to all the data in the study, had critically reviewed the manuscript, and accept responsibility for the decision to submit this manuscript for publication.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.12.039.

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