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Case Report

Two cases of novel coronavirus infection (COVID-19) with transient viral elevation using semi-quantitative real-time reverse transcription PCR and symptom relapse after completion of 10 days of favipiravir treatment☆

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ABSTRACT

The coronavirus disease of 2019 (COVID-19), which began in Wuhan, China, at the end of 2019, is spreading around the world and causing many deaths, mainly from pneumonia. Currently, there are no specific drugs to treat COVID-19, and existing antiviral drugs are being used as an alternative. One of these is favipiravir, a new type of influenza drug. However, its efficacy, dosage, and duration of administration are still under study. In this case study, we administered favipiravir to patients with COVID-19 and determined the viral load of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID-19 pathogen, using semi-quantitative real-time reverse transcription PCR in sputum samples. We report on two patients in whom the viral load increased again after completion of 10 days of favipiravir treatment and a transient relapse of symptoms was observed.

Introduction

A novel coronavirus infection (COVID-19; coronavirus disease 2019), which began as an epidemic in Wuhan, China, in late 2019, has since spread rapidly around the world. On March 11, 2020, the World Health Organization (WHO) declared it a pandemic, and as of December 02, 2020, the number of infected people worldwide has reached 63 million, with over 1,482,000 deaths [1].

COVID-19 is a disease caused by infection with the RNA virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes respiratory distress due to pneumonia and in the worst case, death [2]. Currently, there is no effective treatment for COVID-19, and various drugs such as remdesivir (used to treat Ebola hemorrhagic fever), lopinavir (LPV)/ritonavir (RTV) (used to treat human immunodeficiency virus [HIV] infection), and favipiravir (developed at the time of the H1N1 influenza epidemic) are being used alternatively to determine their efficacy [3,4].

Favipiravir, a nucleic acid analog prodrug that selectively inhibits the RNA-dependent RNA polymerase of RNA viruses, has been considered to be effective against all RNA viral infections, not just influenza viruses [5,6]. Thus, favipiravir is being used for the treatment of COVID-19 in some countries, and the efficacy of favipiravir administration has been reported in China [7]. In Japan, favipiravir is used in compassionate care cases at a dose of 3600 mg on the first day and 1600 mg on the second day and thereafter. The typical duration of administration is within 14 days, but no clear basis has been obtained yet. In addition, antiretroviral therapy (ART) for HIV infection, while initially reducing the viral titer, has been shown to result in a subsequent increase in viral load during treatment [8]. There have not been many reports of transitions in

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Abbreviations

ART	antiretroviral therapy
COVID-19	coronavirus disease 2019
GGO	ground-glass opacity
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
RT-PCR	reverse transcription polymerase chain reaction
SpO2	saturation of percutaneous oxygen
AMPC	amoxicillin
CVA/AMPC	clavulanate/amoxicillin

viral load in the body following favipiravir administration with COVID-19 cases.

In this study, we administered favipiravir to COVID-19 patients admitted to the Department of Infectious Diseases, Kyoto Prefectural University of Medicine, and measured the viral load of SARS-CoV-2 by real-time reverse transcription PCR (RT-PCR) using sputum samples. We report two cases wherein the viral load increased again after the end of favipiravir treatment.

We used sputum as the specimen for determining the viral load by real-time RT-PCR. sputum was basically collected early morning sputum, and only good quality specimens were used, excluding low quality specimens in the Miller–Jones classification and the Geckler classification. Pretreatment of sputum was performed according to the appendix "Pretreatment Methods of Sputum Specimens" of "Manual for the Detection of Pathogen 2019-nCoV" by the National Institute of Infectious Diseases. We used QIAamp® Viral RNA Mini Kit (QIAGEN, Japan) for RNA extraction, and the Cobas® z 480 system (Roche Diagnostics, Switzerland) for real-time RT-PCR. We also used the LightMix® Modular SARS-CoV E-gene kit (TIB Molbiol, Germany) for real-time RT-PCR. A positive control was used to quantify the viral load. The positive control used in this study had a reference Ct value of 29.5 and a concentration of 1000 copies/μL. We calculated the viral load in the patient samples from the difference between the Ct values of the control and patient samples.

Case reports

Case 1

The first patient was a 70-year-old woman. She was a past smoker, with a 40 packs-year, and had comorbidities such as emphysema, dyslipidemia, and an overactive bladder, for which she was being treated with mirabegron (50 mg), rosuvastatin (2.5 mg), benidipine hydrochloride (4 mg), and etizolam (0.5 mg). She had been hospitalized twice for acute exacerbation of chronic obstructive pulmonary disease and pneumonia (COPD). She had no known allergies and lived with three other family members.

Ten days prior to admission to the hospital, the patient had a fever of 38.1 °C, and a dry cough appeared. A chest radiograph was taken, but no abnormality was noted, and an antipyretic medicine was prescribed. Her fever did not subside, and an olfactory disturbance appeared 4 days before admission. She visited her doctor again and was prescribed clavulanate/amoxicillin (CVA/AMPC) and some antiallergic medicine, but her condition did not improve. She was referred to the general hospital. The day before admission, a chest computed tomography (CT) scan was taken, and a specimen of nasopharyngeal swab was taken for PCR analysis because of a suspicion of COVID-19 pneumonia. A positive PCR result was confirmed the next day, and she was admitted to our hospital (day 1). On admission, her body temperature was 36.7 °C,

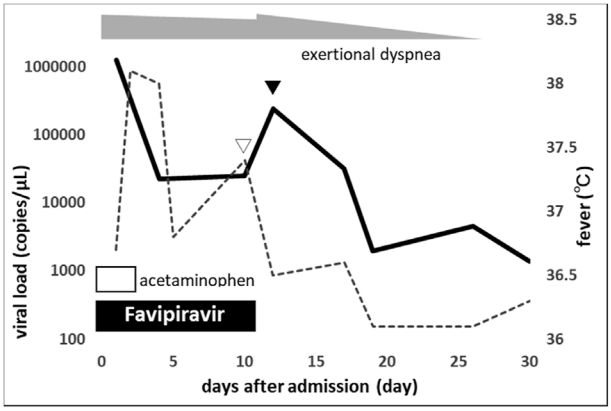

Fig. 1. Changes in viral load of SARS-CoV-2 in sputum of case 1 during and after treatment with favipiravir. The solid line shows the viral load, the dashed line shows the temperature change and the gray bar shows dyspnea on exertion. There was a relapse of fever on day11 (▽), and an increase in viral load was noted on day 12 (▼).

and her SpO_2 was as low as 88% in room air; dyspnea was observed on exertion. Her chest CT scan showed sporadic ground-glass opacity (GGO) and dorsal infiltration in the base of the lung just below the pleura of both lungs, consistent with imaging findings of COVID-19 pneumonia. The administration of favipiravir was started on the day of admission, along with oxygen via a nasal cannula. The amount of virus in sputum at the time of hospitalization, measured by real-time RT-PCR using the E-gene, was 1.26×10^6 copies/μL [9,10].

Antipyretic medication (acetaminophen) was effective within 2 days after admission, and favipiravir therapy resulted in fever reduction and improvement in respiratory status on day 4; treatment was terminated after 10 days because of the general improvement of symptoms. The viral load measured by real-time RT-PCR decreased after treatment with favipiravir, but the viral load increased on day 12, 2 days after the end of treatment (Fig. 1). The patient had a transient fever, exertional dyspnea, and decreased SpO_2 at about the same time, but it did not worsen thereafter. The increase in viral load was also transient and tapered off over 50 days.

Case 2

The second patient was a 61-year-old woman. She was a never-smoker but had hypertension and dyslipidemia, for which she was taking amlodipine (2.5 mg), rosuvastatin (2.5 mg), methylcobalamin (250 mg), and etizolam (0.5 mg). She had been hospitalized for cerebral hemorrhage 10 years prior, but there were no notable sequelae. She had no known allergies, and one family member lived with her.

She developed a mild dry cough and a fever of 38.5 °C 12 days and 10 days prior to admission to the hospital, respectively. She was prescribed an antipyretic and AMPC. Her fever remained, and she visited her doctor again 4 days before admission. A rapid test for influenza was taken at that time, but it was negative. Two days before admission, she was referred to the general hospital, where a chest CT scan was taken, and a specimen of nasopharyngeal wipe fluid was obtained for PCR analysis for SARS-CoV-2 due to interstitial shadows in the lungs. Positive results were confirmed on the day prior to admission, and she was admitted to our hospital on the following day (day 1).

Her temperature on admission was 37.9 °C, and her SpO_2 was 97% under high-dose oxygen; she also had severe dyspnea on exertion. Her chest CT scan at the time showed extensive GGO and

infiltration in both lung areas, mainly just below the pleura. The amount of virus in sputum at the time of hospitalization, measured by real-time RT-PCR using the E-gene, was 2.12×10^5 copies/μL [9,10].

After admission, oxygen administration was continued, and favipiravir treatment was started on the same day. It took 22 days for her respiratory status to improve due to the marked fluctuation of SpO_2 depending on her body position, but her fever subsided on day 11, and her respiratory status gradually improved. Antipyretic medication (acetaminophen) was effective within 3 days after admission, and favipiravir treatment was terminated after 10 days. The viral load obtained by real-time RT-PCR examination showed a decreasing trend during favipiravir treatment, but a transient fever, malaise, dyspnea, and tachypnea on exertion were observed on day 11, the day after the end of treatment, and a transient rise in viral load was observed at the same time (Fig. 2). Her symptoms also resolved for a day, and she had no further exacerbation of symptoms. The viral load also tapered off and became negative on day 32.

Discussion

The efficacy of various antiviral drugs, such as remdesivir, LPV/RTV, and favipiravir, is currently being tested against COVID-19. We report two cases in which favipiravir was effective, but there was a transient viral load elevation and symptom relapse after 10 days treatment. SARS-CoV-2 is a single-stranded RNA virus that belongs to the β-coronavirus genus in the family Coronaviridae [11]. Another single-stranded RNA virus, HIV, has been treated with ART for HIV infection [8]. If the viral load increases again during the course of this treatment, there are various possible causes, including adherence problems and the emergence of drug-resistant viruses [12]. Mild and transient increases in viral load during treatment have also been reported, which have been referred to as “blips” [13].

In the two cases reported herein, the increase in the viral load after the completion of favipiravir treatment was transient; the viral load spontaneously decreased, and the clinical symptoms improved. From this point of view, these two cases may represent “blip”-like phenomena in the antiviral treatment. Although the factors that cause blips in the treatment of HIV infection are not yet fully understood, there are reports that early ART initiation reduces blips [14], and that high viral load before treatment is associated with blips [15]. For COVID-19, it has also been reported that delayed hospitalization after the onset of the disease prolongs the transmission of SARS-CoV-2 [16]. In addition, relapses of symptoms have been reported in cases where PCR testing was positive again after discharge from the hospital [17]. In both cases in this report, the viral load at the time of admission was high and antiviral therapy was started relatively late, 10 days after the onset of the disease, which may have caused the appearance of “blip”-like phenomena. Another possible reason for this phenomena could be related to the effect of specimen quality. Case 1 had COPD and Case 2 had severe pneumonia. Therefore, good quality sputum specimens were obtained and detailed evaluation, including transient viral elevation, was possible. In fact, it has been reported that viruses could be detected more accurately by PCR testing using bronchoalveolar lavage fluid (BALF), which is a more direct lower respiratory tract specimen than sputum, than by using nasal specimens [18]. However, it remains to be determined what other factors cause transient viral re-increase and worsening of symptoms after finishing antiviral therapy for COVID-19. Compared with Case 1, there was a slight time difference between the worsening symptom and transient viral elevation in Case 2. A possible explanation is COVID-19 causes fever and other symptoms due to an immune response when the viral load elevated may be complicated and have various patterns. The other explanation is the quality of the sputum might affect the situation, but the exact reason is unknown.

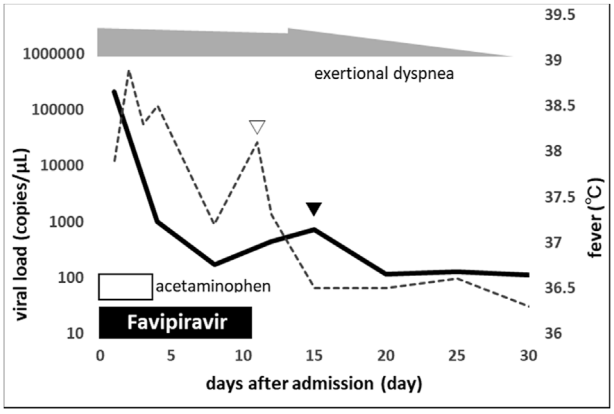

Fig. 2. Changes in viral load of SARS-CoV-2 in sputum of case 2 during and after treatment with favipiravir. The solid line shows the viral load, the dashed line shows the temperature change and the gray bar shows dyspnea on exertion. There was a relapse of symptoms (fever) on day11 (▽), and an increase of viral load was noted on day 15 (▼).

Favipiravir treatment for COVID-19 is thought to inhibit viral growth by selectively inhibiting the RNA-dependent RNA polymerase. However, there is a report that neutralizing antibodies against SARS-CoV-2 are sufficiently produced after 2 weeks of disease onset [19]. Thus, it might be possible to prevent blips or rebound if enough neutralizing antibodies are produced in the body while favipiravir inhibits viral growth. The two patients in the present study completed favipiravir treatment for 10 days, which may have been related to the appearance of “blip”-like phenomena.

There are several limitations in this study. The first is that the number of cases is small. As the number of cases increases in the future, we may be able to reveal in more detail the characteristics and turning points of cases that can cause blips. Second, the collection of patients treated with favipiravir for 14 days would allow a comparison of patient background with patients treated for 10 days and would help ascertain the appropriate duration of treatment. Third, we used sputum to accurately assess the amount of virus in covid-19 pneumonia, but the quality of the specimen is a problem. Although we tried, as much as possible, to use specimens of high quality, the quality of sputum is inevitably inconsistent to some extent. If we could use specimen with higher quality like BALF, it would be possible to capture the variation in viral load, including the “blip”-like phenomena, in more detail. Another limitation is the method of quantification of the virus. It is desirable to measure a reference material at each measurement to create a calibration curve for quantitative analysis with the reagent. However, although LightMix® kit used in this study is for semi-quantitative use, real-time PCR allows for the calculation of Ct values, which has been reported to have a strong correlation in quantification with other tests [20]. Moreover, there are several reports on the usefulness of the quantitative evaluation of semi-quantitative Ct values [21,22]. Moreover, considering the time constraints of the COVID-19 pandemic and the susceptibility to calibration errors in RNA quantification due to pipetting, it is uncommon making a calibration for each test in the actual clinical settings. We think it adequately explainable and informative simply to measure a known reference material and estimated the viral load of the sample from its Ct value in this study.

Here, we report two cases of transient viral elevation and symptom relapse after completion of 10 days of favipiravir

treatment. For patients with risk factors such as older age, high viral load, long time from onset to initiation of treatment, and underlying diseases, one should consider extending the duration of favipiravir treatment.

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Ethical approval

Informed consent was obtained from the patient for publication of this case report.

CRediT authorship contribution statement

Hajime Tsuboi: was the chief investigator and responsible for the data analysis, and. **Yu Kasamatsu:** and, and. **Shin Matsubara:** and. **Akifumi Sasao:** and. **Katsutomo Kunimitsu:** and. **Nana Munakata:** were contributed to collecting data, All authors contributed to the writing of the final manuscript. **Takamasa Ito:** and. **Yasuhiro Tsuchido:** and. **Masanaga Yamawaki:** and. **Naohisa Fujita:** were responsible for the organization and coordination of the trial.

Declaration of competing interest

None.

References

[1] Coronavirus 2019-nCoV CSSE. Coronavirus 2019-nCoV global cases by johns hopkins CSSE. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6. [Accessed 2 December 2020].
[2] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. J Am Med Assoc 2020;323(11):1061–9. https://doi.org/10.1001/jama.2020.1585.
[3] McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res 2020;157:104859. https://doi.org/10.1016/j.phrs.2020.104859. Epub 29 Apr 2020.
[4] Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe Covid-19. N Engl J Med 2020. https://doi.org/10.1056/NEJMoa2007016. NEJMoa2007016.
[5] Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad-spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci 2017;93(7):449–63. https://doi.org/10.2183/pjab.93.027.
[6] Shiraki K, Daikoku T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther 2020;209:107512. https://doi.org/10.1016/j.pharmthera.2020.107512.
[7] Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering 2020. https://doi.org/10.1016/j.eng.2020.03.007. Epub 18 Mar 2020.
[8] Simonetti FR, Kearney MF. Review: influence of ART on HIV genetics. Curr Opin HIV AIDS 2015;10(1):49–54. https://doi.org/10.1097/COH.0000000000000120.
[9] van Kasteren PB, der Veera B, van den Brinka S, Wijsmana L, Jongea J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol 2020;128:104412. https://doi.org/10.1016/j.jcv.2020.104412.
[10] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25:2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.
[11] Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5(4):536–44. https://doi.org/10.1038/s41564-020-0695-z.
[12] Smith C, Phillips A, Dauer B, Johnson MA, Lampe F, Youle M, et al. Factors associated with viral rebound among highly treatment-experienced HIV-positive patient who have achieved viral suppression. HIV Med 2008;10:19–27. https://doi.org/10.1111/j.1468-1293.2008.00650.x.
[13] Farmer A, Wang X, Ganesan A, Deiss RG, Agan BK, O'Bryan TA, et al. Factors associated with HIV viral load "blips" and the relationship between self-reported adherence and efavirenz blood levels on blip occurrence: a case-control study. AIDS Res Ther 2016;13:16. https://doi.org/10.1186/s12981-016-0100-4.
[14] Crowell TA, Pinyakorn S, Sacdalan C, Kroon E, Colby DJ, Puttamaswin S, et al. RV254/SEARCH010 Study Group. Viral blips after treatment initiation during acute HIV infection. Clin Infect Dis 2019:ciz936. https://doi.org/10.1093/cid/ciz936.
[15] Sörstedt E, Nilsson S, Blaxhult A, Gisslén M, Flamholc L, Sönnerborg A, et al. Viral blips during suppressive antiretroviral treatment are associated with high baseline HIV-1 RNA levels. BMC Infect Dis 2016;16:305. https://doi.org/10.1186/s12879-016-1628-6.
[16] Xu K, Chen Y, Yuan J, Yi P, Ding C, Wu W, et al. Factors associated with prolonged viral RNA shedding in patients with COVID-19. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa351. ciaa351. Epub 9 Apr 2020.
[17] Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. PCR assays turned positive in 25 discharged COVID-19 patients. Clin Infect Dis 2020:ciaa398. https://doi.org/10.1093/cid/ciaa398. . Epub 8 Apr 2020.
[18] Ramos KJ, Kapnadak SG, Collins BF, Pottinger PS, Wall R, Mays JA, et al. Detection of SARS-CoV-2 by bronchoscopy after negative nasopharyngeal testing: stay vigilant for COVID-19. Respir Med Case Rep 2020;30:101120. https://doi.org/10.1016/j.rmcr.2020.101120.
[19] van der Heide V. Neutralizing antibody response in mild COVID-19. Nat Rev Immunol 2020;28. https://doi.org/10.1038/s41577-020-0325-2. Apr 2020.
[20] Yip CC-Y, Sridhar S, Cheng AK-W, Leung K-H, Choi GK-Y, Chen JH-K, et al. Evaluation of the commercially available LightMix® Modular E-gene kit using clinical and proficiency testing specimens for SARS-CoV-2 detection. J Clin Virol 2020;129:104476. https://doi.org/10.1016/j.jcv.2020.104476.
[21] Rao SN, Manissero D, Steele VR, Pareja J. A systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther 2020;9:573–86. https://doi.org/10.1007/s40121-020-00324-3.
[22] Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020;25:2001483. https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483.