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Review

Favipiravir (T-705), a novel viral RNA polymerase inhibitor

Yousuke Furuta [a,*], Brian B. Gowen [b], Kazumi Takahashi [a], Kimiyasu Shiraki [c], Donald F. Smee [b], Dale L. Barnard [b]

[a] Toyama Chemical Co., Ltd., 4-1, Shimookui 2-chome, Toyama 930-8508, Japan
[b] Institute for Antiviral Research and Department of Animal, Dairy and Veterinary Sciences, Utah State University, 5600 Old Main Hill, Logan, UT 84322-5600, USA
[c] Department of Virology, Toyama University, 2630 Sugitani, Toyama 930-0194, Japan

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ABSTRACT

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is an antiviral drug that selectively inhibits the RNA-dependent RNA polymerase of influenza virus. It is phosphoribosylated by cellular enzymes to its active form, favipiravir-ribofuranosyl-5′-triphosphate (RTP). Its antiviral effect is attenuated by the addition of purine nucleic acids, indicating the viral RNA polymerase mistakenly recognizes favipiravir-RTP as a purine nucleotide. Favipiravir is active against a broad range of influenza viruses, including A(H1N1)pdm09, A(H5N1) and the recently emerged A(H7N9) avian virus. It also inhibits influenza strains resistant to current antiviral drugs, and shows a synergistic effect in combination with oseltamivir, thereby expanding influenza treatment options. A Phase III clinical evaluation of favipiravir for influenza therapy has been completed in Japan and two Phase II studies have been completed in the United States. In addition to its anti-influenza activity, favipiravir blocks the replication of many other RNA viruses, including arenaviruses (Junin, Machupo and Pichinde); phleboviruses (Rift Valley fever, sandfly fever and Punta Toro); hantaviruses (Maporal, Dobrava, and Prospect Hill); flaviviruses (yellow fever and West Nile); enteroviruses (polio- and rhinoviruses); an alphavirus, Western equine encephalitis virus; a paramyxovirus, respiratory syncytial virus; and noroviruses. With its unique mechanism of action and broad range of antiviral activity, favipiravir is a promising drug candidate for influenza and many other RNA viral diseases for which there are no approved therapies.

Contents

* Corresponding author. Address: Research Administration Department, Toyama Chemical Co., Ltd., 4-1, Shimookui 2-chome, Toyama 930-8508, Japan. Tel.: +81 76 431 8254; fax: +81 76 431 8208.
E-mail address: yousuke_furuta@toyama-chemical.co.jp (Y. Furuta).

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1. Introduction

Favipiravir is a novel antiviral compound that selectively and potently inhibits the RNA-dependent RNA polymerase (RdRP) of influenza and many other RNA viruses. As described below, it has been found to inhibit all serotypes and strains of influenza A, B and C viruses against which it has been tested, including those resistant to currently approved neuraminidase inhibitors. It is also active against a number of arena-, bunya- and flaviviruses, both *in vitro* and in rodent models, and it has shown potent *in vitro* activity against members of the alphavirus, paramyxovirus and norovirus families. This paper reviews current understanding of the antiviral mechanism of action of favipiravir and the breadth of its *in vitro* and *in vivo* inhibitory activity against a broad spectrum of RNA viruses.

2. Structure

Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), originally known as T-705, and the related pyrazinecarboxamide compounds T-1105 and T-1106 were discovered and synthesized by Toyama Chemical Co., Ltd. Their structures are shown in Fig. 1.

Through the screening of a chemical library of Toyama Chemical Co., Ltd. using a plaque reduction assay for anti-viral activity against influenza virus A/PR/8/34, a lead compound, which was designated as T-1105 afterward, was found to be effective. Thereafter, its derivatives were synthesized to evaluate structure–activity studies in terms of *in vitro* and *in vivo* antiviral activities as well as pharmacokinetic properties. Then T-705 and the related compounds were selected for further investigation as drug candidates.

3. Antiviral activity against influenza viruses

Existing anti-influenza virus drugs inhibit the virion M2 ion channel (amantadine and rimantadine) or the viral neuraminidase (oseltamivir, zanamivir). The mechanism of action of favipiravir through direct inhibition of viral replication and transcription is therefore unique among anti-influenza drugs. Since RdRP domains are not present in human cells, and are conserved among RNA viruses, this distinct specific mechanism targeting RNA viral polymerases makes favipiravir an attractive drug candidate. Thus, a number of *in vitro* and *in vivo* studies have been done to demonstrate efficacy in cell culture and in animal models, which are described below.

3.1. In vitro anti-influenza activity

Studies using laboratory strains of influenza virus showed that favipiravir inhibits all types of influenza virus strains of type A, B, and C. Based on plaque reduction in MDCK cells, its 50% effective concentrations (EC_{50}s) were in the range of 0.014–0.55 μg/ml (Furuta et al., 2002). Table 2 summarizes the activity of favipiravir against 53 strains of influenza virus, including seasonal strains A(H1N1), A(H3N2), and influenza B; the A(H1N1)pdm09 pandemic virus; highly pathogenic avian influenza virus A(H5N1) isolated from humans; A(H1N1) and A(H1N2) isolated from swine; and A(H2N2), A(H4N2) and A(H7N2). These included a large number of strains resistant to currently used drugs including amantadine, rimantadine, oseltamivir, and zanamivir. Some strains were also resistant to both M2 and NA inhibitors. Favipiravir has shown a wide range of antiviral activity against all strains including drug-resistant viruses (Sleeman et al., 2010). The 50% cytotoxic concentration (CC_{50}) of favipiravir in host MDCK cells was more than 2000 μg/ml, demonstrating the highly selective inhibition of influenza virus replication (i.e., selective indices of greater than 3000) (Furuta et al., 2002).

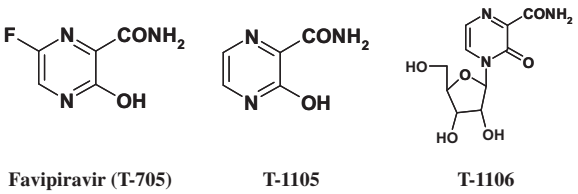

Fig. 1. Chemical structure of favipiravir (T-705), T-1105 and T-1106.

Table 1
Selectivity of favipiravir-RTP on DNA and RNA polymerase activity. (Based on Kiso et al., 2010 and Takahashi et al., 2011.)

Polymerase	Polymerase Type	IC_{50}(μM)
Influenza virus		
RNA polymerase	RNA-dependent RNA polymerase	0.341
Human		
DNA polymerase α	DNA-dependent DNA polymerase	>1000
DNA polymerase β	DNA-dependent DNA polymerase	>1000
DNA polymerase γ	DNA-dependent DNA polymerase	>1000
RNA polymerase II	DNA-dependent RNA polymerase	905

This table shows the 50% inhibitory concentration (IC_{50}) of favipiravir-RTP to influenza virus RNA polymerase, human DNA polymerase (α, β, γ) and human RNA polymerase II. One unit each of human DNA polymerase α, β and γ and human RNA polymerase II were incubated for 1 h with reaction mixture containing ^{3}H-dGTP or ^{3}H-UTP.

3.2. In vivo anti-influenza activity

Favipiravir has also been shown to protect mice against lethal infection by a variety of influenza virus strains (Table 3). For comparison, the dose of oseltamivir was set at 10 mg/kg/day for seasonal A(H3N2) infection in mice and at 20 mg/kg/day for A(H5N1) virus infections. When favipiravir was orally administered 2 or 4 times a day for 5 days in mice infected with lethal doses of influenza virus A/Victoria/3/75(H3N2), A/Osaka/5/70(H3N2) or A/Duck/MN/1525/81(H5N1), improved survival compared to placebo was shown at a dose of 30 mg/kg/day or more. The drug also provided significant protection against the A/Duck/MN/1525/81(H5N1) virus at a dose of 33 mg/kg/day or more, regardless of the number of daily doses. When given 4 times a day, all mice survived. In contrast, oseltamivir therapy failed to impact survival at a dose of 20 mg/kg twice daily for 5 days (Sidwell et al., 2007).

Mice infected with the A/California/04/09(H1N1) virus or A/Anhoi/1/2013(H7N9) virus were also studied for the effect of favipiravir on pulmonary viral load on the third and sixth days after infection. Treatment with 60 and 300 mg/kg/day reduced viral replication in a dose-dependent manner. The inhibitory activity was the same or greater than that of oseltamivir and zanamivir against the A(H1N1)pdm09 virus (Itoh et al., 2009) and A(H7N9) virus (Watanabe et al., 2013). Favipiravir was also found to have a significant therapeutic effect compared to oseltamivir in mice challenged with a 100-fold larger dose of virus, and when treatment was delayed until 96 h post infection (Sidwell et al., 2007; Takahashi et al., 2003).

3.3. Combinations with other drugs

The effect of combining favipiravir and oseltamivir was investigated in mice infected with A/Victoria/3/75(H3N2). The combination of 25 mg/kg of each drug provided significant protection against a lethal challenge dose (Table 4). Synergistic improvements in survival were also achieved with the 20 mg/kg/day dose of

Table 2
Virus susceptibility to favipiravir testing by plaque reduction assays in MDCK cells. (Based on Sleeman et al., 2010).

Viral Type	Favipiravir EC_{50}:μg/ml (μM)	No. of strains	No. of drug-resistant strains[a]		
			A	O	Z
A(H1N1)	0.03–0.79 (0.19–5.0)	15	3	8	2
A(H3N2)	0.07–0.94 (0.45–6.0)	9	7	4	1
B	0.09–0.83 (0.57–5.3)	8	8	4	2
A(H2N2)	0.06 (0.38)	1	0	0	0
A(H4N2)	0.14–0.15 (0.89–0.96)	2	0	0	1
A(H7N2)	0.24–1.60 (1.5–10.2)	2	1	0	0
A(H5N1)[b]	0.20–0.82 (1.3–5.2)	6	3	3	2
A(H1N1)[c]	0.13–0.71 (0.83–4.5)	2	0	0	0
A(H1N2)[c]	0.35 (2.2)	1	0	0	0
A(H1N1)2009	0.13–3.53 (0.83–22.5)	7	7	2	0

EC_{50}, 50% effective concentration.
[a] Number of strains resistant to adamantanes (A), oseltamivir (O), or zanamivir (Z). Changes to M2 and NA were detected by surveillance criteria (Sheu et al., 2008).
[b] Isolated from both humans and birds.
[c] Swine origin which were isolated from human.

Table 3
Therapeutic effects of favipiravir in mouse influenza infection models. (Based on Sidwell et al., 2007; Smee et al., 2007 and Takahashi et al., 2011).

Strain (infectious dose)	Treatment	Dose (mg/kg/day)	Survivors/Total[a]	Regimen
A/Victoria/3/75 (H3N2) [LD_{100}][b]	Control (Placebo)	–	0/20	Every 6 h for 5 days
	Favipiravir	1	0/10	
		3	0/10	
		10	1/10	
		30	7/10**	
		100	10/10**	
		300	10/10**	
	Oseltamivir	10[c]	5/10*	Twice daily for 5 days
A/Osaka/5/70 (H3N2) [3×10^3 PFU/mouse]	Control (Placebo)	–	0/10	Twice daily for 5 days
	Favipiravir	10	1/10	
		30	9/10++	
		100	9/10++	
A/Duck/MN/1525/81 (H5N1) [LD_{100}][b]	Control (Placebo)	–	0/20	Twice daily for 5 days
	Favipiravir	3	0/10	
		10	2/10	
		30	8/10**	
		100	10/10**	
		300	10/10**	
A/Duck/MN/1525/81 (H5N1) [LD_{100}][b]	Control (Placebo)	–	0/20	Every 6 h for 5 days
	Favipiravir	33	10/10**	
		100	10/10**	
		300	10/10**	
	Oseltamivir	20[c]	2/10	Twice daily for 5 days

[a] Number of mice surviving until the end of the experiment (21 days) over the total number of animals in the group.
[b] 100% lethal infectious dose.
[c] Reduced oseltamivir dose.
* $P < 0.01$ compared to control group (Yates-corrected Chi-square test).
** $P < 0.001$ compared to control group (Yates-corrected Chi-square test).
++ $P < 0.001$ compared to control group (Kaplan–Meier method, Log-rank test).

favipiravir combined with 0.1 and 0.3 mg/kg/day of oseltamivir against the A/NWS/33(H1N1) virus. In mice infected with A/Duck/MN/1525/81(H5N1), combining doses of both drugs, which were ineffective as monotherapies, significantly improved survival and body weights (Smee et al., 2010). An increase in the numbers of survivors was also shown using a synergistic combination of 20 mg/kg/day of favipiravir with ineffective low doses of peramivir against the A/California/04/2009(H1N1)pdm infection (Tarbet et al., 2012). These results underline the value of combining favipiravir with NA inhibitors to improve the outcome of severe influenza virus infections, augmenting therapeutic options for outbreak management.

3.4. Clinical evaluation

Favipiravir has been administered to over 1400 subjects in clinical trials in many countries around the world, and has an excellent safety record. A Phase 3 study was completed in Japan and two Phase 2 studies have been completed under a U. S. IND, the latter funded by the US Department of Defense (DOD). Descriptions of

Table 4
Effects of combinations of favipiravir and oseltamivir on an influenza A/Victoria/3/75 (H3N2) virus infection in mice with treatments started 24 h after infection[a]. (Based on Smee et al., 2010.)

Treatment (mg/kg/day)	Survivors/Total	Day of death[b] (mean ± SD)
Control (Placebo)	0/20	8.2 ± 1.1
Favipiravir (100)	7/9*	5.5 ± 0.7
Favipiravir (50)	7/10*	8.3 ± 1.2
Favipiravir (25)	1/10	7.9 ± 1.5
Oseltamivir (50)	6/10*	8.8 ± 1.5
Oseltamivir (25)	1/9	7.5 ± 1.3
Favipiravir (100) + Oseltamivir (50)	10/10*	>21
Favipiravir (100) + Oseltamivir (25)	10/10*	>21
Favipiravir (50) + Oseltamivir (50)	9/10*	8
Favipiravir (50) + Oseltamivir (25)	9/10*	8
Favipiravir (25) + Oseltamivir (50)	10/10*	>21
Favipiravir (25) + Oseltamivir (25)	9/10*,+	8

[a] Oral treatments were given twice a day for 7 days starting 24 h after infection.
[b] Results for day of death are shown for mice that died prior to day 21.
* $P < 0.001$ compared to control group (Fisher's exact test).
+ $P < 0.01$ compared to either compound used alone (Fisher's exact test).

past and current trials of favipiravir under the U. S. program can be found on the website www.clinicaltrials.gov.

4. Mechanism of action

As part of the effort to clarify its mechanism of action, time-of-addition studies were performed by exposing influenza-infected cells to favipiravir at different times during the virus replication cycle and measuring virus yields 10 h after infection were measured by plaque assay. When the drug was added during the viral replication stage (0–2, 2–4, or 4–6 h after infection), yields were significantly reduced (Fig. 2). No inhibitory activity was seen when favipiravir was added at the adsorption stage (at the same time of virus exposure) or at the release stage, 6 or more hours after infection. These results indicate that favipiravir interferes with viral replication (Furuta et al., 2005). Time-of-addition experiments in arenavirus-infected cells also demonstrated that an early to intermediate stage of viral replication is disrupted (Mendenhall et al., 2011a).

The mechanism of inhibition of virus replication by favipiravir was also investigated through competition assays. The simultaneous addition of a 10-fold excess of purine nucleosides and bases reduced its activity against influenza virus, while pyrimidine bases did not (Fig. 3) (Furuta et al., 2005). Similar competitive reversal of antiviral activity specifically by purines was observed with arenavirus infection, using a lymphocytic choriomeningitis virus replicon reporter assay (Mendenhall et al., 2011a). These results suggested that favipiravir acts as a pseudo purine.

To investigate this possibility further, cellular metabolites extracted from Madin Darby canine kidney (MDCK) cells treated with favipiravir were examined by HPLC. Favipiravir ribofuranosyl-5′-triphosphate (favipiravir-RTP) was identified, as was favipiravir-ribofuranose and favipiravir-ribofuranosyl-5′-monophosphate (favipiravir-RMP) (Furuta et al., 2005; Naesens et al., 2013). Metabolism of favipiravir to its triphosphate form was shown to occur in an extracellular concentration-dependent manner (Smee et al., 2009). Favipiravir-RTP was then chemically synthesized and the incorporation of ^{32}P GTP by the influenza viral RNA polymerase in the presence of varying amounts of favipiravir-RTP was measured. Favipiravir-RTP inhibited the polymerase at nanomolar to micromolar concentrations. Favipiravir and favipiravir-RMP did not inhibit influenza RNA polymerase activity. These results

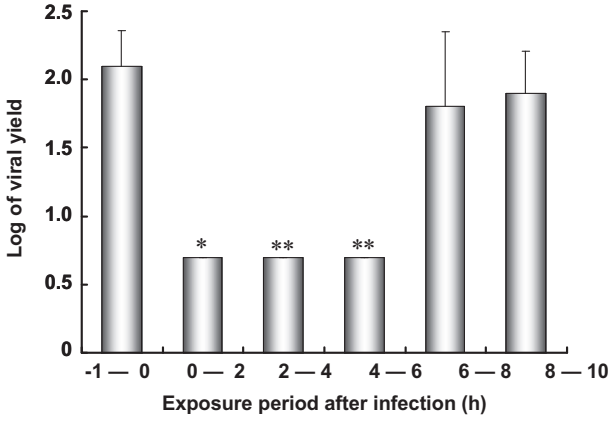

Fig. 2. Favipiravir time-of-addition analysis. MDCK cells were inoculated with influenza A/PR/8/34 virus at a multiplicity of infection of 0.001. Favipiravir was added at the indicated periods. Viral yields were determined at 10 h post-infection by plaque assays. The columns show the mean of viral yield in cells treated with favipiravir (10 μg/ml). Vertical lines represent standard deviations ($n = 3$). Two independent experiments were done, and representative data are shown. *$P < 0.05$, **$P < 0.01$, compared to respective control groups (Student's t test). (Based on Furuta et al., 2005.)

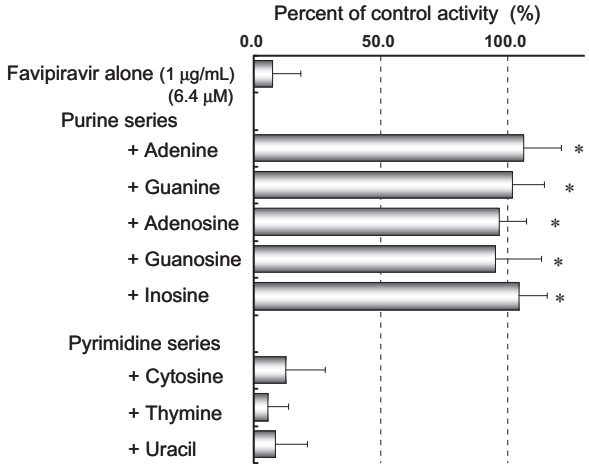

Fig. 3. Competetive inhibition of favipiravir activity by addition of excess nucleic acids and nucleosides. The number of plaque was counted and is given as a percentage of the number of plaques for the nontreated control. All nucleosides and bases were added at 10-fold molarity of the 50% effective concentration (EC_{50}) of favipiravir. *$P < 0.01$ compared to favipiravir alone treated group (Dunnett's test). (Based on Furuta et al., 2005).

indicated that favipiravir is a prodrug that is phosphoribosylated in cells to the active form, favipiravir-RTP, which inhibits influenza viral replication. Thus, favipiravir may be misincorporated in nascent viral RNA, or it may act by binding to conserved polymerase domains, preventing incorporation of nucleotides for viral RNA replication and transcription.

In support of the misincorporation theory, it has recently been shown that favipiravir induces lethal mutagenesis *in vitro* during influenza virus infection. Favipiravir showed significant decrease of viral titer both at a low multiplicity of infection (MOI; 0.0001 PFU/cell) and a high MOI (10 PFU/cell). Sequence analysis of various nucleoprotein (NP) clones revealed an increase in the number of detectable G→A and C→T transversion mutations, along with an increased mutation frequency, and a concomitant shift in the nucleotide profiles of the NP gene analyzed from various clones. However, no viable T-705 resistant mutants could be isolated. These results suggested that favipiravir has virucidal effect (Baranovich et al., 2013). It is assumed that a similar mechanism might occur with other viruses inhibited by favipiravir, which may account for the broad-spectrum virus inhibition by favipiravir.

Recently, two studies have been reported for the mechanism of inhibition of favipiravir-RTP against influenza virus RdRP. Jin et al., developed the assay of adding the RNA template, which was constructed to form the panhandle structure, to the recombinant influenza virus RNA polymerase PA/PB1/PB2 complex for observing incorporation by influenza virus RdRP in the elongation mode (Jin et al., 2013). They indicated that favipiravir-RTP was efficiently incorporated into nascent RNA strand and the single incorporation of favipiravir-RTP molecule partially prevents further extension of the RNA strand and then consecutive incorporation of favipiravi-RTP thoroughly prevent further extension of the RNA strand. On the other hand, Sangawa et al., indicated that a single molecule of favipiravir-RTP was incorporated into the nascent RNA strand and inhibits further strand extension by observing the cap snaching and initiation reaction of transcription of influenza virus RNA polymerase by adding ^{32}P-labeled 5′cap1 RNA into virus- derived RNA complex (Fig. 4) (Sangawa et al., 2013).

To investigate the impact of favipiravir on host cell and viral nucleotide metabolism, the inhibitory activity of favipiravir-RTP was examined against human RNA and DNA polymerases and on viral RdRP. As shown in Table 1, favipiravir-RTP inhibited the influenza viral RdRP activity at a 50% inhibitory concentration (IC_{50}) of 0.341 μM, but 50% inhibition of the human DNA polymerase α, β or γ was not observed even at 1000 μM (Kiso et al., 2010). The IC_{50} for the human RNA polymerase II was 905 μM (Takahashi et al., 2011); favipiravir was therefore 2650 times more selective for the influenza virus RdRP, consistent with the lack of inhibition of host-cell DNA and RNA synthesis (Furuta et al., 2005).

The mechanism of action of favipiravir was also compared with that of ribavirin, a guanosine analog that also has antiviral activity against a wide range of RNA viruses after phosphorylation. Ribavirin monophosphate (ribavirin-MP) inhibited inosine monophosphate dehydrogenase (IMPDH) in host cells (Streeter et al., 1973), resulting in reduced GTP levels. Hence, cellular and viral RNA polymerase activities were reduced due to limited substrate. When the inhibitory activities of favipiravir-RMP and ribavirin-MP against IMPDH were directly compared, the IC_{50} values were 601 μM and 3.9 μM, respectively. Since favipiravir-RMP was 150 times weaker than ribavirin-MP in its IMPDH inhibitory effect, IMPDH does not seem to be a major target enzyme for favipiravir (Furuta et al., 2005). In confirmation of these results, GTP levels in MDCK cells were reduced to a much greater extent by ribavirin than by favipiravir at equimolar extracellular concentrations (Smee et al., 2009). Collectively, the data thus far indicated that the primary mechanism of action of favipiravir was specific inhibition of viral RNA polymerase (Furuta et al., 2005; Mendenhall et al., 2011a). Fig. 5 shows a schematic representation of the intracellular conversion and the mechanism of action of favipiravir, and the influenza virus replication cycle showing the stages targeted by favipiravir-RTP and existing drugs (Furuta et al., 2009).

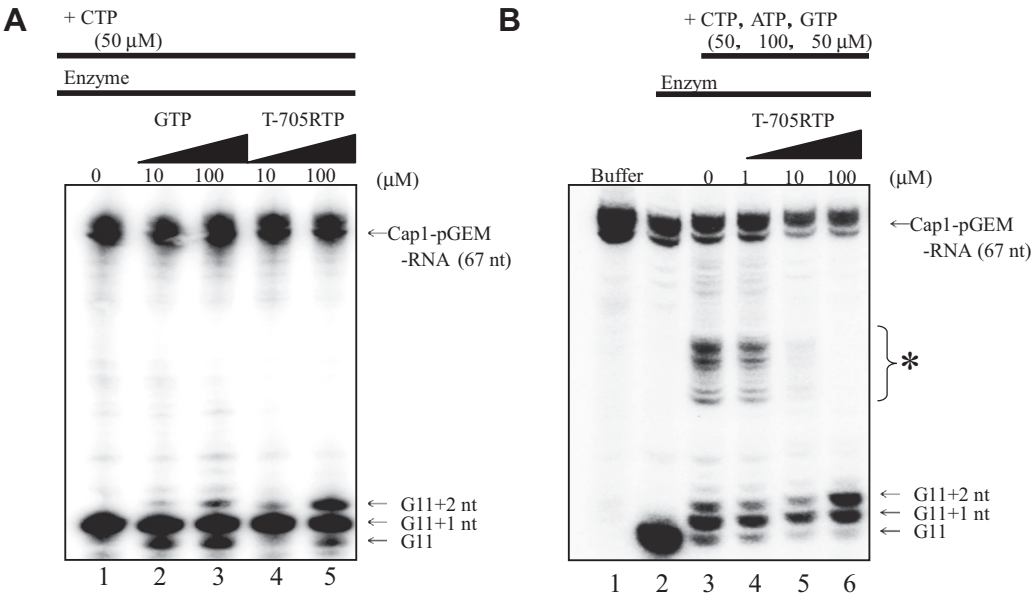

Fig. 4. Incorporation and inhibition of T-705RTP against influenza virus RdRP. (A) Incorporation of GTP and T-705RTP at the position of G11 + 2. The 32P-labeled pGEM-7zf (+) DNA run-off transcript with a 5′Cap1 structure (Cap1-pGEM-mRNA), crude influenza virus RdRp containing a viral genome, and nucleotides including T-705RTP were incubated. Reaction products were then electrophoresed. Lane 1–5: Cap1-pGEM-mRNA and crude enzyme solution +50 μM CTP; Lane 2, 3: Conditions of lane 1 + 100 and 1000 μM GTP; Lane 4, 5: Conditions of lane 2 + 100 and 1000 μM T-705RTP. (B) Inhibition of T-705RTP against influenza virus RdRp. The 32P-labeled pGEM-7zf (+) DNA run-off transcript with a 5′Cap1 structure (Cap1-pGEM-mRNA), crude influenza virus RdRp containing a viral genome, and nucleotides including T-705RTP were incubated. Reaction products were then electrophoresed. Lane 1: Cap1-pGEM-mRNA; Lane 2–6: Cap1-pGEM-mRNA + crude enzyme solution; Lane 3–6: Conditions of lane 2 + 50 μM CTP, 100 μM ATP, 50 μM GTP; Lanes 4–6: Conditions of lane 3 + 10, 100, and 1000 μM T-705RTP *Elongated RNA was detected when GTP, ATP, and CTP were added to the reaction mixture.

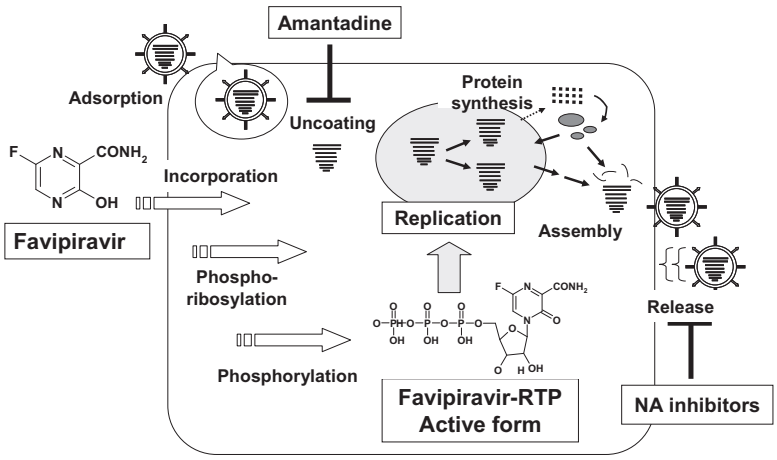

Fig. 5. Mechanism of action of favipiravir. Favipiravir is converted to favipiravir-RTP by host cell enzymes and selectively inhibits the activity of the influenza viral RNA polymerase. (Furuta et al., 2009.)

Table 5
In vitro inhibitory effects of favipiravir and ribavirin against arenavirsues. (Based on Gowen et al., 2007 and Mendenhall et al., 2011a).

Virus[a]	Strain	Favipiravir[b]			Ribavirin[b]		
		CC_{50} ± SD	$EC_{50\ or\ 90}$ ± SD	SI	CC_{50} ± SD	$EC_{50\ or\ 90}$ ± SD	SI
JUNV	Candid 1	188 ± 53 (1197 ± 337)	0.79 ± 0.47 (5 ± 3)	239	51 ± 15 (209 ± 61)	2.7 ± 2.2 (11 ± 9)	19
PICV	An 4763	175 ± 63 (1114 ± 401)	0.94 ± 0.47 (6 ± 3)	186	38 ± 21 (156 ± 86)	3.2 ± 2.2 (13 ± 9)	12
TCRV	TRVL 11573	214 ± 31 (1362 ± 197)	0.94 ± 0.63 (6 ± 4)	227	68 ± 8.1 (278 ± 33)	2.4 ± 0.73 (10 ± 3)	28
GTOV	S-26764	>157 (>1000)	6.8 ± 3.1 (43 ± 20)	>23	>244 (> 1000)	74 ± 52 (303 ± 228)	>3.3
JUNV	Romero	>157 (>1000)	3.3 ± 3.0 (21 ± 19)	>48	>244 (> 1000)	12 ± 20 (71 ± 81)	>20
MACV	Carvallo	>157 (>1000)	8.4 ± 1.7 (53 ± 11)	>19	>244 (> 1000)	17 ± 5.1 (122 ± 13)	>14

[a] JUNV, Junin virus; PICV, Pichinde virus; TCRV, Tacaribe virus; GTOV, Guanarito virus; MACV, Machupo virus.
[b] 50% cytotoxic concentration (CC_{50}) and 50% effective concentration (EC_{50}) values are in μg/ml for JUNV (Candid 1), PICV and TCRV determined by CPE reduction assays using Vero cells, and SI (selectivity index) is calculated as CC_{50}/EC_{50}. Ninety percent effective concentration (EC_{90}) values are in μg/mL for GTOV, JUNV (Romero) and MACV by plaque and focus-forming unit reduction assays using Vero E6 cells. The values noted in brackets are in μM.

5. Activity against other pathogenic RNA viruses

A number of arena-, bunya-, flavi-, and alphaviruses cause hemorrhagic fever (HF) and/or encephalitis, with high case fatality rates. No vaccines or approved antiviral therapies are available for most of these severe diseases, underscoring the urgent need for effective broad-spectrum antiviral agents. Ribavirin is the only licensed drug that has been shown to be effective against arenaviral HF, but its use is off-label and activity is based on comparison with historical controls (McCormick et al., 1986). The following sections review efficacy data for favipiravir against a number of these pathogenic agents and related viruses, including several picornaviruses and murine norovirus.

5.1. Arenaviruses

An increasing number of arenaviruses have been found to cause severe disease in humans (Moraz and Kunz, 2011). Aside from ribavirin, which has toxicity concerns, there are no small-molecule drugs approved for therapeutic use. *In vitro* studies have compared the inhibitory activity of favipiravir and ribavirin against pathogenic arenaviruses (Table 5). In assays measuring the reduction of cytopathic effect (CPE), the EC_{50}s for favipiravir were 0.79–0.94 μg/ml (5–6 μM) against the Candid #1 strain of Junin virus (JUNV), Pichinde virus (PICV), and Tacaribe virus. In plaque and focus-forming unit (FFU) reduction assays, the EC_{90}s against highly pathogenic strains of Guanarito virus, JUNV (Romero), and Machupo virus were 3.3–8.4 μg/ml (21–53 μM).

Twice-daily treatment regimens prevented the death of PICV-infected hamsters, reduced serum and tissue viral loads and prevented the development of liver disease (Gowen et al., 2007). Favipiravir also protected hamsters challenged with PICV even when treatments were initiated on days 4, 5 or 6 of infection (late stages of infection), in the face of substantial viral loads (Gowen et al., 2008). Remarkably, the drug was also effective in PICV-infected guinea pigs, even when treatment was delayed until after the onset of signs of illness (Mendenhall et al., 2011b). Fig. 6 shows the effect of favipiravir treatment of PICV-infected guinea pigs beginning 7 days after infection. A dose of 300 mg/kg/day showed effects on survival (Fig. 6A), preservation of body weight (Fig. 6B), and reduction of fever (Fig. 6C), equal to or better than those obtained with 50 mg/kg/day of ribavirin. Serum aspartate aminotransferase (AST), a prognostic marker of disease severity in Lassa fever (McCormick et al., 1986), was significantly reduced in a dose-dependent manner when assessed on day 10 (Fig. 6D). Viremia was reduced by a mean of 2.1, 1.3, and 1.6 $\log_{10}$ $CCID_{50}$/ml in the high- and intermediate-dose groups and in the ribavirin-treated group, respectively (Fig. 6E). These studies are guiding the further evaluation of favipiravir in guinea pigs challenged with JUNV, the highly pathogenic agent of Argentine HF.

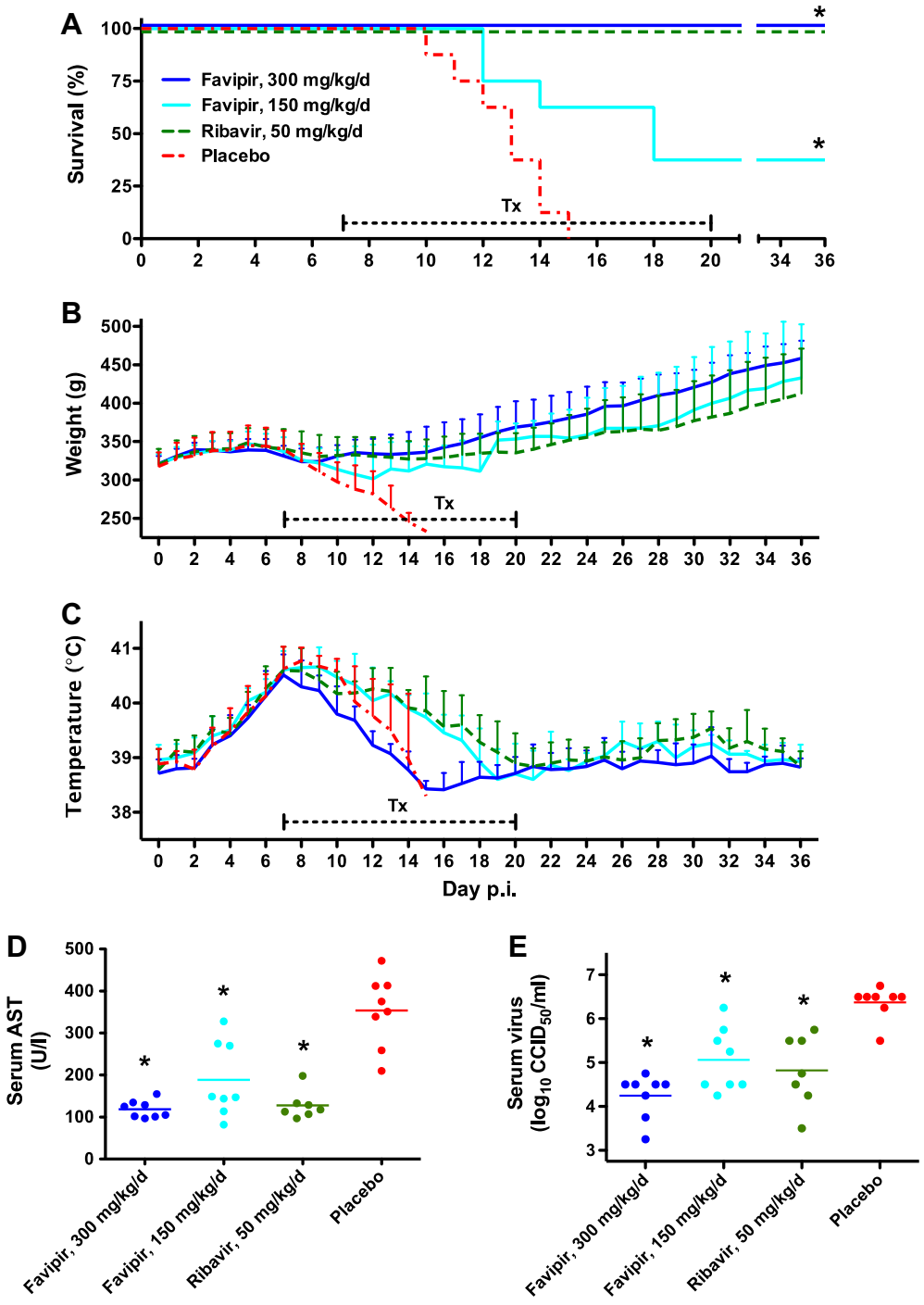

Fig. 6. Favipiravir treatment of advanced PICV infection in guinea pigs. Guinea pigs (n = 7–8/group) challenged with 500 PFU of p19 PICV were treated with the indicated dosages of favipiravir, ribavirin, or placebo beginning on day 7 of infection. The 150-mg/kg/d group received a loading dose of 300 mg/kg/d on the first day of treatment. Drugs were administered twice daily for 14 days (capped hashed line) and (A) survival, (B) body weights, and (C) temperatures were monitored for 36 days. Serum was collected on day 10 for analysis of (D) AST and (E) viremia. *$P < 0.05$ compared to placebo-treated animals. (Mendenhall et al., 2011b).

5.2. Bunyaviruses

In the family *Bunyaviridae*, there are many viruses like La Crosse virus (LACV), Rift Valley fever virus (RVFV), Crimean-Congo HF virus and hantavirus. They cause severe symptoms in humans such as hemorrhagic fever, severe fever with thrombocytopenia, and re-anl or pulmonary syndrome. When tested against a range of bunyaviruses in cell culture, favipiravir has greater activity and selectivity than ribavirin (Table 6). Its EC_{50}s against LACV, several phleboviruses including RVFV, Punta Toro virus (PTV), and sandfly fever virus, and the Dobrava, Maporal, and Prospect Hill hantaviruses were in the range of 5–30 μg/ml (32–191 μM), as measured by CPE or FFU reduction assays. Twice-daily treatment of mice and hamsters infected with PTV prevented death, reduced serum and tissue viral loads, and prevented liver dysfunction (Gowen et al., 2007, 2010).

5.3. Flaviviruses

Favipiravir inhibits several pathogenic flaviviruses including yellow fever virus (YFV) and West Nile virus (WNV) (Julander et al., 2009a; Morrey et al., 2008). However, considerably higher

Table 6
In vitro inhibitory effects of favipiravir and ribavirin against bunyavirsues. (Based on Gowen et al., 2007 and Buys et al., 2011).

Virus[a]	Strain	Favipiravir[b]			Ribavirin[b]		
		CC_{50} ± SD	EC_{50} ± SD	SI	CC_{50} ± SD	EC_{50} ± SD	SI
LACV	—	>1000 ± 0 (>6365 ± 0)	5.0 ± 2.0 (32 ± 13)	>199	877 ± 211 (3595 ± 864)	17 ± 12 (70 ± 49)	51
PTV	Adames	>1000 ± 0 (>6365 ± 0)	30 ± 5.0 (191 ± 32)	>33	898 ± 88 (3681 ± 360)	42 ± 22 (172 ± 90)	21
RVFV	MP-12	>980 ± 29 (>6257 ± 185)	5.0 ± 0.9 (32 ± 6)	>196	>906 ± 161 (>3714 ± 659)	13 ± 4 (53 ± 16)	>70
SFNV	Naples	>1000 ± 0 (>6365 ± 0)	18 ± 26 (115 ± 166)	>55	>729 ± 220 (>2989 ± 901)	22 ± 12 (90 ± 49)	>33
DOBV	Sotkamo	756 ± 104 (4816 ± 662)	10 ± 1.1 (93 ± 18)	52	296 ± 153 (1215 ± 628)	18 ± 0.6 (72 ± 2.4)	17
MPRLV	HV9021050	753 ± 186 (4795 ± 1186)	15 ± 2.8 (65 ± 17)	74	256 ± 33 (1051 ± 135)	11 ± 0.7 (47 ± 2.9)	22
PHV	MP40	600 ± 10 (3819 ± 64)	10 ± 4.1 (66 ± 26)	58	248 ± 211 (1018 ± 866)	5.6 ± 0.5 (23 ± 1.9)	44

[a] LACV, La Crosse virus; PTV, Punta Toro virus; RVFV, Rift Valley fever virus; SFNV, Sandfly fever virus; DOBV, Dobrava virus; MPRLV, Maporal virus; PHV, Prospect Hill virus.
[b] 50% cytotoxic concentration (CC_{50}) and 50% effective concentration (EC_{50}) values are in μg/ml as determined by CPE reduction assays using Vero cells, and SI (selectivity index) was calculated as CC_{50}/EC_{50}. The values noted in brackets are in μM.

concentrations of favipiravir were required, compared to influenza virus. The EC_{90} in YFV-infected Vero cells was 51.8 μg/ml (330 μM) in a yield-reduction assay and a confirmatory luciferase-based cell-viability assay (Julander et al., 2009a). The drug was effective when added 4, 8, or 12 h after virus challenge. In YFV-infected hamsters, favipiravir administered orally at 200 or 400 mg/kg/d for 8 days, beginning 4 h prior to virus exposure, significantly protected the animals against death (Julander et al., 2009a). Complete protection was also observed with a dose of 400 mg/kg/day when treatment initiated as late as 2 days after virus inoculation, and 8/10 animals survived when treatment was begun on day 3 ($P < 0.05$).

Favipiravir was also active against WNV *in vitro* and in rodents (Morrey et al., 2008). In Vero cells, the EC_{50}s were 53 ± 4 μg/ml (337 ± 25 μM). Twice-daily oral treatment with 200 mg/kg started 4 h after subcutaneous viral challenge protected nine out of ten mice from death ($P < 0.01$) and reduced viral protein expression and viral RNA in brain tissue at day 6 post exposure (Morrey et al., 2008). The minimal effective oral dose was calculated to be 20–65 mg/kg when given twice daily, beginning the day after challenge. More impressively, treatment begun on day 2 resulted in 8/10 survivors ($P < 0.05$). Prophylactic favipiravir also protected against WNV-infected hamsters (8/10 survivors, $P < 0.01$) (Morrey et al., 2008). Importantly, WNV envelope protein was not detected in brain tissue of treated animals 7 days after virus challenge.

5.4. Alphaviruses

Favipiravir has also demonstrated activity in Vero cells infected with Western equine encephalitis virus (WEEV), with an EC_{90} of 49 μg/ml (312 μM) (Julander et al., 2009b). Oral treatment of WEEV-infected mice with 400 mg/kg/d (twice daily for 7 days) begun 4 h prior to virus challenge resulted in significant improvement in survival ($P < 0.01$) and a prolongation of the time to death. However, the mean viral brain titer on day 4 was not significantly reduced, with only a 10-fold reduction in treated animals, and although signs of disease were relatively mild, they were not eliminated. However, a modest improvement in clinical signs such as the amelioration of weight loss and a significant protection against death suggest that favipiravir may be an effective treatment for other severe alphavirus infections.

In unpublished work presented at the recent 26th International Conference on Antiviral Research, (Jochmans et al., 2013) evaluated the activity of favipiravir against Chikungunya virus (CHIKV), and found that it was highly active against the virus in Vero cells and that it protected mice against lethal CHIKV infection. They also investigated genetic mutations related to the development of resistance of CHIKV cultured in the presence of favipiravir, and identified some mutations in the RdRp associated with several-fold changes in the EC50s.

5.5. Picornaviruses

Favipiravir inhibited the replication of foot-and-mouth disease virus (FMDV) *in vitro* with an EC_{50} equal to 14 μg/ml (89 μM) (Furuta et al., 2009). However, the analogues T-1106 and T-1105 were more active against this virus *in vitro*. Favipiravir also selectively inhibited poliovirus in Vero cells, with an EC_{50} of 4.8 μg/ml (31 μM) and a selectivity index of 29, and inhibited rhinovirus replication in HeLa cells, with an EC50 of 29 μg/ml (186 μM and an SI > 43) (Furuta et al., 2002).

5.6. Noroviruses

Favipiravir was recently shown to be active against murine norovirus, modestly inhibiting the development of CPE in cell culture with EC_{50}s of 39 ± 4 μg/ml (248 ± 25 μM) (Rocha-Pereira et al., 2012). Viral RNA synthesis, as measured by RT-qPCR, was also inhibited (EC_{50}s = 19 ± 6 μg/ml [121 ± 38 μM]). However, cytotoxicity in the RAW 264.7 mouse macrophage cell line was higher than that in Vero cells, resulting in an SI of 4.4 by CPE assay and 8.7 by RT-qPCR. Time-of-addition studies found that favipiravir exerted its maximum antiviral activity when added up to 4 h postinfection, correlating with the onset of viral RNA synthesis, suggesting that the viral RdRp was the target of inhibition.

6. Conclusion

As described above, studies in cell culture and in mouse models have shown that favipiravir has potent therapeutic efficacy against a broad range of influenza viruses, including highly pathogenic influenza A(H5N1) virus and viruses resistant to NA inhibitors. Synergistic activity was demonstrated in combination with NA inhibitors, suggesting that favipiravir will broaden the therapeutic options for the management of infections by highly pathogenic avian strains and for severely ill patients.

Favipiravir also inhibits the replication of many other RNA viruses including agents in the arenavirus, bunyavirus and

flavivirus families, including agents of viral hemorrhagic fever and encephalitis. The endemic areas for many of these pathogens have been gradually expanding, due to the effects of global warming and the increasing rate and speed of international travel. The limited treatment option for these diseases is a major concern for public health officials, especially since many of them could be used for bioterrorism. Ongoing and planned efficacy studies with favipiravir will therefore target a number of highly pathogenic bunyaviruses, including Rift Valley fever, Andes and Sin Nombre hantaviruses and Crimean Congo hemorrhagic fever virus. Evaluations of favipiravir's inhibitory activity against pathogenic arenaviruses, including Junin and Lassa, are currently under way or in the planning stages. The development of favipiravir as a novel therapy for influenza and for diseases caused by a range of highly pathogenic RNA viruses will bolster national security and global public health.

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References

Baranovich, T., Wong, S.S., Armstrong, J., Marjuki, H., Webby, R.J., Webster, R.G., Govorkova, E.A., 2013. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. J. Virol. 87, 3741–3751.

Buys, K.K., Jung, K.H., Smee, D.F., Furuta, Y., Gowen, B.B., 2011. Maporal virus as a surrogate for pathogenic New World hantaviruses and its inhibition by favipiravir. Antivir. Chem. Chemother. 21, 193–200.

Furuta, Y., Takahashi, K., Fukuda, Y., Kuno, M., Kamiyama, T., Kozaki, K., Nomura, N., Egawa, H., Minami, S., Watanabe, Y., Narita, H., Shiraki, K., 2002. In vitro and in vivo activities of anti-influenza virus compound T-705. Antimicrob. Agents Chemother. 46, 977–981.

Furuta, Y., Takahashi, K., Kuno-Maekawa, M., Sangawa, H., Uehara, S., Kozaki, K., Nomura, N., Egawa, H., Shiraki, K., 2005. Mechanism of action of T-705 against influenza virus. Antimicrob. Agents Chemother. 49, 981–986.

Furuta, Y., Takahashi, K., Shiraki, K., Sakamoto, K., Smee, D.F., Barnard, D.L., Gowen, B.B., Julander, J.G., Morrey, J.D., 2009. T-705 (favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. Antiviral Res. 82, 95–102.

Gowen, B.B., Smee, D.F., Wong, M.H., Hall, J.O., Jung, K.H., Bailey, K.W., Stevens, J.R., Furuta, Y., Morrey, J.D., 2008. Treatment of late stage disease in a model of arenaviral hemorrhagic fever: T-705 efficacy and reduced toxicity suggests an alternative to ribavirin. PLoS One 3, e3725.

Gowen, B.B., Wong, M.H., Jung, K.H., Sanders, A.B., Mendenhall, M., Bailey, K.W., Furuta, Y., Sidwell, R.W., 2007. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. Antimicrob. Agents Chemother. 51, 3168–3176.

Gowen, B.B., Wong, M.H., Jung, K.H., Smee, D.F., Morrey, J.D., Furuta, Y., 2010. Efficacy of favipiravir (T-705) and T-1106 pyrazine derivatives in phlebovirus disease models. Antiviral Res. 86, 121–127.

Itoh, Y., Shinya, K., Kiso, M., Watanabe, T., Sakoda, Y., Hatta, M., Muramoto, Y., Tamura, D., Sakai-Tagawa, Y., Noda, T., Sakabe, S., Imai, M., Hatta, Y., Watanabe, S., Li, C., Yamada, S., Fujii, K., Murakami, S., Imai, H., Kakugawa, S., Ito, M., Takano, R., Iwatsuki-Horimoto, K., Shimojima, M., Horimoto, T., Goto, H., Takahashi, K., Makino, A., Ishigaki, H., Nakayama, M., Okamatsu, M., Takahashi, K., Warshauer, D., Shult, P.A., Saito, R., Suzuki, H., Furuta, Y., Yamashita, M., Mitamura, K., Nakano, K., Nakamura, M., Brockman-Schneider, R., Mitamura, H., Yamazaki, M., Sugaya, N., Suresh, M., Ozawa, M., Neumann, G., Gern, J., Kida, H., Ogasawara, K., Kawaoka, Y., 2009. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 460, 1021–1025.

Jin, Z., Smith, L.K., Rajwanshi, V.K., Kim, B., Deval, J., 2013. The ambiguous base-pairing and high substrate efficiency of T-705 (favipiravir) ribofuranosyl 5′-triphosphate towards influenza A virus polymerase. PLoS One 8, e68347.

Jochmans, D., Segura, G.N., Delang, L., Pastprino, B., Querat, G., Dallmeier, K., Bello, F., Tas, A., Snijder, E., de Lamballerie, X., Martina, B., Froeyen, M., Neyts, J., van Hemert, M., Leyssen, P., 2013. Inhibition of Chikungunya virus replication by T-705 (favipiravir) and identification of resistance associated mutations in the RNA-dependent RNA polymerase. 26th International Conference on Antiviral Research Poster 169.

Julander, J.G., Shafer, K., Smee, D.F., Morrey, J.D., Furuta, Y., 2009a. Activity of T-705 in a hamster model of yellow fever virus infection in comparison with that of a chemically related compound, T-1106. Antimicrob. Agents Chemother. 53, 202–209.

Julander, J.G., Smee, D.F., Morrey, J.D., Furuta, Y., 2009b. Effect of T-705 treatment on western equine encephalitis in a mouse model. Antiviral Res. 82, 169–171.

Kiso, M., Takahashi, K., Sakai-Tagawa, Y., Shinya, K., Sakabe, S., Le, Q.M., Ozawa, M., Furuta, Y., Kawaoka, Y., 2010. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. Proc. Natl. Acad. Sci. USA 107, 882–887.

McCormick, J.B., King, I.J., Webb, P.A., Scribner, C.L., Craven, R.B., Johnson, K.M., Elliott, L.H., Belmont-Williams, R., 1986. Lassa fever. Effective therapy with ribavirin. N. Engl. J. Med. 314, 20–26.

Mendenhall, M., Russell, A., Juelich, T., Messina, E.L., Smee, D.F., Freiberg, A.N., Holbrook, M.R., Furuta, Y., de la Torre, J.C., Nunberg, J.H., Gowen, B.B., 2011a. T-705 (favipiravir) inhibition of arenavirus replication in cell culture. Antimicrob. Agents Chemother. 55, 782–787.

Mendenhall, M., Russell, A., Smee, D.F., Hall, J.O., Skirpstunas, R., Furuta, Y., Gowen, B.B., 2011b. Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. PLoS Negl. Trop. Dis. 5, e1342.

Moraz, M.L., Kunz, S., 2011. Pathogenesis of arenavirus hemorrhagic fevers. Expert Rev. Anti. Infect. Ther. 9, 49–59.

Morrey, J.D., Taro, B.S., Siddharthan, V., Wang, H., Smee, D.F., Christensen, A.J., Furuta, Y., 2008. Efficacy of orally administered T-705 pyrazine analog on lethal West Nile virus infection in rodents. Antiviral Res. 80, 377–379.

Naesens, L., Guddat, L., Keough, D., van Kuilenburg, A.B., Meijer, J., Voorde, J.V., Balzarini, J., 2013. Role of human hypoxanthine guanine phosphoribosyltransferase in activation of the antiviral agent T-705 (favipiravir). Mol. Pharmacol.. http://dx.doi.org/10.1124/mol.113.087247.

Rocha-Pereira, J., Jochmans, D., Dallmeier, K., Leyssen, P., Nascimento, M.S., Neyts, J., 2012. Favipiravir (T-705) inhibits in vitro norovirus replication. Biochem. Biophys. Res. Commun. 424, 777–780.

Sangawa, H., Komeno, T., Nishikawa, H., Yoshida, A., Takahashi, K., Nomura, N., Furuta, Y., 2013. Mechanism of action of T-705 ribosyl triphosphate against influenza virus RNA polymerase. Antimicrob. Agents Chemother. 2013. PMID:23917318 [Epub ahead of print] <http://aac.asm.org/content/early/2013/07/30/AAC.00649-13.full.pdf+html>.

Sheu, T.G., Deyde, V.M., Okomo-Adhiambo, M., Garten, R., Xu, X., Bright, R., Butler, E., Wallis, T.R., Klimov, A.I., Gubareva, L.V., 2008. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide in 2004–2008. Antimicrob. Agents Chemother. 52, 3284–3292.

Sidwell, R.W., Barnard, D.L., Day, C.W., Smee, D.F., Bailey, K.W., Wong, M.H., Morrey, J.D., Furuta, Y., 2007. Efficacy of orally administered T-705 on lethal avian influenza A (H5N1) virus infections in mice. Antimicrob. Agents Chemother. 51, 845–851.

Sleeman, K., Mishin, V.P., Deyde, V.M., Furuta, Y., Klimov, A.I., Gubareva, L.V., 2010. In vitro antiviral activity of favipiravir (t-705) against drug-resistant influenza and 2009 A(H1N1) viruses. Antimicrob. Agents Chemother. 54, 2517–2524.

Smee, D.F., Bailey, K.W., Wong, M.H., Barnard, D.L., Furuta, Y., Sidwell, R.W., 2007. In vivo virus-inhibitory effects of T-705 against influenza A and B virus strains. In: IX International Symposium on Respiratory Viral Infections. Hong Kong 2007; March. 3–6.

Smee, D.F., Hurst, B.L., Egawa, H., Takahashi, K., Kadota, T., Furuta, Y., 2009. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virus-infected cells. J. Antimicrob. Chemother. 64, 741–746.

Smee, D.F., Hurst, B.L., Wong, M.H., Bailey, K.W., Tarbet, E.B., Morrey, J.D., Furuta, Y., 2010. Effects of the combination of favipiravir (T-705) and oseltamivir on influenza A virus infections in mice. Antimicrob. Agents Chemother. 54, 126–133.

Streeter, D.G., Witkowski, J.T., Khare, G.P., Sidwell, R.W., Bauer, R.J., Robins, R.K., Simon, L.N., 1973. Mechanism of action of 1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. Proc. Natl. Acad. Sci. USA 70, 1174–1178.

Takahashi, K., Furuta, Y., Fukuda, Kuno M., Kamiyama, T., Kozaki, K., Nomura, N., Egawa, H., Minami, S., Shiraki, K., 2003. In vitro and in vivo activities of T-705 and oseltamivir against influenza virus. Antivir. Chem. Chemother. 14, 235–241.

Takahashi, K., Smee, D.F., Shiraki, K., Kawaoka, Y., Furuta, Y., 2011. Pharmacological effects of favipiravir, an anti-influenza viral drug.. J. Med. Pharm. Sci. 66, 429–441 (Japanese) Jpn..

Tarbet, E.B., Maekawa, M., Furuta, Y., Babu, Y.S., Morrey, J.D., Smee, D.F., 2012. Combinations of favipiravir and peramivir for the treatment of pandemic influenza A/California/04/2009 (H1N1) virus infections in mice. Antiviral Res. 94, 103–110.

Watanabe, T., Kiso, M., Fukuyama, S., Nakajima, N., Imai, M., Yamada, S., Murakami, S., Yamayoshi, S., Iwatsuki-Horimoto, K., Sakoda, Y., Emi Takashita, E., McBride, R., Noda, T., Hatta, M., Imai, H., Zhao, D., Kishida, N., Shirakura, M., de Vries, R.P., Shichinohe, S., Okamatsu, M., Tamura, T., Tomita, Y., Fujimoto, N., Goto, K., Katsura, H., Kawakami, E., Ishikawa, I., Watanabe, S., Ito, M., Yuko Sakai-Tagawa, Y., Sugita, Y., Uraki, R., Yamaji, R., Eisfeld, A.J., Zhong, G., Fan, S., Ping, J., Maher, E.A., Hanson, A., Uchida, Y., Saito, T., Ozawa, M., Neumann, G., Kida, H., Odagiri, T., Paulson, J.C., Hasegawa, H., Tashiro, M., Kawaoka, Y., 2013. Characterization of H7N9 influenza A viruses isolated from humans. Nature. http://dx.doi.org/10.1038/nature12392, Published online 10 July 2013.