Effect of Favipiravir on some epidemic infections: A mini review

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Abstract. Favipiravir is a modern antiviral drug that can be used in developing viral pandemics, such as the Ebola virus, H1N1 flu, Lassa fever, and haemorrhagic fever outbreaks in Argentina in 2009. A chain conclusion Favipiravir is transformed to favipiravir-RTP and inserted into the RNA strand that is elongating. Then, since favipiravir acts as a chain terminator, chain elongation slows down at incorporation site of favipiravir and does not continue. The proofreading enzyme cannot repair this complex of RNA-favipiravir and it will be discarded as excessive RNA, which causes the extinction of the viral genome. Mutagenesis with lethal consequences Ribavirin is used in the RNA elongating process until completion. The single strand of RNA with several ribavirin incorporation sites incorporates ribavirin or acts as mRNA for the production of viral proteins. Due to incorporation of ribavirin onto viral RNA, a mismatching of the base pairs (transition mutation) will occur. Translation of this mutated RNA will then cause mutations in the sequence of amino acid residues of the protein, result in impaired of protein function. Viral proteins that have lost their function are unable to replicate or generate infectious viral particles, and the virus is no longer infectious. As a result, lethal mutagenesis ends viral infection in a different way than chain termination. Favipiravir's clinical trial against Ebola virus-infected patients has not yet been formally approved, and further research is required. Favipiravir, on the other hand, poses a risk of embryo toxicity and teratogenicity. As a result, the Japanese Ministry of Health, Welfare and Labor have been approved this medication with recommendations for more clinical use until August 15, 2014, when they approved the use of favipiravir due to the Ebola virus epidemic in West Africa. The aim of this review is to investigate the potential impact of favipiravir on infections especially RNA-virus infections, according to previous published investigations, and thus to encourage rapid approval of this interesting antivirus in emergencies.

Keyword: Favipiravir, influenza viruses, picornaviruses, Ebola virus, Lassa Virus, distemper virus

1. Introduction

Favipiravir is an inhibitor for influenza virus infections that is currently in clinical research. It is basically first discovered as a selective inhibitor of the replication of RNA of influenza A virus. Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide, Figure 1, a nucleobase mimic also known as T-705, is currently in clinical test-Phase 3 in the USA and recently approved in Japan for the treatment of seasonal influenza where it stops the replication of non-RNA viruses, including influenza [1, 2]. During the 20th century, three types of influenza pandemics occurred at several decades, according to a 2019 WHO report. Of which, the severest one was called 'Spanish Flu' (caused by an A (H1N1) virus), which caused 20-50 million deaths during 1918-1919.

During 1957-1958, a milder pandemics 'Asian Flu' caused by an A(H2N2) virus) in 1968, another virus was indicated called 'Hong Kong Flu' caused by an A(H3N2) virus; each was caused of around 1-4 million deaths. Although most pandemics of the H1N1 were mild, in 2009, it was globally estimated to cause 100,000-400,000 deaths [3,4]. This review is to explore the potential of favipitavir to act against other types of epidemic infections or pathogenic RNA viruses, such as the very recent COVID-19, and to get insight into the mechanism of favipitavir action.

2. Synthesis of favipiravir

Favipiravir was first synthesized from the starting material 2-aminoypyrazine, which is commercially
available. The proposed synthesis method (Scheme 1) consisted of seven steps and was identified as a novel and efficient 3,6-dichloropyrazine-2-carbonitrile synthesis method. The Sandmeyer reaction was diazotized/chlorinated in four stages: regioselective pyrazine ring chlorination, brominating, Pd-catalyzed cyanation, and diazotization/chlorination. This technique avoided the hazardous POCl₃ used in previous synthetic methods and yielded 1.3 times more (48%) than a recently published procedure. This protocol removed the hazardous POCl₃ of previous synthetic methods and produced a yield of 48%; 1.3 times higher than a recently published procedure. Following nucleophilic fluorination, the desired product (nitrile hydration and hydroxyl substitution) was effectively produced. Another previous synthetic process was investigated to overcome the allergy-causing dichloro using the same starting material. However, the crucial step of mono fluorination at the intermediate's pyrazine C6 has yet to be completed [5, 6].

![Scheme 1: The suggested synthetic method to prepare favipiravir and the preferred route is red highlighted](image)

### 3. The activity against influenza viruses

The viron M2 (amantadine and rimantadine) or viral neuraminidase ion channels are blocked by all anti-influenza drugs currently available (oseltamivir, zanamivir). Favipiravir works through a specific mechanism that targets viral RNA polymerases and inhibits RNA replication and transcription directly. As a result of this unique mechanism, favipiravir has become a promising candidate drug. Lots of studies have been reported to indicate its efficacy in cell culture, *in vitro* and *in vivo* [7, 8].

### 4. Mechanism of inhibition

Competition assays were assisted to explore the mechanism of inhibition of virus replication by favipiravir. Favipiravir’s efficacy against the influenza virus was reduced by adding a 10-fold excess of purine nucleosides and bases at the same time. Pyrimidine bases, on the other hand, did not [9]. Similarly, another competitive reversal of antiviral activity (specifically by purines) used lymphocytic choriomeningitis virus replicon reporter assays was indicated with arenavirus infections [10]. Based on that, Favipiravir found to be active as a pseudo purine.
5. **Activity of favipiravir against other RNA viruses**

A variety of arena-, bunya-, flavi-, and alphaviruses cause hemorrhagic fever (HF) and/or encephalitis, with high mortality rates. The majority of these severe diseases currently lack vaccines or licensed antiviral therapies, emphasizing the need for effective antiviral agents. Ribavirin is the only FDA-approved medication shown to be successful in the treatment of arena viral HF, but it is used off-label and requires historical monitoring [11]. The efficacy of favipiravir against a variety of pathogenic agents and associated viruses, such as many picornaviruses and murine norovirus, is discussed on the following sections.

5.1. **Picornaviruses**

*In vitro*, favipiravir showed inhibitory effect to replication of the foot-and-mouth disease virus (FMDV) with an EC50 of lg/ml [10]. However, *in vitro*, the analogues were more effective against this virus. By EC50 of lg/ml, favipiravir selectively inhibited poliovirus in Vero cells. In HeLa cells, favipiravir selectivity inhibited rhinovirus replication with an indexed EC50 of lg/ml [12].

5.2. **Lassa Virus Infection in Macaques**

Lassa virus (LASV; family Arenaviridae, genus Mammarenavirus) is the cause of Lassa fever, a serious hemorrhagic disease. About 300,000 people are infected with LASV per year, with 20% of those infected developing life-threatening clinical symptoms such as edema, hemorrhage, and multi-organ...
failure, resulting in 5,000 deaths. Most human diseases are acquired from the multimammate rat, which is a natural rodent reservoir [12] (Mastomys natalensis). There is human-to-human transmission, which is mainly nosocomial. LASV is only found in West Africa and has a well-defined endemic area. The highest prevalence of LASV infections is in Nigeria, Sierra Leone, Liberia, and Guinea [13,14], although moderate outbreaks of Lassa fever have been recorded in several other West African countries.

The etiological factor of Lassa fever, a severe hemorrhagic disease, is Lassa virus (LASV; Arenaviridae family, Mammarenavirus genus). About 300,000 people are infected with LASV per year, with 20% of those infected developing life-threatening clinical symptoms such as edema, hemorrhage, and multi-organ failure, and 5,000 people dying. Most human infections develop from a natural source (the multimammate rat Mastomys natalensis) [12]. Human-to-human transmission, which is often nosocomial, occurs.

5.3. The epidemic of Ebola virus (EBOV)

The epidemic of Ebola Virus (EBOV) disease prevalence in western Africa in 2013-2016 highlighted the need for more efficient pharmacological therapy for viral replication and treatment of the consequences of infection, including more than 28,000 confirmed or suspected cases and more than 11,000 deaths. No licensed medicines for treatment of the EBOV and other filovirus diseases exist at the moment, despite efforts to develop vaccines and antivirals [15]. When assessed with Usamriid and others, Favipiravir has demonstrated its efficacy in EBOV mouse models [16,17]. Favipiravir was evaluated in the phase II clinical trial for West Africa in the 2013-2016 EBOV outbreak and administered to several patients, including one combined with convalescent plasma, in compassionate use protocols [18, 19]. The retrospective clinical case study in Sierra Leone has demonstrated survival benefits and reduced viral stress associated with the treatment of favipiravir [22,23].

5.4. Canine distemper virus

Canine distemper virus (CDV), a highly contagious pathogen in the Morbillivirus - Paramyxoviridae family, causes a multi-systemic disease in carnivores and extreme immunosuppression [24, 25]. Canidae, Felidae, Mustelidae, Procyonidae, Ailuridae, Mephitidae, Hyaenidae, Phocidae and Viverridae, are among the animal families infected by CDV [26, 27]. It causes some of health disorders, in the respiratory, gastrointestinal, and neurological systems. It effectively blocked viral replication in CDV-infected Vero and DH82 cells, suggest that it could be used to treat CDV infections in the future. Indirect immunofluorescence monitoring was used for characterization the growth properties of the CDV-3 and CDV-11 strains in Vero and DH82 cells (IFA). The cell lines inoculated with CDV-3 or CDV-11 both Vero and DH82 revealed strong positive reaction signal with anti-CDV N monoclonal antibody (figure 1c-f); however, mock cells did not indicate positive reaction with anti-CDV N monoclonal antibody (figure 1a and b). The viral titers of the culture viruses were also assessed with a 50% tissue culture infectious dose per milliliter test (TCID50) (figure 1g). CDV-3 and CDV-11 viral titers in Vero cells peaked at 105.5 and 106.6 TCID50/ml after 72 hours, respectively, and then plateaued between 72 and 96 hours. CDV showed a steady increase in viral titers in DH82 cells over the course of the study, with viral titers of CDV-3 and CDV-11 peaking at around 105.5 TCID50/ml at 96 hours. Interestingly, favipiravir at a concentration from 2.441 g/ml to 1250 g/ml was previously inhibited CDV-3 and CDV-11 replication in Vero and DH82 cells. When given at fixed time intervals after virus infection, favipiravir showed efficacious antiviral effects. Vero cells reported a slight decrease in viability after favipiravir treatment, but favipiravir-treated DH82 cells showed no cytotoxicity. Anti-CDV polyclonal serum only inhibited CDV in the supernatant, nevertheless, favipiravir directly reduced viral replication in cells, as well as, indirectly minimized the number of virions in the supernatant.
Favipiravir and anti-CDV polyclonal serum were then combined and potentially rapidly inhibited the virions in supernatant and virus replication in cells [28].

**Figure. 1.** CDV-3 and CDV-11 growth characteristics in Vero and DH82 cells. At a MOI of 0.1, cells were infected with CDV-3 or CDV-11 and incubated at 37 °C. At 0, 24, 48, 72, and 96 hours, viruses were collected. Both experiments were done in triplicate. Controls included Vero cells (A) and DH82 cells (B) with no CDV infection. At a MOI of 0.1, Vero cells were contaminated with CDV-3 (C) and CDV-11 (E). CDV-3 (D) and CDV-11 (F) were infected at a MOI of 0.1 in DH82 cells. After three days of incubation, the cells were fixed and stained with specific antibody for nucleoprotein (CDV monoclonal) and curves of CDV growth in Vero and DH82 cells were studied [28]. Images=200x magnification.

6. Conclusion
This review shades light on the effectiveness of favipiravir to suppress viral replication inside the infected cells, suggests that it has a lot of promise as a treatment for other RNA viruses like COVID-19. Because of the potential for enhanced efficacy in humans, the availability of oral administration, and advanced preclinical reports (announced by the US Food and Drug Administration), it is recommended that favipiravir should be clinically tested as a COVID-19 virus treatment.

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7. References
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