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Short communication

Antiviral effect of favipiravir (T-705) against measles and subacute sclerosing panencephalitis viruses

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Summary

Subacute sclerosing panencephalitis (SSPE) is a late-onset, intractable, and fatal viral disease caused by persistent infection of the central nervous system with a measles virus mutant (SSPE virus). In Japan, interferon-α and ribavirin are administered intracerebroventricularly to patients with SSPE. However, as the therapeutic effect exists but is insufficient, more effective drugs are needed. Favipiravir, which is used clinically as an anti-influenza drug, is widely active against RNA viruses. In this study, the antiviral effect of favipiravir against measles virus (Edmonston strain) and SSPE virus (Yamagata-1 strain) was examined in vitro. The 50% effective concentrations of favipiravir (inhibiting viral plaque formation by 50%) for Edmonston and Yamagata-1 strains were 108.7 ± 2.0 μM (17.1 ± 0.3 μg/mL) and 38.6 ± 6.0 μM (6.1 ± 0.9 μg/mL), respectively, which were similar to those of ribavirin. The antiviral activity of favipiravir against the SSPE virus was demonstrated, for the first time, in this study.
Subacute sclerosing panencephalitis (SSPE) is a late-onset viral infection characterized by persistent infection of the central nervous system with a measles virus mutant (SSPE virus) after measles, leading to severe cognitive impairment and vegetative death in a short period. We reported the clinical efficacy of intracerebroventricular administration of interferon-α and ribavirin in a small number of patients with SSPE without serious adverse reactions (1, 2). Because the therapeutic effect exists but is insufficient, more effective drugs are needed.

Favipiravir (T-705) is an anti-influenza drug classified as an RNA polymerase inhibitor. Favipiravir is phosphorylated in the cell and incorporated into viral RNA as a nucleic acid analog during the mRNA elongation reaction. This inhibits the mRNA transcription process by stopping the elongation reaction (3, 4). Favipiravir has a broad spectrum of activity against RNA viruses other than influenza including Orthomyxoviridae, Bunyaviridae, Arenaviridae, Filoviridae, Paramyxoviridae, Flaviviridae, Togaviridae, Picornaviridae, and Caliciviridae (5, 6). Antiviral activity of favipiravir against the SSPE virus, which is a mutant strain of the measles virus in the Paramyxoviridae family, is expected, but has not yet been reported. The antiviral activity of favipiravir (Hubei Tianyao Pharmaceutical, Xianfeng, China) against a measles virus laboratory strain (Edmonston strain) and a SSPE virus clinical isolate (SSPE Yamagata-1 strain) (7) was examined by conducting the viral plaque reduction assay using African green monkey (Vero) cells in vitro (8). Vero/SLAM cells, in which human SLAM is expressed (9), were used for virus propagation. Vero cells were grown in minimum essential
medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 μg/mL gentamicin, 100 IU/mL penicillin, 1 μg/mL amphotericin B, and 0.3 mg/mL L-glutamine (10% FBS-MEM). Vero/SLAM cells were grown in 10% FBS-MEM supplemented with 0.4 mg/mL Geneticin for selection. The Edmonston strain of measles virus was used to inoculate Vero/SLAM cells, and harvested after freeze thawing the infected cells. The SSPE Yamagata-1 strain was propagated in Vero/SLAM cells, harvested by trypsinization, prepared as an infected cell suspension in Vero/SLAM cell medium supplemented with 10% dimethyl sulfoxide, and stored at −80°C until use. The plaque reduction assay was performed as follows. After discarding the cell culture medium from monolayers of Vero cell in a 12-well microplate, 50 μL of virus in 10% FBS-MEM were added to each well and shaken at room temperature for 1 h to adsorb the virus to the cells. The Edmonston strain was used to produce cell-free virions. This yielded a reproducible number of syncytia (generally 200–300 plaque-forming units) in the control wells. The Yamagata-1 strain was also used to yield a reproducible number of syncytia (generally 70–150 plaque-forming units) in control wells. Then, 1 mL of 0.75% methylcellulose-10% FBS-MEM containing serially diluted drugs was overlaid on each well, and the cells were cultured at 35°C in 5% CO₂. After incubation for an appropriate number of days, the plates were fixed in 10% formalin and the number of typical plaques was counted. The 50% effective concentration (EC50) (i.e., the concentration required to inhibit viral plaque formation by 50%) was determined on day 5 or 6, and day 3
or 4, post inoculation for the Edmonston and Ymagata-1 strains, respectively. The 50% cytotoxic concentration (CC$_{50}$), defined as the concentration required to reduce cell viability by 50% relative to that of untreated control cells, was determined by the WST-1 assay (Cell Proliferation Reagent WST-1, Sigma-Aldrich, Tokyo, Japan). The selectivity index (SI) was defined as the ratio of the CC$_{50}$ relative to the EC$_{50}$ value. Ribavirin (Adooq Bioscience, Irvine, CA, USA) and interferon-α (IFN-α) (Sumiferon Injection®, Sumitomo Dainippon Pharma, Osaka, Japan) were used as reference drugs. EC$_{50}$s of favipiravir for the Edmonston and Yamagata-1 strains were 108.7 ± 2.0 μM (17.1 ± 0.3 μg/mL) and 38.6 ± 6.0 μM (6.1 ± 0.9 μg/mL), respectively. EC$_{50}$s of ribavirin for each virus were 172 ± 49.5 μM (42.0 ± 12.1 μg/mL) and 38.1 ± 1.6 μM (9.3 ± 0.4 μg/mL), respectively. The CC$_{50}$s and SIs of both drugs were similar. Moreover, IFN-α showed high antiviral activity and SI against both viruses (Table 1). The antiviral activity of favipiravir against the SSPE virus was demonstrated, for the first time, in this study, Jochmans et al. (6) analyzed the antiviral activity of favipiravir against a broad range of paramyxoviruses and reported EC$_{90}$s of 9 ± 2 μM, 10 ± 3 μM, and 13 ± 7 μM for the Edmonston strain of the measles virus when favipiravir was added 24 h before inoculation, simultaneously with inoculation, and 24 h after inoculation, respectively. However, they did not analyze the effect of favipiravir against SSPE. The different antiviral activity of favipiravir that we found against the Edmonston strain may be partly due to differences in the assays, cells, and viruses used for the evaluation, but not the timing of cell
treatment with favipiravir relative to virus inoculation. For example, they performed a virus yield reduction assay and used a GFP-expressing Edmonston strain (MeV-Edm-GFP) and Vero/SLAM cells(6), while we performed a plaque reduction assay and used the Edmonston strain and Vero cells. In addition, the antiviral activity of favipiravir against the severe fever with thrombocytopenia syndrome virus was confirmed previously in *in vitro* and *in vivo* studies, and the antiviral effect was reported to be higher than that of ribavirin (10).

The SSPE virus has gene mutations compared with the wild-type measles virus genome. The M gene, which encodes matrix proteins, is more frequently mutated than other genes in human clinical cases, but not genes related to the mRNA elongation reaction (11). Therefore, an effect of favipiravir against the SSPE virus *in vivo* is expected. Despite this potential *in vivo* effectiveness, there is a key point that must be resolved before favipiravir can be used clinically. Favipiravir is only administered orally, but this route may not achieve a sufficient drug concentration in the cerebrospinal fluid (CSF) to treat SSPE. Because SSPE is caused by proliferation of the SSPE virus in the central nervous system, antiviral effects cannot be expected unless orally ingested favipiravir crosses the blood-brain barrier and maintains a sufficient drug concentration in the central nervous system. For ribavirin, because the oral route did not increase its concentration in the CSF sufficiently to provide antiviral activity against the SSPE virus, a liquid formulation was administered intracerebroventricularly (1). Therefore, it is necessary to investigate whether oral administration of favipiravir reaches a
sufficient concentration in the CSF. If not, it will be necessary to develop a liquid formulation and perform intravenous or intracerebroventricular administration. The measles epidemic continues in developing countries (12), and sporadic cases of measles occur in developed countries including Japan (13). The development of new SSPE drugs is an urgent task because there are no drugs that show sufficient clinical effects on SSPE.

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Conflict of interest:

The authors have no financial relationships relevant to this article to disclose. The authors have no other conflicts of interest to disclose. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
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Table 1. *In vitro* activity of T-705 against measles and SSPE viruses

| Compounds | EC$_{50}$ | CC$_{50}$ | SI  | EC$_{50}$ | CC$_{50}$ | SI  |
|-----------|-----------|-----------|-----|-----------|-----------|-----|
| Favipiravir | 108.7 ± 2.0 μM | > 1000 μM | > 9.1 | 38.6 ± 6.0 μM | > 1000 μM | > 25.9 |
| Ribavirin | 172.3 ± 49.5 μM | > 1000 μM | > 5.8 | 38.1 ± 1.6 μM | > 1000 μM | > 26.2 |
| IFN-α | 69.7 ± 60.3 IU/mL | > 100,000 IU/mL | > 1434.7 | 64.9 ± 6.7 U/mL | > 10,000 IU/mL | > 1540.8 |

EC$_{50}$, 50% effective concentration; CC$_{50}$, 50% cytotoxic concentration, SI, selectivity index. EC$_{50}$s and CC$_{50}$s are presented as means ± SD.